

Development of a non-invasive technique to determine reproductive hormones in cetaceans

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This thesis is submitted to the University of Sydney in fulfilment of the requirement for the Degree of Doctor of Philosophy.

The work presented in this thesis is, to the best of my knowledge and belief, original except as acknowledged in the text. I hereby declare that I have not submitted this material, either in full or in part, for a degree at this or any other institution.

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31 August 2005



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Executive Summary

Reproductive physiology plays a vital role in population growth and vitality. Baseline data on reproductive physiology and a comprehensive knowledge of breeding biology are essential to conservation management. Great whales have been hunted from the 16th century to the present day. Although many populations are increasing there are populations with low or declining reproductive rates. In 2001 it was recommended to the International Whaling Commission that new techniques be developed to assess the internal physiology of great whales. This study, based on this recommendation, aims to develop analytical methods to assess reproductive hormones in cetacean blow samples and determine the feasibility of its use with free-swimming great whales.

A method for the assessment of steroid hormone concentrations using liquid chromatography-mass spectrometry (LC-MS) was developed and validated. These methods were then used to determine testosterone and progesterone concentrations in saliva and blow of bottlenose dolphins. The stability of testosterone and progesterone was found to be a major issue. Without inhibitors, hormone concentrations increased by up to 65% over three hours at 21°C. Storing samples at low temperatures (-20°C or -80°C) slowed but did not cease the rate of change. The addition of inhibitors, manganese chloride and amoxicillin potassium/clavulanate, improved the stability of testosterone and progesterone. It is proposed that when using dolphin saliva and blow samples to measure reproductive hormones the samples are extracted as soon as possible after collection to prevent degradation. This study highlighted the need to address steroid hormone stability prior to any long-term biological program, to ensure that changes seen in hormone concentration are due to biological activity rather than storage.

A technique to collect blow samples from free-swimming great whales was developed. This technique, in conjunction with the specially developed LC-MS methods allowed for the determination of testosterone and progesterone concentrations in humpback whale blow. The techniques developed in this study to determine reproductive hormones in cetacean saliva and blow have applications for both captive and wild population studies. In captive institutions, saliva and/or blow can be used to monitor reproductive cycling in both females and males. As it is non-invasive it can be used on a daily basis with minimal stress to the animals. The use of blow sampling has the capacity to improve our understanding of reproductive cycling in great whales as it can be used to sample animals in both the breeding and feeding areas. This technique may allow us to now examine whether reproductive dysfunction is playing a role in the slow recovery of critically endangered species such as the North Atlantic right whale.

Table of Contents

Acknowledgements	i
Executive Summary	iii
Table of Contents	v
List of Tables	x
List of Figures	xii
Abbreviations Used	xv
Scientific Names	xvii
Chapter 1 - Introduction	
1.1 Introduction	1
1.2 Whales and whaling	5
1.3 Hormones	9
1.3.1 Steroid hormones.....	10
1.3.1.1 Cholesterol.....	10
1.3.1.2 Steroid synthesis.....	13
1.3.1.3 Enzymes in steroid synthesis	16
1.3.1.4 Transport.....	16
1.3.2 Androgens	22
1.3.3 Progestins and oestrogens	25
1.4 Cetacean reproduction	27
1.4.1 Females.....	28
1.4.2 Males	30
1.5 Current hormonal sampling techniques	31
1.5.1 Blood	32
1.5.2 Faecal.....	33
1.5.3 Urine	33
1.5.4 Saliva.....	34
1.5.5 Other infrequently used hormone sampling methods	35
1.6 Aims of this study	36

Chapter 2 – General Methodology

2.1 Immunoassays	38
2.2 Mass spectrometry	39
2.2.1 History	39
2.2.2 Chromatography-Mass spectrometry	40
2.2.3 Mass spectrometric analysis	41
2.3 Immunoassays vs. LC-MS	43
2.4 General method development	46
2.4.1 Stability	50
2.4.2 Internal standard.....	50
2.4.3 Other validation criteria	54

Chapter 3 – Testosterone

3.1 Introduction	55
3.2 Materials and Methods	57
3.2.1 Chemicals and solutions	57
3.2.2 LC analysis	58
3.2.3 LC-MS methods.....	58
3.2.4 Sample collection and preparation	61
3.2.5 Validation.....	62
3.2.6 Statistical analysis.....	63
3.3 Results	64
3.3.1 Validation.....	64
3.3.2 Dolphin samples (endogenous testosterone concentrations)	68
3.4 Discussion	71
3.4.1 Validation.....	71
3.4.2 Dolphin samples (endogenous testosterone concentrations)	72
3.5 Conclusion	73

