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**MILK COMPOSITION OF THE
NEW ZEALAND SEA LION
AND
FACTORS THAT INFLUENCE IT**

**A thesis presented in partial fulfilment of
the requirements for the degree of
Doctor of Philosophy
in
Zoology**

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ABSTRACT

The objectives of the present study were to: 1) describe the gross chemical milk composition of the New Zealand sea lion (NZSLs), *Phocarctos hookeri*, in early lactation; 2) validate an analytical method for sea lion milk composition; 3) investigate a series of temporal, individual and dietary factors that influence the milk composition of the NZSL and; 4) investigate the temporal and spatial differences in the fatty acids signatures of sea lion milk.

A comprehensive literature review revealed that data on milk composition in otariid species is either missing or limited, that to be able to fully describe their milk composition extensive sampling was required and that the temporal, maternal and offspring factors that influence milk composition in pinnipeds are poorly understood. The review identified that considerable work has been conducted to infer diet via the application of fatty acids signature analysis of milk and blubber. There are many factors (i.e. metabolism, *de novo* synthesis and endogenous sources) that contribute to the differences in fatty acid composition between the diet and milk or blubber.

Milk samples from NZSL were used to test whether a new method would give similar results as the standard methods of milk analysis. Agreement between analytical methods for milk components was assessed using different measures of statistical fitness and the results indicated that the new method was comparable to the standard methods and applicable to the milk of sea lions, pinnipeds and to ecological studies of lactation. Milk from NZSLs was collected over a period of seven years (1997, 1999 to 2003, and 2005) in early lactation to describe the composition of milk of NZSL and to test for differences between years. The results indicated that: i) the milk protein concentration was comparable to other species of pinnipeds; ii) the milk fat concentration and the milk energy content of NZSL is the lowest reported for otariids in early lactation; however iii) the milk fat concentration was significantly different between years. These results suggested that the milk composition of NZSLs was influenced by annual changes in the environment; however, there may be other unidentified factors. Month, maternal body

condition, age, body weight and length, offspring sex and age, and attendance pattern were compared with milk components. The results identified that month, maternal body condition and age significantly affected milk fat concentration. These results and the fact that maternal body condition varied significantly between years and mothers nursing male pups had lower body condition and produced milk lower in energy content suggested that local food resources along with other unidentified factors have an effect on the reproductive success of NZSLs. To test whether the fatty acid signature analysis (FASA) of lipid rich tissues (milk, blubber and serum) of otariids could be used to infer diet a mixture of vegetable oil (with distinctive fatty acid signature) was fed to 24 lactating NZSL and tissue samples were collected at different time intervals. Significant increases in the concentration of specific fatty acids in serum and milk were observed with peaks within 12hrs and 24hrs respectively of ingestion. Concentrations in milk remained elevated for up to 72hrs and there were differential rates of incorporation into milk. These findings confirm the potential of FASA to infer the composition of the diet. The variation in milk fatty acid signatures from lactating NZSL from four years (1997, 2003, 2004 and 2005) of sampling were measured in order to test whether differences occurred between years. Fatty acids signatures from five potential prey species including the commercially important arrow squid were incorporated into the analysis to associate the changes in milk fatty acids with a shift in prey choice. The results indicated that milk fatty acid signatures were different in 1997 and 2003; however, it was not possible to relate these differences to the five prey species. The variability in the annual arrow squid catch data suggested that local food resources around the Auckland Islands may also be variable.

In conclusion, the milk produced by the NZSL has the lowest concentration of fat and energy in early lactation reported for any otariid species. The main factors that contributed to changes in milk quality were stage of lactation, year and maternal body condition. The yearly variation in the quality of milk appears to be a result of their lactation strategy or to variable local food conditions that also affect maternal body condition. Therefore monitoring the annual milk quality may be a means to monitor the health of a pinniped

population and potential management tool for pinniped species. This thesis has shown that annual changes in the diet of NZSL can be assessed with milk fatty acid signatures.

PREFACE

A lack of understanding of the lactation strategies adopted by New Zealand sea lions (NZSL) was the keystone that initiated the work presented in this thesis. *A priori* there were some questions that were proposed such as what's the gross chemical milk composition of the milk?; Does it vary in relation to environmental conditions?; What are the factors that are determining the milk quality produced by NZSLs?; Do these factors have detrimental effects on the quality of the milk of NZSLs and thus on their reproductive success as measured by pup survival?

The interaction with the commercial squid fisheries is evident and thus the question that comes to mind is whether both fisheries and NZSLs are targeting the same food source, and if so would the competition adversely impact on the lactating NZSL? Would this interaction reduce the quality and quantity of milk produced by the NZSL?

I started this project with the idea of analysing the gross chemical composition of milk of NZSL and relating its composition to a number of maternal and offspring characteristics and temporal factors. Although there has been some work in this area but not necessarily on sea lions, I realize that there were many bias incorporated in these studies, for instance, methodologies were not standardized for the analysis of milk composition.

