

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.

**Faecal steroid measurements for the assessment  
of reproductive function in Japanese quail  
(*Coturnix coturnix japonica*) and kakapo (*Strigops  
habroptilus*).**

A thesis presented in partial fulfilment of the requirements for the  
degree of  
Masters of Science  
in Physiology  
at Massey University, Palmerston North,  
New Zealand.

**Emma Jane Hawke**

**2002**

---

## Abstract

The kakapo (*Strigops habroptilus*) is an endangered parrot endemic to New Zealand and little is known of its reproductive physiology. Reproductive function is conventionally determined by the measurement of reproductive steroids in plasma samples. This is impractical and invasive in endangered, free-living species. However, the measurement of reproductive steroids in avian faecal samples is practiced. Few studies have documented strong relationships between faecal and plasma steroid concentrations. The objectives of this study were to develop and validate a faecal extraction method for the measurement of oestradiol, progesterone and testosterone in Japanese quail (*Coturnix coturnix japonica*); determine the relationships between steroid concentrations and gonadal development in quail; and define annual faecal hormone cycles of kakapo in relation to their breeding status.

Groups of male and female quail were held on different photoperiodic and temperature regimes to produce birds with a range of gonad sizes and steroid concentrations. Steroid concentrations were measured in faeces and plasma by radioimmunoassay. Positive relationships were demonstrated between plasma and faecal steroid concentrations. Faecal steroid concentrations had strong positive relationships with ovary and testis size in female and male quail respectively.

The extraction method developed was then applied to faecal samples, which were collected from kakapo in their free-living environment on Whenua Hou (Codfish Island). The samples were collected from identified birds over three potential breeding seasons. There were annual cycles of hormone concentrations that corresponded with cycles of breeding activity in females and males. No significant differences were found between breeding and non-breeding years for faecal concentrations of all three hormones, suggesting that kakapo undergo a degree of gonadal development each year. Annual hormone profiles for individual birds supported this finding.

This study quantifies the value of collecting multiple faecal samples in both captive and wild situations and demonstrates the power and value of faecal steroid analysis.



*Author with Sirocco, photo: R.Cole.*

---

## Acknowledgements

I would like to thank my supervisor Dr. John Cockrem and IVABS for their support throughout this project and especially for allowing me the freedom to pursue my other passion - climbing.

I would also like to thank the Department of Conservation, in particular Paul Jansen and Graeme Elliot for the opportunity to work with such a special bird and experience the culture of a conservation island community. Special thanks to Ros Cole, who taught me so much about kakapo and Whenua Hou, Phred Dobbins and the 'vollies' who made my 'island experience' filled with hilarity and enjoyment.

Thanks to my fellow CERG students for your help and perspectives and a special thanks to Wei – at least Peter will get some work done now huh? Thanks to Jane Candy for your help with the world of RIA, and Heather Hesterman for your advice on faecal extraction methods.

Thanks to Dr. Kate Maguire and Dr. Bruce MacKay for your statistical expertise, especially Kate for being patient and explaining things to me more than once. Now I know why you work on apples rather than endangered wild animals, aside from the fact that you can snack while you work!

Special thanks to my family and climbing friends – although somewhat annoying at times, your relentless “how’s your thesis going?” was encouraging when I needed it. Most of all, I’d like to thank James. Thank you for putting up with me and looking after me. And to Mum, thanks for gifting me with your determination and perseverance.

Funding for this project came from the IVABS Postgraduate Research Fund, Comalco and the Kakapo Recovery Programme, Department of Conservation.