Chapter 4 – Testosterone Stability

4.1 Introduction	74
4.2 Materials and Methods	76
4.2.1 Sample preparation.....	76
4.2.2 LC-MS assay	77
4.2.3 Inhibitors	77

4.2.4	Freeze-thaw experiments.....	77
4.2.5	Short-term storage.....	78
4.2.6	Long-term storage.....	78
4.2.7	Statistical analysis.....	79
4.3	Results	79
4.3.1	Inhibitors	79
4.3.2	Freeze-thaw experiments.....	85
4.3.3	Short-term storage	85
4.3.3.1	Saliva at 21°C	85
4.3.3.2	Blow at 21°C	88
4.3.4	Long-term storage.....	88
4.3.4.1	Saliva at -20°C	88
4.3.4.2	Blow at -20°C	89
4.3.4.3	Saliva at -80°C	89
4.3.4.4	Blow at -80°C	89
4.4	Discussion	90
4.5	Conclusion.....	95

Chapter 5 – Female Reproductive Hormones

5.1	Introduction	96
5.1.1	Oestrous cycle	96
5.2	Materials and Methods.....	101
5.2.1	Chemicals and solutions	101
5.2.2	LC analysis	102
5.2.3	LC-MS methods.....	102
5.2.4	Sample collection and preparation	102
5.2.5	Validation.....	103
5.2.6	Statistical analysis.....	104
5.3	Results	105
5.3.1	Progesterone validation	105
5.3.2	Oestradiol validation	109
5.3.3	Oestrone validation	109
5.3.4	Dolphin samples (endogenous progesterone concentrations).....	112
5.4	Discussion	115
5.5	Conclusion.....	118

Chapter 6 – Progesterone Stability

6.1 Introduction	119
6.2 Materials and Methods	121
6.2.1 Sample preparation.....	121
6.2.2 LC-MS assay	121
6.2.3 Inhibitors	121
6.2.4 Freeze-thaw experiments.....	121
6.2.5 Short-term storage	122
6.2.6 Long-term storage.....	122
6.2.7 Statistical analysis.....	122
6.3 Results	123
6.3.1 Freeze-thaw experiments.....	123
6.3.2 Short-term storage	123
6.3.2.1 Saliva at 21°C	123
6.3.2.2 Blow at 21°C	126
6.3.3 Long-term storage.....	127
6.3.3.1 Saliva at -20°C	127
6.3.3.2 Blow at -20°C	127
6.3.3.3 Saliva at -80°C	129
6.3.3.4 Blow at -80°C	129
6.4 Discussion	131
6.5 Conclusion	133

Chapter 7 – Whale Blow Collection: is it feasible?

7.1 Introduction	135
7.1.1 Humpback whale reproduction.....	136
7.2 Materials and Methods	139
7.2.1 Sample collection materials	139
7.2.2 Blow sample collection.....	140
7.2.3 Blow sample analysis.....	146
7.2.4 0-100% gradient analysis.....	148
7.3 Results	151
7.3.1 Sample collection materials	151
7.3.2 Blow sample analysis.....	151
7.3.3 0-100% gradient analysis.....	152

7.4 Discussion	159
7.4.1 Sample collection materials	159
7.4.2 Blow sample analysis.....	159
7.4.3 0-100% gradient analysis.....	161
7.5 Conclusion.....	162

Chapter 8 – General Discussion

8.1 Analytical chemistry.....	163
8.2 Stability	164
8.3 Whale blow collection	167
8.4 Conclusion.....	169

References	171
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Appendices

Appendix 1.....	190
Appendix 2.....	199

Tables

Chapter 2

Table 2.1: Substances tested as internal standards	52
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Chapter 3

Table 3.1: Intra-batch and inter-batch precision and accuracy for testosterone in dolphin saliva and blow	67
--	----

Table 3.2: Endogenous testosterone concentrations of male bottlenose dolphins	70
--	----

Chapter 4

Table 4.1: Changes in spiked testosterone concentration in bottlenose dolphin saliva and blow at 21°C with no inhibitors or inhibitors added.....	87
--	----

Chapter 5

Table 5.1: Intra- and inter-batch precision and accuracy for progesterone in dolphin saliva and blow	108
---	-----

Chapter 6

Table 6.1: Changes in spiked progesterone concentration in bottlenose dolphin saliva and blow at 21°C with no inhibitors or inhibitors added	125
---	-----