The first step in this project was to validate for the milk of NZSL an analytical method based on infra-red technology that is usually applied in the analysis of milk of dairy animals. Next it was evident that the factors such as maternal characteristics that influence the milk composition needed to be investigated and eventually the long and short term effects of diet. It soon became obvious that the complex mechanisms of milk fat synthesis/secretion related to the physiology of sea lions and the factors governing this mechanism would make it difficult to fully understand the relation between milk and diet and to draw objective conclusions. I found that there was little known about the mechanism of transfer of dietary lipids to milk lipids, in particular in pinnipeds.

Finally, my study focused on the milk composition and the factors that affect it because of its importance in the dynamics and recovery of the population of the species. Information on the quality of the milk can be used as an indirect index of the reproductive success and as a measure of the health of the population.

DEDICATION

"I dedicate this thesis to both my grandmother, Pochola (Jorgelina Bustamante de Riet), and my grandfather Hector (Hector Maria Sapriza) that passed away while doing this thesis, and of course to my parents. They more than anyone, have been the best support and encouragement I could have hoped for. Any accomplishment of mine is due in no small part to their support."

"Quiero dedicar esta tesis de doctorado a mi abuela Pochola (Jorgelina Bustamante de Riet) y a mi abuelo Héctor (Héctor Maria Sapriza), que fallecieron en el periodo de esta tesis, y por supuesto a mis padres. Ellos, más que nadie, han sido el mejor apoyo y ánimo que yo haya podido esperar. Cualquiera de mis logros, son en parte, debido a su apoyo."

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Duncan's M. understanding of the physiology of lactation in dairy animals has made enormous contribution to this thesis. During this project he had make me think further and challenge me by making many valuable suggestion and constructive criticisms.

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The research was conducted under permit from the Animal Ethics Committee-Approval AEC86 (1 July 1999) of the Department of Conservation approved the handling and capture of NZSL and collection of milk samples. Massey University Ethics committee approved the handling and experiments conducted in Chapter 6 of this thesis.

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TABLE OF CONTENTS

ABSTRACT	III
PREFACE	VI
DEDICATION	VIII
ACKNOWLEDGMENTS	IX
LIST OF TABLES	XVI
LIST OF FIGURES	XIX
LIST OF ABBREVIATIONS	XXV
CHAPTER 1	1
GENERAL INTRODUCTION AND LITERATURE REVIEW	1
FOREWORD	2
GENERAL INTRODUCTION	2
LACTATION STRATEGIES IN PINNIPEDS	4
MILK COMPOSITION: A COMPARATIVE REVIEW	15
FACTORS THAT INFLUENCE MILK COMPOSITION	23
FATTY ACIDS: A SOURCE OF INFORMATION	42
GENERAL DISCUSSION	57
LIFE HISTORY OF NEW ZEALAND SEA LION	60
THESIS RATIONALE	62
THESIS OUTLINE	64
THESIS AIMS	65
REFERENCES	73
CHAPTER 2	109
DETERMINING THE GROSS COMPOSITION OF THE MILK OF NEW ZEALAND SEA LIONS USING FOURIER TRANSFORM INFRARED SPECTROMETRY	109
ABSTRACT	110

INTRODUCTION	111
MATERIALS AND METHODS	113
RESULTS	119
DISCUSSION	128
CONCLUSION	134
REFERENCES	135

CHAPTER 3 **141**

GROSS COMPOSITION AND INTER-ANNUAL VARIATION OF THE MILK OF NEW ZEALAND SEA LIONS	141
ABSTRACT	142
INTRODUCTION	143
MATERIALS AND METHODS	144
RESULTS	147
DISCUSSION	152
CONCLUSION	161
REFERENCES	162

CHAPTER 4 **169**

RELATIONSHIP BETWEEN THE GROSS MILK COMPOSITION AND MATERNAL CHARACTERISTICS IN NEW ZEALAND SEA LIONS	169
ABSTRACT	170
INTRODUCTION	171
MATERIALS AND METHODS	173
RESULTS	176
DISCUSSION	187
CONCLUSION	195
REFERENCES	196

CHAPTER 5 **203**

ABSORPTION AND DISTRIBUTION OF DIETARY FATTY ACIDS IN TISSUES OF LACTATING NEW ZEALAND SEA LIONS	203
ABSTRACT	204
INTRODUCTION	205

MATERIALS AND METHODS	208
RESULTS	217
DISCUSSION	236
CONCLUSION	244
REFERENCES	245
CHAPTER 6	255
<hr/>	
TEMPORAL AND SPATIAL MILK FATTY ACID SIGNATURE ANALYSIS OF LACTATING NEW ZEALAND SEA LIONS	255
ABSTRACT	256
INTRODUCTION	257
MATERIALS AND METHODS	259
RESULTS	263
DISCUSSION	278
CONCLUSION	283
REFERENCES	284
CHAPTER 7	289
<hr/>	
GENERAL DISCUSSION	289
INTRODUCTION	290
CONSTRAINTS ON THE RESEARCH	290
LACTATION STRATEGY IN NEW ZEALAND SEA LIONS	291
MANAGEMENT AND CONSERVATION: NEW ZEALAND SEA LIONS AT AUCKLAND ISLANDS	293
FUTURE RESEARCH	299
SOLUTIONS TO CONSTRAINTS ON THE RESEARCH	300
SOME DIRECTIONS FOR FUTURE RESEARCH	300
GENERAL CONCLUSIONS	301
REFERENCES	303
APPENDIX	307
<hr/>	
REFERENCES	318