# Contents

1	General Introduction.....	1
1.1	Kakapo.....	1
1.2	The timing of seasonal breeding in birds.....	3
1.3	Reproduction and the gonadal axis in birds.....	3
1.4	Role of gonadal steroids in birds.....	4
1.5	Steroid metabolism.....	5
1.5.1	Biosynthesis.....	5
1.5.2	Steroid action and transport.....	6
1.5.3	Catabolism and excretion.....	6
1.6	Faecal steroids.....	7
1.6.1	History and development of faecal steroid analysis.....	8
1.6.2	Applications of faecal steroid analysis.....	9
1.6.2.1	Sex determination and gender differences.....	9
1.6.2.2	Evaluation of breeding potential and reproductive activity.....	9
1.6.2.3	Correlation of endocrine changes with behavioural changes.....	10
1.6.2.4	Glucocorticoid responses to stress.....	10
1.6.3	Limitations of urinary and faecal steroid analysis.....	11
1.7	Thesis outline.....	14
2	Development of methods to measure faecal steroids in Japanese quail.....	17
2.1	Introduction.....	17
2.2	Methods.....	21
2.2.1	Birds.....	21
2.2.2	Experimental design.....	21
2.2.3	Sample collection.....	21
2.2.3.1	Faeces.....	21
2.2.3.2	Plasma.....	22
2.2.3.3	Reproductive system.....	23
2.2.4	Steroid extraction from plasma.....	23
2.2.5	Steroid extraction from faecal samples.....	25
2.2.5.1	Original extraction method.....	25
2.2.5.2	Refinement of original extraction method.....	26

---

2.2.5.2.1	Results .....	27
2.2.5.3	Faecal extraction method.....	28
2.2.5.4	Further development of faecal preparation methods .....	30
2.2.5.4.1	Grinding methods .....	30
2.2.5.4.2	Extraction methods .....	31
2.2.5.4.2.1	Results .....	32
2.2.6	Radioimmunoassay of testosterone .....	35
2.2.6.1	Intra- and inter-assay variation .....	35
2.2.6.2	Parallelism and hormone recovery .....	36
2.2.7	Radioimmunoassay of oestradiol.....	36
2.2.7.1	Intra- and inter-assay variation .....	37
2.2.7.2	Parallelism and hormone recovery .....	37
2.2.8	Radioimmunoassay of progesterone.....	37
2.2.8.1	Intra- and inter-assay variation .....	38
2.2.8.2	Parallelism and hormone recovery .....	38
2.2.9	Statistical analyses.....	40
2.3	Results .....	41
2.3.1	Gonad weight.....	41
2.3.2	Egg laying and cloacal foam.....	42
2.3.3	Dropping weight.....	42
2.3.4	Plasma testosterone.....	45
2.3.5	Faecal testosterone.....	45
2.3.6	Relationship between plasma and faecal testosterone.....	46
2.3.7	Plasma oestradiol.....	51
2.3.8	Faecal oestradiol .....	51
2.3.9	Relationship between plasma and faecal oestradiol .....	52
2.3.10	Plasma progesterone .....	55
2.3.11	Faecal progesterone .....	55
2.3.12	Relationship between plasma and faecal progesterone .....	56
2.4	Discussion.....	59
2.4.1	Gonad weight.....	59
2.4.2	Dropping weight.....	60
2.4.3	Testosterone.....	61
2.4.4	Oestradiol.....	64

---

2.4.5	Progesterone .....	66
2.5	Conclusion .....	68
3	Plasma and faecal steroids and gonadal function in Japanese quail .....	71
3.1	Introduction.....	71
3.2	Methods .....	74
3.2.1	Birds.....	74
3.2.2	Experimental design .....	74
3.2.3	Sample collection.....	74
3.2.3.1	Faeces .....	74
3.2.3.2	Plasma samples.....	75
3.2.3.3	Reproductive system.....	75
3.2.4	Steroid extraction from plasma.....	76
3.2.5	Steroid extraction from faecal samples.....	76
3.2.6	Radioimmunoassay of testosterone .....	78
3.2.6.1	Intra- and inter-assay variation .....	78
3.2.6.2	Parallelism and hormone recovery .....	78
3.2.7	Radioimmunoassay of oestradiol.....	79
3.2.7.1	Intra- and inter-assay variation .....	79
3.2.7.2	Parallelism and hormone recovery .....	79
3.2.8	Radioimmunoassay of progesterone.....	80
3.2.8.1	Intra- and inter-assay variation .....	80
3.2.8.2	Parallelism and hormone recovery .....	80
3.2.9	Statistical analyses .....	82
3.3	Results.....	84
3.3.1	Gonad weight.....	84
3.3.2	Egg laying, cloacal foam and cloacal measurements .....	84
3.3.3	Faecal production.....	85
3.3.4	Plasma testosterone.....	88
3.3.5	Faecal testosterone.....	89
3.3.6	Relationship between plasma and faecal testosterone .....	90
3.3.7	Plasma oestradiol .....	115
3.3.8	Faecal oestradiol .....	116
3.3.9	Relationship between plasma and faecal oestradiol .....	117
3.3.10	Plasma progesterone .....	133



