

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

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

Lisa Shelley Argilla

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.

1.5	Study Aims	21
2.	Chapter 2: High Prevalence of <i>Leucocytozoon</i> spp. In the endangered yellow-eyed penguin (<i>Megadyptes antipodes</i>) in the sub-Antarctic regions of New Zealand	22
2.1	Abstract	22
2.2	Introduction	23
2.3	Materials and Methods	25
2.3.1	Study sites	25
2.3.2	Collection of blood samples and blood smear preparation	25
2.3.3	Post-mortem sample analysis and collection	26
2.3.4	Molecular Studies	26
2.3.5	Phylogenetic Analysis of <i>Leucocytozoon</i> isolates	27
2.3.6	Statistical Analysis	27
2.3.7	Ethics Approval and permits	28
2.4	Results	28
2.4.1	Clinical Findings	28
2.4.2	Pathological Findings	28
2.4.3	Blood smears	31
2.4.4	Molecular Analysis	31
2.4.5	Phylogenetic Analysis of <i>Leucocytozoon</i> isolates	32
2.5	Discussion	36
3.	Chapter 3: Risk factors for mortality in endangered yellow-eyed penguin (<i>Megadyptes antipodes</i>) chicks at three separate breeding locations during the 2008/09 breeding season	40
3.1	Abstract	40
3.2	Introduction	40
3.3	Materials and Methods	43
3.3.1	Locations	43
3.3.2	Regions within locations	43
3.3.3	Age at death	44
3.3.4	Temperature	44
3.3.5	<i>Leucocytozoon</i> infection status	44

3.3.6	Body Condition	46
3.3.7	Nest Monitoring	46
3.3.8	Human Impact	47
3.3.9	Diphtheritic Stomatitis	47
3.3.10	Statistical Analysis	47
3.4	Results	48
3.4.1	Location	48
3.4.2	Chick Age	48
3.5	Otago Peninsula	51
3.5.1	Temperature	51
3.5.2	Nest Vegetation Type	54
3.5.3	Nest Cover	55
3.5.4	Human Impact	56
3.5.5	Diphtheritic Stomatitis	57
3.5.6	Regions	58
3.6	Enderby Island	60
3.6.1	Temperature	60
3.6.2	<i>Leucocytozoon</i> infection status	60
3.6.3	Parental <i>Leucocytozoon</i> infection status	60
3.6.4	Parental Body Condition Score	60
3.6.5	Regions	61
3.7	Discussion	63
4.	Chapter 4: General Discussion	69
4.1	Introduction	69
4.1.1	<i>Leucocytozoon</i> in yellow-eyed penguins across their range	69
4.1.2	Assessment of risk factors for mortality in yellow-eyed penguin chicks	70
4.2	Scope and limitations	70
4.3	Implications of the study	71
5.	References	77
6.	Appendices	84

List of pictures and figures	Page
 CHAPTER 1	
Figure 1.1: Population distribution of yellow-eyed penguins (<i>Megadyptes antipodes</i>)	5
 CHAPTER 2	
Figure 2.1: Distribution of number and date of death (A) and age of death (B) for yellow eyed penguin chicks (<i>Megadyptes antipodes</i>) on Enderby Island from November 2008 to January 2009	29
Figure 2.2: Haematoxylin and eosin-stained tissues from a yellow-eyed penguin chick (<i>Megadyptes antipodes</i>). Mature exo-erythrocytic meronts of <i>Leucocytozoon</i> spp. in the spleen (A), liver (B) and thyroid (C)	30
Figure 2.3: Gametocytes of <i>Leucocytozoon</i> spp. in host cells identified from Giemsa-stained blood smear of a yellow-eyed penguin (<i>Megadyptes antipodes</i>)	31
Figure 2.4: Phylogenetic analysis of <i>Leucocytozoon</i> spp. isolated from yellow-eyed penguins (<i>Megadyptes antipodes</i>)	35
 CHAPTER 3	
Figure 3.1: Yellow-eyed penguin (<i>Megadyptes antipodes</i>) breeding sites and study regions for the 2008/09 breeding season on the South Island of New Zealand	45
Figure 3.2: Yellow-eyed penguin (<i>Megadyptes antipodes</i>) breeding sites and study regions for the 2008/09 breeding season on Enderby Island	45
Figure 3.3: The effect of location on mortality of yellow-eyed penguin chicks (<i>Megadyptes antipodes</i>) in the 2008/09 breeding season	49

