

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.

**Microbial Infection of Avian Eggs:
A Threat to All Synchronously Incubating Species?
Case Study of New Zealand's Little Blue Penguin
(*Eudyptula minor*)**

A thesis presented in partial fulfilment of the requirements for the degree of
Master of Science in Conservation Biology
at Massey University, Auckland
New Zealand

Anne-Sophie Boyer
2010



Tiritiri Matangi Island, New Zealand

Abstract

Microbial infection of eggs was originally investigated in terms of human health only. Recently, however, it was found that it can also cause early embryo mortality in birds, mainly through trans-shell infection prior to incubation. Trans-shell infection is highly dependent upon environmental conditions, egg temperature and egg properties such as shell quality and antimicrobial defences. Microbial infection of eggs is more likely to occur in synchronously incubating species as first laid eggs can be exposed for up to several days prior to full incubation. One example of a population that seems at particular risk of egg microbial infection is New Zealand's little blue penguin (*Eudyptula minor*) from Tiritiri Matangi Island. This bird lays two eggs on average three days apart, and is believed to begin full incubation only after the second egg has been laid. Both eggs are laid in particularly humid and soiled nests and contain only low levels of lysozyme, an important antimicrobial protein. The aims of this study were therefore to 1) obtain a first examination of the rates of shell and trans-shell microbial infection of chicken eggs in New Zealand and assess the effects of cleaning on those rates, 2) investigate the role of microbes in hatching failure of little blue penguin eggs and 3) investigate other factors affecting little blue penguin egg viability. This study revealed that shell infection in chicken eggs significantly increased with exposure and significantly decreased with cleaning; however, trans-shell infection was only marginally affected by exposure and cleaning. On Tiritiri Matangi Island, Hauraki Gulf, New Zealand, nest type, egg order and shell cleaning did not affect hatching success, suggesting that nest conditions and microbial infection prior to incubation were not a major cause of egg mortality in this population. Temporary abandonment during incubation, however, was very frequent in the second half of the breeding season and

fatal to most eggs. These temporary abandonments seemed to be caused by resource limitations, an aspect that should be investigated in future studies.

Acknowledgements

A great thank you to all the people who helped and supported me during this year. Thanks to my supervisor Dianne Brunton who encouraged me to study little blue penguins. Thank you for your support, your advice, your words of encouragement and thank you for finding two great field assistants to help me! Naomi and Peter, this study would not have been the same without you. Thank you very much for your patience, your long hours of walking and searching on Tiri and your devotement to this project. I couldn't have asked for better volunteers.

Thank you to my co-supervisor Paul Rainey for his advice and for allowing me to use his lab and to Xue-Xian Zhang for patiently showing me how to plate my samples. And of course, a big thank you to Chris Rodley for going out of his way to find me incubators...

Also, thank you to all the people who helped on Tiri. Thanks Mary-Ann, Dave and James for keeping an eye on the penguins and for making sure my volunteers and I returned safely each night.

Great thanks to Dianne Brunton, Mark Seabrook-Davison and Sarah Dwyer for reviewing my drafts and providing useful comments. A big thank you to Marleen Baling and the ecology lab for their help, support and cakes, with a special thanks to the building 86 crowd. Finally, a big thank you to Chris and my family (and Inghams Chicken!) for their support.

This research was approved by the Department of Conservation (permit AK-25488-FAU) and funded in part by the Ecology and Conservation Group, Massey University.

Table of Contents

ABSTRACT	III
ACKNOWLEDGMENTS.....	V
LIST OF PLATES.....	X
LIST OF FIGURES.....	XI
LIST OF TABLES.....	XII
CHAPTER 1. General overview	1
1.1 Introduction	2
1.2 Conservation significance of this research	5
1.3 Aims of this research.....	7
CHAPTER 2. Avian egg structure and microbial	8
Abstract.....	9
2.1 Introduction	9
2.2 Egg structure	11
2.2.1 Shell	11
2.2.2 Membranes	12
2.2.3 Egg contents	13
2.3 Microbial infection.....	15
2.3.1 Routes of transmission	15
2.3.2 Factors affecting transmission	17
2.3.2.1 Quantity and types	17
2.3.2.2 Temperature and humidity	19
2.3.2.3 Shell and membrane quality	19
2.3.2.4 Chemical defences	21
2.3.4 Consequences of infection.....	22
CHAPTER 3. Shell and trans-shell microbial infection of chicken eggs	25
Abstract.....	26
3.1 Introduction	27
3.2 Aims	29
3.3 Methods.....	30
3.3.1 Study species, site and time	30
3.3.2 Egg collection and artificial nests	30
3.3.3 Experimental design.....	31
3.3.3.1 Egg swabbing	32
3.3.3.2 Egg cleaning.....	33
3.3.4 Egg content analysis.....	33
3.3.4.1 Egg opening	33
3.3.4.2 Targeted microbes	34