Table 6.2: Changes in spiked progesterone concentration in bottlenose dolphin saliva and blow at -20°C with no inhibitors or inhibitors added	128
--	-----

Table 6.3: Changes in spiked progesterone concentration in bottlenose dolphin saliva and blow at -80°C with no inhibitors or inhibitors added	130
--	-----

Chapter 7

Table 7.1: a) Humpback whale blow samples collected from Peregian Beach in 2003	149
b) Humpback whale blow samples collected from Peregian Beach in 2004	150
Table 7.2: Positive ($[M+]$) and negative ($[M+H]$) mass-to-charge ratios (m/z) for some of the precursors and metabolites of testosterone and progesterone.....	158

List of Figures

Chapter 1

Figure 1.1: Six areas of the Antarctic as designated by the International Whaling Commission (IWC)	6
Figure 1.2: Five ring cholesterol structure and its derivatives	11
Figure 1.3: Relationship between peptide hormones and steroid hormones.....	14
Figure 1.4: The principal pathways of human steroid hormone synthesis	17
Figure 1.5a: 21-Carbon pathway for steroid metabolism.	20
Figure 1.5b: Androgen and oestrogen metabolism.....	21
Figure 1.6: Negative feedback system of the hypothalamic-pituitary-gonadal.....	24
Figure 1.7: a) The progestins. b) The oestrogens.....	26

Chapter 2

Figure 2.1: Chromatogram of dolphin blow in scan mode.	42
Figure 2.2: Chromatogram and mass spectrum of dolphin blow in SIM mode.	44
Figure 2.3: Formic acid has the same m/z as testosterone.....	48
Figure 2.4: Acetic acid has the same m/z as oestradiol	49
Figure 2.5: a) MS chromatogram of lidocaine standard. b) MS chromatogram of lidocaine, testosterone standard and mixed dolphin saliva.	53

Chapter 3

Figure 3.1: Determination of testosterone m/z by direct infusion into the mass spectrometer	59
Figure 3.2: MS chromatogram of saliva sample from dolphin Sirius showing the native testosterone peak.	60
Figure 3.3: Retention times of testosterone and internal standard.	65
Figure 3.4: Direct infusion of cotton gauze into the mass spectrometer	66
Figure 3.5: a) MS chromatogram of endogenous testosterone in dolphin saliva. (b) MS chromatogram of endogenous testosterone in dolphin blow.	69

Chapter 4

Figure 4.1: a) Changes in 50 ng/ml spiked testosterone in bottlenose dolphin saliva and blow at 21°C. b) Changes in 0.5 ng/ml spiked testosterone in bottlenose dolphin saliva and blow at 21°C	80
Figure 4.2: Differences in testosterone concentration over six hours using different MnCl ₂ concentrations	82
Figure 4.3: (a) Stability of saliva spiked with testosterone (5 ng/ml) at 21°C with Augmentin [®] and MnCl ₂ over 18 hours. (b) Stability of blow spiked with testosterone (50 ng/ml) at 21°C with Augmentin [®] and MnCl ₂ over 18 hours.	83
Figure 4.4: a) Three cycles of freezing-thawing saliva and blow with 50 ng/ml testosterone. b) Three cycles of freezing-thawing saliva and blow with 5 ng/ml testosterone	86

Chapter 5

Figure 5.1: Outline of the oestrous cycle	98
Figure 5.2: Determination of progesterone <i>m/z</i> by direct infusion	106
Figure 5.3: Retention times of progesterone and internal standard.....	107
Figure 5.4: a) Determination of oestrone <i>m/z</i> by direct infusion. b) Determination of oestradiol <i>m/z</i> by direct infusion	110
Figure 5.5: Retention times of a) oestradiol and b) oestrone	111
Figure 5.6: a) MS chromatogram of progesterone in pregnant dolphin saliva; and b) progesterone in pregnant dolphin blow	113
Figure 5.7: a) MS chromatogram of progesterone in dolphin saliva. b) MS chromatogram of progesterone in dolphin blow.....	114

Chapter 6

Figure 6.1: a) Three cycles of freezing-thawing saliva and blow with 50 ng/ml progesterone. b) Three cycles of freezing-thawing saliva and blow with 0.5 ng/ml progesterone	124
--	-----