Figure 3.4:	Epidemic curve of yellow eyed penguin (<i>Megadyptes antipodes</i>) chick mortality for Enderby Island during the 2008/2009 breeding season	49
Figure 3.5:	Epidemic curve of yellow eyed penguin (<i>Megadyptes antipodes</i>) chick mortality for the Otago Peninsula during the 2008/2009 breeding season	50
Figure 3.6:	Epidemic curve of yellow-eyed penguin (<i>Megadyptes antipodes</i>) chick mortality for The Catlins during the 2008/2009 breeding season	50
Figure 3.7:	Survival probability for yellow eyed penguin (<i>Megadyptes antipodes</i>) chicks at three locations (Catlins, Otago Peninsula and Enderby Island) during the 2008/2009 breeding season. The shaded areas represent the (95% confidence intervals)	52
Figure 3.8:	Hazard function curves for mortality of yellow eyed penguin (<i>Megadyptes antipodes</i>) chicks in the 2008/2009 breeding season at three locations (Catlins, Otago Peninsula and Enderby Island).	52
Figure 3.9:	Survival probability (A) and Hazard Function curves (B) for yellow-eyed penguin (<i>Megadyptes antipodes</i>) 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.	53
Figure 3.10:	Effect of nest type on mortality rate of yellow-eyed penguin (<i>Megadyptes antipodes</i>) 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)	54
Figure 3.11:	Effect of nest cover (open vs closed) on mortality rate of yellow-eyed penguin (<i>Megadyptes antipodes</i>) 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).	55

- 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 ($P<0.001$). 56
- 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 ($P<0.001$) 57
- 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 ($P<0.001$). 58
- 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 ($P<0.011$). 61

List of Tables

Page

CHAPTER 1

Table 1.1:	Suggested Classification of Yellow-eyed Penguin Breeding Seasons in Terms of Survival and Breeding Success (Modified from Moore, 1994).	16
-------------------	---	----

CHAPTER 2

Table 2.1:	Prevalence of <i>Leucocytozoon</i> spp. by light microscopy, and PCR of blood and tissue samples	33
-------------------	--	----

Table 2.2:	The sequence divergence (as a percentage) between 15 mitochondrial cytochrome b gene lineages of avian <i>Leucocytozoon</i> spp.	34
-------------------	--	----

CHAPTER 3

Table 3.1:	Odds ratios for factors implicated in the mortality of yellow eyed penguin chicks (<i>Megadyptes antipodes</i>) on Otago Peninsula in the 2008/2009 breeding season.	59
-------------------	--	----

Table 3.2:	Odds ratios for factors implicated in the mortality of yellow eyed penguin chicks (<i>Megadyptes antipodes</i>) on Enderby Island in the 2008/2009 breeding season.	62
-------------------	---	----

List of Appendices		Page
Appendix A	Additional images of haematoxylin and eosin stained tissues showing histopathological stages (meronts) of <i>Leucocytozoon</i> spp. in multiple organs from a dead yellow-eyed penguin chick.	84
Appendix A1	Histological sections of the heart (I –II), small intestine (III-IV) and kidney (V-VI) of a yellow-eyed penguin (<i>Megadyptes antipodes</i>) chick showing mature exo-erythrocytic meronts of <i>Leucocytozoon</i> spp.	84
Appendix A2	Histological sections of the liver (I –II), lung (III-IV) and pancreas (V-VI) of a yellow-eyed penguin (<i>Megadyptes antipodes</i>) chick showing mature exo-erythrocytic meronts of <i>Leucocytozoon</i> spp.	85
Appendix B	Avian malaria (<i>Leucocytozoon</i>) nested PCR	86
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.	87
Appendix C1	Data from Enderby Island including <i>Leucocytozoon</i> analysis	87
Appendix C2	Risk factor data from all 3 locations	89
Appendix C3	Survival data with weather data	108
Appendix D	Summary of necropsy findings of dead chicks from Otago and Endeby Island	116
Appendix D1	Table summarizing gross and histopathological findings.	116
Appendix D2	Gross pathology images from selected Otago penguin chicks	117
Appendix D3	Gross pathology images from selected Enderby Island penguin chicks	118
Appendix E	Information required for investigation of mass mortality events in yellow-eyed penguins	120