3.3.4.3 Plating and colony counts	35
3.3.5 Shell size and thickness	35
3.3.6 Statistical analysis	36
3.4 Results	37
3.4.1 Nest conditions	37
3.4.2 Infection at laying	38
3.4.3 Treatments	38
3.4.3.1 Impact of exposure	38
3.4.3.1.1 Shell infections	38
3.4.3.1.2 Membrane, albumen and yolk infections.....	40
3.4.3.2 Impact of cleaning	41
3.4.3.2.1 Shell infections	41
3.4.3.2.2 Membrane, albumen and yolk infections.....	42
3.4.4 Shell and trans-shell infection.....	43
3.4.5 Impact of temperature and humidity on shell and trans-shell infections.....	45
3.4.6 Shell size and thickness	46
3.4.7 Fungal infections.....	46
3.5 Discussion	47
3.5.1 Exposure and cleaning.....	47
3.5.2 Nest types and environmental conditions	48
3.5.3 Shell thickness	49
3.5.4 Fungal infections.....	50
3.5.5 Conclusions	51
3.6 Appendix	52
3.6.1 Shell and membrane microbial infection	52
3.6.2 Correlation between temperature, relative humidity and microbial infection	53
 CHAPTER 4. Effect of nest type, microbial infection and exposure on the viability of little blue penguin (<i>Eudyptula minor</i>) eggs on Tiritiri Matangi Island.....	54
Abstract.....	55
4.1 Introduction	56
4.1.1 Background information about little blue penguins	56
4.1.1.1 Sub species and status.....	56
4.1.1.2 Breeding ecology	57
4.1.1.3 Feeding ecology.....	59
4.1.1.4 Threats	60
4.1.2 Reasons for choosing this study species	60
4.2 Aims.....	61
4.3 Methods.....	62
4.3.1 Study site	62
4.3.1.1 Study site and time	62
4.3.1.2 Sampling area.....	62
4.3.2 Survey methods.....	64

4.3.2.1 Nest searches	64
4.3.2.2 Potential and breeding nests	64
4.3.2.3 Nest types	65
4.3.3 Data collection	67
4.3.3.1 Nest conditions	67
4.3.3.2 Nest monitoring	67
4.3.3.3 Egg and chick removal	68
4.3.4 Definitions	69
4.3.4.1 Lay, hatching and fledging dates and incubation period	69
4.3.4.2 Failed nests	71
4.3.5 Experimental design	73
4.3.5.1 Egg extraction, labeling, measuring and swabbing	74
4.3.5.2 Treatments	75
4.3.5.3 Targeted microbes and plating	75
4.3.5.4 Egg analysis	75
4.3.6 Data analysis	76
4.3.6.1 Defining reproductive success	76
4.3.6.2 Microbial infection	77
4.3.6.3 Statistical analysis	77
4.4 Results	78
4.4.1 Lay date and conditions	78
4.4.2 Nesting attempts	79
4.4.2.1 Potential nests, breeding nests and RDB	79
4.4.2.2 Nest location and type	80
4.4.3 Egg laying and hatching	81
4.4.3.1 Incubation length	81
4.4.3.2 Laying and hatching intervals	82
4.4.3.3 Egg properties	82
4.4.4 Nest conditions	83
4.4.5 Breeding success	83
4.4.6 Egg failure	85
4.4.6.1 Stage of failed eggs	86
4.4.6.2 Factors affecting egg failure	87
4.4.7 Causes of chick mortality	91
4.5 Discussion	93
4.5.1 Lay date and conditions	93
4.5.2 Breeding attempts	94
4.5.3 Egg laying, hatching and incubation	95
4.5.4 Egg size	96
4.5.5 Nest types	97
4.5.6 Microbes and cleaning	98
4.5.7 Parental abandonment and food limitations	98