Chapter 7

Figure 7.1: Study site at Peregrine Beach, Queensland, Australia.....	141
Figure 7.2: 13-metre carbon fibre pole used to collect blow samples from whales, the pole is bracket mounted to the vessel (a) and steered by a crew member (b).	142
Figure 7.3: Blow collection device was a 5-inch bamboo double ring with nylon stocking that had been sonicated with 100% acetonitrile stretched across it	144
Figure 7.4: a) The blow collection device was mounted to the top of the pole b) Collection of a blow that was graded as a medium quality sample	145
Figure 7.5: MS chromatograms of scans of different collection materials	153
Figure 7.6: MS chromatogram showing presence of testosterone in humpback whale blow	156
Figure 7.7: Chromatogram showing presence of progesterone in humpback whale blow	157

Abbreviations Used

ANOVA	Analysis of variance
ART	Assisted reproductive technology
CL	corpus luteum
Cr	Creatinine
Da	Daltons
DHT	5 α -dihydrotestosterone
EDCs	Endocrine disrupting chemicals
EIA	Enzyme immunoassay
ESI	Electrospray ionisation
FSH	Follicle stimulating hormone
GC	Gas chromatography
GC-MS	Gas chromatography-mass spectrometry
GnRH	Gonadotropin releasing hormone
H	Proton
hCG	Human chorionic gonadotropin
HPLC	High performance liquid chromatography
hrs	Hours
IWC	International Whaling Commission
IS	Internal standard
LC	Liquid chromatography
LC-MS	Liquid chromatography-mass spectrometry
LC-MS-MS	Liquid chromatography-mass spectrometry-mass spectrometry
LH	Luteinising hormone
LOD	Limit of detection

LOQ	Limit of quantification
M	Molecular mass
MeOH	Methanol
min	Minute
mins	Minutes
MnCl ₂	Manganese chloride
MS	Mass spectrometry
mw	Molecular weight
<i>m/z</i>	Mass-to-charge ratio
NI	No inhibitors
PdG	Pregnanediol-3 α -glucuronide
PTP	Plasma transport protein
RE	Relative error
RIA	Radioimmunoassay
RSD	Relative standard deviation
RT	Retention time
scc	Side chain cleavage
secs	Seconds
SIM	Selected-ion monitoring mode
SPE	Solid phase extraction
SRM	Selected-reaction monitoring mode
TOF	Time-of-flight mass spectrometry

Scientific Names

Asian elephant	<i>Elephas maximus</i>
Bandicoot	<i>Isoodon macrourus</i>
Black Rhinoceros	<i>Diceros bicornis</i>
Bottlenose dolphin	<i>Tursiops truncatus</i>
Bowhead whale	<i>Balaena mysticetus</i>
California sea lion	<i>Zalophus californianus</i>
Capuchin monkeys	<i>Cebus apella</i>
Cattle	<i>Bos sp.</i>
Chimpanzee	<i>Pan troglodytes schweinfurthii</i>
Common dolphin	<i>Delphinus delphis</i>
Dairy cow	<i>Bos taurus</i>
False killer whale	<i>Pseudorca crassidens</i>
Feral horses	<i>Equus caballus</i>
Fin whale	<i>Balaenoptera physalus</i>
Goat	<i>Capra hircus</i>
Gorillas	<i>Gorilla gorilla</i>
Harbour seal	<i>Phoca vitulina</i>
Horse	<i>Equus caballus</i>
Human	<i>Homo sapiens</i>
Humpback whale	<i>Megaptera novaeangliae</i>
Killer whale	<i>Orcinus orca</i>
Minke whale	<i>Balaenoptera acutostrata</i>
Monk seal	<i>Monachus schauinslandi</i>
Muriquis	<i>Brachyteles arachnoides</i>
Nile hippopotamus	<i>Hippopotamus amphibius</i>

North Atlantic right whale	<i>Eubalaena glacialis</i>
One-humped camel	<i>Camelus dromedarius</i>
Pig	<i>Sus scrofa</i>
Polar bear	<i>Ursus maritimus</i>
Rat	<i>Rattus norvegicus</i>
River buffalo	<i>Bubalus bubalis</i>
Sei whale	<i>Balaenoptera borealis</i>
Sheep	<i>Ovis aries</i>
Short-finned pilot whale	<i>Globicephala macrorhynchus</i>
Siberian tigers	<i>Panthera tigris altaica</i>
Southern right whale	<i>Eubalaena australis</i>
Sperm whale	<i>Physeter macrocephalus</i>
Timber Wolf	<i>Canis lupis</i>
Western lowland gorilla	<i>Gorilla gorilla gorilla</i>
White-tailed deer	<i>Odocoileus virginianus</i>

"Instead of predicting new events with great precision, or explaining what has already been found, a great deal of scientific progress is linked to what is deemed possible and likely. Expectations, however vague and intuitive, lay the groundwork for discovery....."

Frans de Waal, 2001.