---

3.3.11	Faecal progesterone .....	134
3.3.12	Relationship between plasma and faecal progesterone .....	135
3.3.13	Hormone concentration interactions in plasma .....	151
3.3.14	Hormone concentration interactions in faecal samples .....	153
3.4	Discussion.....	155
3.4.1	Gonad weight.....	155
3.4.2	Faecal production.....	157
3.4.3	Testosterone.....	157
3.4.4	Oestradiol.....	161
3.4.5	Progesterone .....	163
3.4.6	Hormone concentration interactions in plasma and faeces.....	166
3.5	Conclusions .....	167
4	Faecal reproductive steroids in Kakapo.....	171
4.1	Introduction .....	171
4.2	Methods .....	175
4.2.1	Bird information and habitat.....	175
4.2.2	Sample collection .....	175
4.2.3	Steroid extraction from faecal samples.....	176
4.2.4	Radioimmunoassay of testosterone .....	178
4.2.4.1	Intra- and inter-assay validation .....	179
4.2.4.2	Parallelism and hormone recovery .....	179
4.2.5	Radioimmunoassay of oestradiol.....	179
4.2.5.1	Intra- and inter-assay validation .....	180
4.2.5.2	Parallelism and hormone recovery .....	180
4.2.6	Radioimmunoassay of progesterone.....	181
4.2.6.1	Intra- and inter-assay validation .....	181
4.2.6.2	Parallelism and hormone recovery .....	182
4.2.7	Statistical analyses.....	184
4.3	Results .....	186
4.3.1	Breeding activity.....	186
4.3.2	Faecal testosterone.....	187
4.3.3	Faecal oestradiol .....	200
4.3.4	Faecal progesterone .....	211
4.4	Discussion.....	223

---

4.4.1	Testosterone .....	223
4.4.2	Oestradiol and progesterone .....	226
4.5	Conclusion .....	231
5	General discussion .....	235
5.1	Conclusions.....	236
5.2	Directions for future research .....	238

## List of Figures

<b>Figure 2.1.</b> Parallelism demonstrated for quail faecal and plasma samples. ....	39
<b>Figure 2.2.</b> Gonad weight in Japanese quail held on different photoperiods.....	41
<b>Figure 2.3.</b> Dropping weight in Japanese quail held on different photoperiods.....	43
<b>Figure 2.4.</b> Relationships between gonad weight and dropping weight. ....	43
<b>Figure 2.5.</b> Relationship between dropping weight and total faecal hormone content per dropping.....	44
<b>Figure 2.6.</b> Plasma and faecal testosterone concentrations.....	47
<b>Figure 2.7.</b> Mean faecal testosterone concentrations for individual birds.....	49
<b>Figure 2.8.</b> Plasma and faecal oestradiol concentrations .....	53
<b>Figure 2.9.</b> Mean faecal oestradiol concentrations for individual birds .....	54
<b>Figure 2.10.</b> Plasma and faecal progesterone concentrations .....	57
<b>Figure 2.11.</b> Mean faecal progesterone concentrations for individual birds.....	58
<b>Figure 3.1.</b> Parallelism demonstrated for quail faecal and plasma samples. ....	81
<b>Figure 3.2.</b> Gonad weight in Japanese quail held on different photoperiods.....	86
<b>Figure 3.3.</b> Faecal production in Japanese quail held on different photoperiods. ....	88
<b>Figure 3.4.</b> Plasma and faecal testosterone concentrations.....	91
<b>Figure 3.5.</b> Plasma testosterone concentrations on one day.....	92
<b>Figure 3.6.</b> Relationships between gonad weight and plasma hormone concentrations.. ..	94
<b>Figure 3.7.</b> Mean plasma testosterone concentrations for individual birds. ....	95
<b>Figure 3.8.</b> Changes in plasma testosterone concentrations over time in individual females on short days.....	97
<b>Figure 3.9.</b> Changes in plasma testosterone concentrations over time in individual females on long days. ....	98