4.5.8 Considerations and limitations.....	101
4.5.9 Conclusions	101
CHAPTER 5. General summary and recommendations for future management.....	103
5.1 General summary.....	104
5.1.1 Causes and consequences of microbial infection	104
5.1.2 Effect of other factors on LBP egg viability	105
5.2 Future studies and management recommendations.....	105
5.2.1 Little blue penguins.....	105
5.2.1.1 Foraging studies	105
5.2.1.2 Egg removal	107
5.2.1.3 Eggshell structure and chemical defences	108
5.2.1.4 Incubation behaviour	108
5.2.2 Microbial infection in other avian species	109
REFERENCES	111

LIST OF PLATES

PLATE 1.1 LITTLE BLUE PENGUIN ATTEMPTING TO INCUBATE AN EGG WHILE RAISING A CHICK ON TIRITIRI MATANGI ISLAND, NEW ZEALAND	1
PLATE 2.1 PHOTO OF A CHICKEN EGG SHOWING ITS POROUS SHELL	8
PLATE 3.1 CHICKEN EGGS AND DATALOGGER IN ARTIFICIAL BURROWS	25
PLATE 3.2 ARTIFICIAL BURROWS USED FOR THIS STUDY	31
PLATE 3.3 EXAMPLE OF A TSA PLATE (LEFT) AND A MAC PLATE (RIGHT)	35
PLATE 4.1 LITTLE BLUE PENGUIN INCUBATING EGGS IN A SOIL NEST ON TIRITIRI MATANGI ISLAND	54
PLATE 4.2 EVIDENCE OF WEIGHT DIFFERENCES BETWEEN THE YOUNGEST AND OLDEST CHICKS FROM THE SAME CLUTCH THAT HATCHED ONE DAY APART	92
PLATE 4.3 LITTLE BLUE PENGUIN THAT HAS PUSHED THE YOUNGEST CHICK AWAY FROM THE NEST AND IS BROODING THE OTHER CHICK, ONE DAY OLDER	92
PLATE 5.1 LITTLE BLUE PENGUIN CHICK ON TIRITIRI MATANGI ISLAND	102

