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.

## The time course of corticosterone responses in kororā (little penguin, *Eudyptula minor*)

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

Master of Science
In Zoology at
Massey University

Kar Hui (Kelly) Long 2017

## Abstract

When birds and other vertebrates perceive a situation to be threatening the hypothalamopituitary-adrenal (HPA) axis is activated and glucocorticoid hormones are secreted from the
adrenal gland. Activation of the HPA axis in response to a stimulus perceived to be
threatening is called a stress response. The main glucocorticoid hormone in birds is
corticosterone. Corticosterone responses of birds are typically measured by the collection of
an initial blood sample when a bird is captured or picked up, then the collection of further
blood samples until 30 to 60 minutes has elapsed, at which time the bird is released. Whilst
this standard sampling protocol provides information on the size of the corticosterone
response, it does not provide any indication of how long it takes for corticosterone
concentrations to return to initial values. The main objective of this thesis was to characterise
the total duration of the corticosterone response of free-living kororā (little penguins,

Eudyptula minor).

Little penguins at Oamaru were picked up from their nestboxes and initial blood samples collected. Birds were handled and then restrained by being placed in a box. Further blood samples were collected 15, 30 and 60 min after the birds were first picked up. Birds were then returned to their nest boxes and an additional blood sample collected 15, 30, 60, 120, 240, or 360 min later. Mean corticosterone concentrations declined to initial values two hours after birds were returned to nest boxes. The rates at which corticosterone concentrations increased when a stressor was present and then decreased when the stressor was no longer present were positively correlated. Seasonal changes in corticosterone responses in little penguins were also investigated in this study. Mean corticosterone responses were similar in winter and in the pre-laying period, whereas mean responses were lower in birds during early chick rearing. Corticosterone responses during the pre-laying

period were greater in male than female little penguins. The current study is the first to document the complete corticosterone responses of free-living penguins and provides information about changes of corticosterone concentrations after a stressor is removed from the free-living individuals. It is also the first to reveal that free-living penguins with relatively high corticosterone responses to a stressor had relatively high rates of corticosterone decline.

## Acknowledgements

First of all, I would like to thank Professor John Cockrem and Professor Murray Potter for providing guidance and help throughout this study. Thank you for your guidance on experimental design, write-up and statistical analysis. Thank you for teaching me how to do and think like a scientist, a researcher. Special thanks to Professor John Cockrem providing this special chance and financial support of working with little penguins.

I would also like to thank Philippa Agnew and the staff from the Oamaru Blue Penguin Colony. Thank you for allowing us to work in the colony and providing a warm and welcoming environment. Thank you Philippa for providing support and advice regarding birds handling skills and experimental design. The penguin shows were awesome and your passion in conservation work inspires me a lot.

Thank you my group mates, Rachel Choi, Shelley Ogle and Henry Elsom for providing help in this study. Thank you for the company down in Oamaru and helping me to collect blood samples from the little penguins. Those were great memories and experience that I will never forget for my whole life.

A special thanks to Jane Candy for doing the corticosterone assay for us. Without your help, I will not able to produce the result section of this thesis.

Thank you for the Ecology Bursary for granting me a scholarship, so that I could cut down on my working hours and focus on my assignments. Thank you to the Institute of Veterinary and Biomedical Sciences for funding for this project and for attendance at the NZ bird conference in 2016. It was my first conference and I learnt a lot from the conference.

Thank you to my family for encouraging me throughout the process and always having faith in me. Thank you to everyone who provided mental support and helped push me through. Specially thank you to my mom for coming to New Zealand just so that I can focus on writing my thesis.

I would also like to thank you my boyfriend, Alan, for providing great support throughout this study. Without you in the first year of my Master, I would not be able to continue and finish this study. Thank you for letting me rant, cooking for me when I am depressed and being unreasonable. Thank you for reminding me I have to take a break from time to time, which helped me in progressing and working more efficiently.

Thank you Xue Qi, Rachel Choi, Mari Nakano, Angel Pat, and Lewis Ho for spending time with me in the library. Thank you to my flatmates for keeping the flat a good environment for study. I would also like to thank all my friends in Malaysia and New Zealand who encouraged me through email and messages.

Finally, thank you to all the little penguins from Oamaru that provided blood samples (although involuntarily) for the study. Thank you for your invaluable contribution to science.

## **Table of contents**

Abstract	i
Acknowledgements	iii
Table of contents	V
Chapter 1: General introduction	1
1.1 Introduction	1
1.2 Stress in animals	3
1.2.1 Stress	3
1.2.2 Stressor	3
1.2.3 Stress system	4
1.2.4 Stress responses	5
1.3 Hypothalamic-pituitary-adrenal (HPA) axis	7
1.3.1 Overview	7
1.3.2 The hypothalamus	7
1.3.3 The pituitary gland	8
1.3.4 The adrenal gland	9
1.3.5 Glucocorticoids	9
1.4 The avian corticosterone response	14
1.4.1 Individual variation and repeatability of corticosterone responses	16
1.4.2 Seasonal changes in corticosterone responses	18
1.4.3 Other factors that affect corticosterone responses	21
1.5 Negative feedback of corticosterone	22
1.5.1 Physiological mechanism	23

1.5.2 The efficacy of negative feedback	30
1.6 Corticosterone responses of penguins	37
1.7 Outline of thesis	42
Chapter 2: Experimental studies of the corticosterone responses of kororā	43
2.1 Introduction	43
2.2 Materials and methods	45
2.2.1 Study site and animals	45
2.2.2 Blood sampling	46
2.2.3 Study design	47
2.2.4 Corticosterone radioimmunoassay	48
2.2.5 Statistics	48
2.3 Results	50
2.3.1 Corticosterone concentrations in initial samples	50
2.3.2 Individual variation in corticosterone responses to 60 min handling and resfollowed by return of birds to their nestbox	
2.3.3 Duration of corticosterone responses	51
2.3.4 Corticosterone in last sample in relation to corticosterone at 60 min	58
2.3.5 Rate of change of corticosterone and integrated corticosterone responses	59
2.3.6 Breeding stages	59
2.3.7 Corticosterone responses of male and female penguins	63
2.4 Discussion	65
2.4.1 Sampling time and initial corticosterone concentrations	65
2.4.2 Total duration of corticosterone responses of little penguins	67
2.4.3 Individual variation in corticosterone responses	70
2.4.4 The rate of decline of corticosterone concentrations	71
2.4.5 Corticosterone responses of pre-breeding and breeding birds	72

2.4.6 Sex differences in corticosterone responses	74
2.5 Conclusion	75
Chapter 3 General discussion	76
3.1 Introduction	76
3.2 Major conclusions	78
3.3 Future studies	79
Appendix	81
References	86