---

<b>Figure 3.10.</b> Changes in plasma testosterone concentrations over time in individual immature males on long days..	99
<b>Figure 3.11.</b> Changes in plasma testosterone concentrations over time in individual males on long days..	100
<b>Figure 3.12.</b> Change in mean plasma testosterone concentrations over time in quail held on different photoperiods.....	101
<b>Figure 3.13.</b> Daily faecal testosterone production. ....	103
<b>Figure 3.14.</b> Faecal testosterone concentrations for one day .....	103
<b>Figure 3.15.</b> Relationships between gonad weight and faecal hormone concentrations. ....	104
<b>Figure 3.16.</b> Mean faecal testosterone concentrations for individual birds.....	105
<b>Figure 3.17.</b> Changes in faecal testosterone concentrations over time in individual females on short days. ....	107
<b>Figure 3.18.</b> Changes in faecal testosterone concentrations over time in individual females on long days. ....	108
<b>Figure 3.19.</b> Changes in faecal testosterone concentrations over time in individual immature males on long days. ....	109
<b>Figure 3.20.</b> Changes in faecal testosterone concentrations over time in individual males on long days. ....	110
<b>Figure 3.21.</b> Change in mean faecal testosterone concentrations over time in quail held on different photoperiod regimes .....	111
<b>Figure 3.22.</b> Correlations between plasma concentrations and faecal testosterone concentrations or production. ....	113
<b>Figure 3.23.</b> Plasma and faecal oestradiol concentrations. ....	118
<b>Figure 3.24.</b> Plasma oestradiol concentrations for one day. ....	119
<b>Figure 3.25.</b> Mean plasma oestradiol concentrations for individual birds.....	120
<b>Figure 3.26.</b> Changes in plasma oestradiol concentrations over time in individual females on short days. ....	121

---

<b>Figure 3.27.</b> Changes in plasma oestradiol concentrations over time in individual females on long days..	122
<b>Figure 3.28.</b> Changes in plasma oestradiol concentrations over time in individual immature males on long days..	123
<b>Figure 3.29.</b> Changes in plasma oestradiol concentrations over time in individual males on long days.....	124
<b>Figure 3.30.</b> Change in mean plasma oestradiol concentrations over time in quail held on different photoperiods.....	125
<b>Figure 3.31.</b> Daily faecal oestradiol production.....	125
<b>Figure 3.32.</b> Faecal oestradiol concentrations for one day .....	126
<b>Figure 3.33.</b> Mean faecal oestradiol concentrations for individual birds. ....	126
<b>Figure 3.34.</b> Changes in faecal oestradiol concentrations over time in individual females on short days.....	127
<b>Figure 3.35.</b> Changes in faecal oestradiol concentrations over time in individual females on long days. ....	128
<b>Figure 3.36.</b> Changes in faecal oestradiol concentrations over time in individual immature males on long days. ....	129
<b>Figure 3.37.</b> Changes in faecal oestradiol concentrations over time in individual males on long days.....	130
<b>Figure 3.38.</b> Change in mean faecal oestradiol concentrations over time in quail held on different photoperiods.....	131
<b>Figure 3.39.</b> Correlations between plasma concentrations and faecal oestradiol concentrations or production. ....	131
<b>Figure 3.40.</b> Plasma and faecal progesterone concentrations .....	136
<b>Figure 3.41.</b> Plasma progesterone concentrations for one day .....	137
<b>Figure 3.42.</b> Mean plasma progesterone concentrations for individual birds.....	138
<b>Figure 3.43.</b> Changes in plasma progesterone concentrations over time in individual females on short days.....	139