LIST OF FIGURES

FIGURE 2.1. DRAWING OF A RADIAL SECTION OF AN EGG SHELL.....	12
FIGURE 2.2. DRAWING OF CUT-AWAY SECTIONS SHOWING THE DIFFERENT LAYERS INSIDE A CHICKEN EGG	14
FIGURE 2.3. DIAGRAM OF THE YELLOW AND WHITE YOLK LAYERS IN AN UNINCUBATED EGG	15
FIGURE 2.4. DIAGRAM SHOWING THE STAGES OF EGG FORMATION, TIME SPENT AT EACH SITE AND STAGES AT WHICH MICROBIAL INFECTION CAN OCCUR.....	16
FIGURE 2.5. SEQUENCE OF EVENTS LEADING TO THE GENERALIZED INFECTION OF EGG CONTENTS	17
FIGURE 2.6. IMAGE (X965) OF THE INNER (I) AND OUTER (O) SHELL MEMBRANES AND CALCIUM DEPOSITS (C) ASSOCIATED WITH THE LATTER	21
FIGURE 3.1. MAP OF NEW ZEALAND SHOWING THE LOCATION OF THIS STUDY	30
FIGURE 3.2. MEAN TEMPERATURE (GREY) AND RELATIVE HUMIDITY (BLACK) IN EACH NEST, OVER THE WHOLE STUDY PERIOD (\pm SE)	37
FIGURE 3.3. PERCENTAGE OF EGGS SHOWING INFECTION ON EITHER TSA PLATES OR MAC PLATES (A) IN EACH LAYER, (B) AFTER EACH EXPOSURE PERIOD	39
FIGURE 3.4. EFFECTS OF DURATION OF EXPOSURE ON MICROBIAL GROWTH ON THE EGG SHELL (a,b) AND MEMBRANE (A,B) OF CLEANED (GREY) AND UNCLEANED (BLACK) EGGS.	41
FIGURE 3.5 MICROBIAL GROWTH ON EGG SHELLS ON CLEANED (GREY) AND UNCLEANED (BLACK) EGGS AFTER 1 (A, a), 3 (B, b) AND 5 (C, c) DAYS OF EXPOSURE	43
FIGURE 3.6. SHELL (BLACK) AND MEMBRANE (GREY) CFU COUNTS AFTER 1, 3, AND 5 DAYS OF EXPOSURE	44
FIGURE 3.7. RELATIONSHIP BETWEEN MEAN CFU COUNTS (LOG TRANSFORMATION) ON EGG SHELLS OF CLEANED (C-WHITE POINTS) AND UNCLEANED (N-GREY POINTS) AND TEMPERATURE (A,a) AND RELATIVE HUMIDITY (B,b).	45
FIGURE 4.1 MAP OF NEW ZEALAND (A) SHOWING A CLOSE UP OF THE HAURAKI GULF (B) AND TIRITIRI MATANGI ISLAND (C) WITH ITS SURVEYED AREAS.	63
FIGURE 4.2 LBP ARTIFICIAL NEST BOX (A), SOIL NEST (B), ROCK NEST (C) AND VEGETATION NEST (D).	66
FIGURE 4.3 LBP EMBRYONIC DEVELOPMENTAL STAGES	72
FIGURE 4.4 LBP EGG LABELLING, SWABBING AND WEIGHING	74
FIGURE 4.5 LBP NEST COUNT ACCORDING TO THEIR LAY DATE AND AVERAGE SST ASSOCIATED WITH EACH TWO-WEEK PERIOD PRE-LAYING, DURING LAYING AND POST LAYING.	78
FIGURE 4.6 MONTHLY AVERAGE SST CORRESPONDING TO THE INITIAL LAY DATE OF LBP ON TIRITIRI MATANGI ISLAND FROM 1975 TO 2009.....	79
FIGURE 4.7 DATES OF FIRST, SECOND AND THIRD BREEDING ATTEMPTS OF REPLACEMENT BREEDING LITTLE BLUE PENGUINS	80
FIGURE 4.8 MAP OF TIRITIRI MATANGI ISLAND SHOWING THE LOCATION OF LBP NESTS	80
FIGURE 4.9 COMPARISON OF NEST TYPES BETWEEN EARLY (GREY) AND LATE (BLACK) LBP NESTS	74
FIGURE 4.10 AVERAGE (\pm SE) LBP INCUBATION LENGTH IN SOIL NESTS (N=4), ROCK NESTS (N=10) AND VEGETATION NESTS (N=2)	82
FIGURE 4.11 CAUSES OF EGG FAILURE OF EARLY (GREY) AND LATE (BLACK) LBP EGGS (N=56) OVER THE 2009 BREEDING SEASON ON TIRITIRI MATANGI ISLAND	86
FIGURE 4.12 EMBRYONIC DEVELOPMENTAL STAGE OF EARLY (GREY) AND LATE (BLACK) FAILED LBP EGGS	87
FIGURE 4.13 AVERAGE (\pm SE) INITIAL SHELL CFU COUNTS FROM TSA (GREY) AND MAC (BLACK) PLATES FOR EACH NEST TYPE	88

LIST OF TABLES

TABLE 3.1 DISTRIBUTION OF EGGS BETWEEN CLEANING TREATMENTS, EXPOSURE PERIODS AND NESTS	32
TABLE 4.1 NEST TYPES USED BY LBP AND THEIR DESCRIPTION AND LOCATION ON TIRITIRI MATANGI	67
TABLE 4.2 DEFINITION REPRODUCTIVE PARAMETERS USED IN THIS STUDY	76
TABLE 4.3 COMPARISONS OF LENGTH, WIDTH, WEIGHT AND VOLUME OF FIRST-LAID AND SECOND-LAID EGGS IN TWO-EGG CLUTCHES OF LITTLE BLUE PENGUINS ..	83
TABLE 4.4 COMPARISONS OF LBP APPARENT BREEDING SUCCESS ON TIRITIRI MATANGI ISLAND OVER THE LAST FIVE YEARS.	84
TABLE 4.5 LITTLE BLUE PENGUIN REPRODUCTIVE SUCCESS (% \pm 95% CONFIDENCE INTERVALS) ON TIRITIRI MATANGI ISLAND OVER THE 2009 BREEDING SEASON.	85
TABLE 4.6 COMPARISONS OF AVERAGE CFU COUNTS (LOG) BETWEEN CLEANED AND UNCLEARED LBP EGGS THAT HATCHED AND FAILED.	89
TABLE 4.7 COMPARISONS OF LENGTH, WIDTH, WEIGHT, VOLUME AND SHELL THICKNESS OF HATCHED AND FAILED LITTLE BLUE PENGUIN EGGS	90
TABLE 4.8 NUMBER OF DAYS THAT EGGS WERE INCUBATED, TEMPORARILY ABANDONED, AND INCUBATED AGAIN AND THE ESTIMATED AGE AT WHICH THE EMBRYO CEASED TO DEVELOP	91