---

<b>Figure 3.44.</b> Changes in plasma progesterone concentrations over time in individual females on long days.. .....	140
<b>Figure 3.45.</b> Changes in plasma progesterone concentrations over time in individual immature males on long days.. .....	141
<b>Figure 3.46.</b> Changes in plasma progesterone concentrations over time in individual males on long days. ....	142
<b>Figure 3.47.</b> Change in mean plasma progesterone concentrations over time in quail held on different photoperiods.....	143
<b>Figure 3.48.</b> Daily faecal progesterone production.....	143
<b>Figure 3.49.</b> Faecal progesterone concentrations for one day .....	144
<b>Figure 3.50.</b> Mean faecal progesterone concentrations for individual birds. ....	144
<b>Figure 3.51.</b> Changes in faecal progesterone concentrations over time in individual females on short days. ....	145
<b>Figure 3.52.</b> Changes in faecal progesterone concentrations over time in individual females on long days. ....	146
<b>Figure 3.53.</b> Changes in faecal progesterone concentrations over time in individual immature males on long days. ....	147
<b>Figure 3.54.</b> Changes in faecal progesterone concentrations over time in individual males on long days. ....	148
<b>Figure 3.55.</b> Change in mean faecal progesterone concentrations over time in quail held on different photoperiods.....	149
<b>Figure 3.56.</b> Correlations between plasma concentrations and faecal progesterone concentrations or production. ....	149
<b>Figure 3.57.</b> Canonical scores for the first two canonical discriminant functions (CDFs) of plasma hormone concentrations in four groups of quail held on different photoperiods .....	152
<b>Figure 3.58.</b> Canonical scores for the first two canonical discriminant functions (CDFs) of faecal hormone concentrations in four groups of quail held on different photoperiods .....	154

<b>Figure 4.1.</b> Parallelism demonstrated for kakapo faecal samples.....	183
<b>Figure 4.2.</b> Faecal testosterone concentrations in male kakapo.....	190
<b>Figure 4.3.</b> Faecal testosterone concentrations in male kakapo.....	192
<b>Figure 4.4.</b> Variation in faecal testosterone concentration between fresh droppings from individual birds from the same day or roost site.....	193
<b>Figure 4.5.A</b> Faecal testosterone profiles for individual male kakapo. ....	194
<b>Figure 4.5.B</b> Faecal testosterone profiles for individual male kakapo. ....	195
<b>Figure 4.5.C</b> Faecal testosterone profiles for individual male kakapo. ....	196
<b>Figure 4.6.</b> Relationship between faecal testosterone concentration and faecal testosterone extraction efficiency.. ....	197
<b>Figure 4.7.</b> Faecal testosterone extraction efficiencies for different scores of physical characteristics of individual droppings.....	198
<b>Figure 4.8.</b> Faecal testosterone concentrations for different scores of physical characteristics of individual droppings.....	199
<b>Figure 4.9.</b> Faecal oestradiol concentrations in female kakapo.....	202
<b>Figure 4.10.</b> Faecal oestradiol concentrations in female kakapo.....	204
<b>Figure 4.11.</b> Variation in faecal oestradiol concentration between fresh droppings from individual birds from the same day or roost site.....	205
<b>Figure 4.12.A</b> Faecal oestradiol profiles for individual female kakapo. ....	206
<b>Figure 4.12.B</b> Faecal oestradiol profiles for individual female kakapo.....	207
<b>Figure 4.13.</b> Relationship between faecal oestradiol concentration and faecal oestradiol extraction efficiency.. ....	208
<b>Figure 4.14.</b> Faecal oestradiol extraction efficiencies for different scores of physical characteristics of individual droppings.....	209
<b>Figure 4.15.</b> Faecal oestradiol concentrations for different scores of physical characteristics of individual droppings.....	210
<b>Figure 4.16.</b> Faecal progesterone concentrations in female kakapo.....	213
<b>Figure 4.17.</b> Faecal progesterone concentrations in female kakapo. ....	215

<b>Figure 4.18.</b> Variation in faecal progesterone concentration between fresh droppings from individual birds from the same day or roost site.....	216
<b>Figure 4.19.A</b> Faecal progesterone profiles for individual female kakapo. ....	217
<b>Figure 4.19.B</b> Faecal progesterone profiles for individual female kakapo.....	218
<b>Figure 4.20.</b> Relationship between faecal progesterone concentration and faecal testosterone extraction efficiency.. ....	219
<b>Figure 4.21.</b> Faecal progesterone extraction efficiencies for different scores of physical characteristics of individual droppings.....	220
<b>Figure 4.22.</b> Faecal progesterone concentrations for different scores of physical characteristics of individual droppings.....	221



## List of Tables

<b>Table 2.1.</b> Dropping consistency grading scale. ....	22
<b>Table 2.2.</b> Volumes and solvents used for testosterone, oestradiol and progesterone extraction from quail plasma samples. ....	24
<b>Table 2.3.</b> Mean quail faecal oestradiol extraction efficiencies for five different extraction methods. ....	27
<b>Table 2.4.</b> Mean faecal oestradiol extraction efficiencies for male and female quail for five different extraction methods. ....	28
<b>Table 2.5.</b> Scores for grinding capabilities for various instruments. ....	30
<b>Table 2.6.</b> Mean quail faecal steroid extraction efficiencies for eight different extraction. ....	34
<b>Table 2.7.</b> Summary of one-way ANOVA of plasma and faecal hormone concentrations across birds grouped by photoperiod. ....	48
<b>Table 2.8.</b> Relationships between gonad weight and plasma and faecal hormone concentrations in males (paired testis weight and testosterone) and females (ovary weight and oestradiol or progesterone). ....	48
<b>Table 2.9.</b> Summary of one-way ANOVA tests of variation in faecal hormone concentration between birds within each treatment group. ....	49
<b>Table 2.10.</b> Variation between birds in individual and mean faecal steroid concentrations. ....	50
<b>Table 2.11.</b> Correlations between plasma and mean faecal hormone concentrations. ....	51
<b>Table 3.1.</b> Body weight and cloacal diameter (females) or cloacal area (males) at the start and at the end of the sampling period. ....	87
<b>Table 3.2.</b> Summary of one-way ANOVA of plasma and faecal hormone concentrations across birds grouped by photoperiod. ....	93
<b>Table 3.3.</b> Relationships between gonad weight and plasma and faecal hormone concentrations in males (paired testis weight and testosterone) and females (ovary weight and oestradiol or progesterone). ....	95

---

<b>Table 3.4.</b> Summary of one-way ANOVA for comparisons of plasma hormone concentrations between birds in each treatment group.....	96
<b>Table 3.5.</b> Variation between birds in plasma steroid concentrations .....	102
<b>Table 3.6.</b> Summary of one-way ANOVA for comparisons of faecal hormone concentration between birds within each treatment group. ....	106
<b>Table 3.7.</b> Variation between birds in faecal steroid concentrations .....	112
<b>Table 3.8.</b> Correlations between plasma and faecal testosterone concentrations and production.....	114
<b>Table 3.9.</b> Correlations between plasma and faecal oestradiol concentrations and production.....	132
<b>Table 3.10.</b> Correlations between plasma and faecal progesterone concentrations and production.....	150
<b>Table 3.11.</b> Standardised canonical coefficients (SCC) from the first two canonical discriminant functions (CDFs) developed from plasma hormone concentrations on four groups of birds .....	152
<b>Table 3.12.</b> Standardised canonical coefficients (SCC) from the first two canonical discriminant functions (CDFs) developed from faecal hormone concentrations on four groups of birds .....	154
<b>Table 4.1.</b> Fresh kakapo dropping characteristic grading scheme. ....	176
<b>Table 4.2.</b> Egg-laying by female kakapo on Whenua Hou, during the 1996-1997 season and on Pearl Island, 1998-1999 season. ....	187
<b>Table 4.3.</b> Parameter estimates of cubic equation (Equation 4.1) fitted to mean testosterone concentrations by month for two seasons, 1996-97 and 1997-98. ...	191
<b>Table 4.4.</b> Extraction efficiencies using the same extraction method for testosterone, oestradiol and progesterone in quail and kakapo.....	197
<b>Table 4.5.</b> Summary of one-way ANOVA and t-tests of faecal testosterone concentrations and extraction efficiencies across different scores of physical characteristics of individual droppings.....	200
<b>Table 4.6.</b> Parameter estimates of quadratic equation (Equation 4.2) fitted to mean oestradiol concentrations by month for two seasons, 1996-97 and 1997-98.....	203

---

<b>Table 4.7.</b> Summary of one-way ANOVA and t-test of faecal oestradiol concentrations and extraction efficiencies across different scores of physical characteristics of individual droppings. ....	211
<b>Table 4.8.</b> Parameter estimates of quadratic equation (Equation 4.2) fitted to mean progesterone concentrations by month for two seasons, 1996-97 and 1997-98...	214
<b>Table 4.9.</b> Summary of one-way ANOVA and t-tests of faecal progesterone concentrations and extraction efficiencies across different scores of physical characteristics of individual droppings. ....	222