LIST OF TABLES

CHAPTER 1

Table 1.	Duration of lactation period in fur seals, sea lions and walruses.	66
Table 2.	Duration of lactation in phocids.	67
Table 3.	Milk composition in phocids.	68
Table 4.	Milk composition of otariids and walrus.	69
Table 5.	Mineral constituents of milk of different species with emphasis on that of marine mammals.	70
Table 6.	Temporal parameters of attendance pattern in otariids.	72

CHAPTER 2

Table 7.	Values corresponding to the concordance correlation coefficient (CCC) and the description and significance.	119
Table 8.	Descriptive statistics of New Zealand sea lion, <i>Phocarctos hookeri</i> , milk composition determined with Roesse Gottlieb, Kjeldahl, gravimetric standard methods and the Milkoscan and ASE test methods.	120
Table 9.	Measures of statistical fitness to assess agreement between Roesse Gottlieb, Kjeldahl and Total solids gravimetric standard methods and alternative Milkoscan (FT 120) and Accelerated Solvent Extraction (ASE) test methods to determine the composition of New Zealand sea lion's, <i>Phocarctos hookeri</i> , milk fat, protein and total solids.	125

CHAPTER 3

Table 10.	Number of lactating New Zealand sea lions, <i>Phocarctos hookeri</i> , sampled either once or twice in early lactation and collected over seven summer seasons at Sandy Bay, Enderby Island, Auckland Islands and the number of milk samples analysed.	148
Table 11.	Mean gross chemical composition of whole milk of New Zealand sea lions, <i>Phocarctos hookeri</i> , collected in early lactation over seven seasons at the breeding site of Sandy Bay, Enderby Island, Auckland Islands.	149
Table 12.	Concentrations of protein and major cations in skim milk of New Zealand sea lions, <i>Phocarctos hookeri</i> , in the early lactation period collected over seven seasons at Sandy Bay, Enderby Island, Auckland Islands.	149

CHAPTER 4

Table 13.	Number of lactating New Zealand sea lions, <i>Phocarctos hookeri</i> , in early lactation at Enderby Island, Auckland islands from 1997,	
-----------	------------------------------------------------------------------------------------------------------------------------------------------	--

	1999 to 2003 and 2005 for which age was known, VHF fitted, body condition index (BCI kg/cm) calculated, length and weight recorded and pups sexed and their age was recorded in each of the seven years of the study	177
Table 14.	Descriptive statistics for maternal characteristics, pup age, attendance pattern, and milk composition of New Zealand sea lions, <i>Phocarctos hookeri</i> , in early lactation at Enderby Island, Auckland islands from 1997, 1999 to 2003 and 2005. Means values are expressed with standard deviation, sample size and with maximum and minimum values.	177
Table 15.	Morphometric data of lactating female New Zealand sea lions, <i>Phocarctos hookeri</i> , in early lactation at Enderby Island, Auckland islands from 1997, 1999 to 2003 and 2005. Body length (cm) was adjusted for age, random effects of animal, month and year whereas age (years) was adjusted for random effects of animal, month and year. Mean values are expressed with standard errors and with sample sizes (n).	178
Table 16.	Correlation coefficients ¹ between maternal body condition index (BCI; kg/cm), maternal body weight (kg), pup age (days), maternal attendance pattern (time ashore and time at sea, hrs), and the concentration of milk components (lipid g/kg, protein in whole milk g/kg, protein in skim milk g/kg, total solids g/kg, energy content kJ/g) of New Zealand sea lions, <i>Phocarctos hookeri</i> , in early lactation at Enderby Island, Auckland islands from 1997, 1999 to 2003 and 2005. Level of significance **P<0.01, ***P<0.001 after Bonferroni correction was applied.	182
Table 17.	Least squares means (\pm SE) of maternal weight (kg) and length (cm), body condition (kg) and milk composition for each consecutive month in early lactation, for each age class (young mothers 3 to 10 years old; and old mother 11 to 26 years old) and offspring sex of New Zealand sea lions, <i>Phocarctos hookeri</i> , Enderby Island, Auckland islands.	183

CHAPTER 5

Table 18.	Identification number (ID), mass, body length and age of the 25 branded female NZ sea lions and the sex of their pup, at first capture and the interval to second capture.	217
Table 19.	Actual intervals (hrs) at which females (n=25) were captured and the number of samples of milk, blubber and serum collected together with the pooled time intervals used for statistical analysis.	219

Table 20.	Fatty acid composition (mass %) of New Zealand sea lion milk, blubber, serum and a cocktail of natural vegetable oils (CoNVO) at time 0. Values are means with standard errors. Fatty acids in bold indicate that a significant difference occurred after the CoNVO was administered.	225
-----------	---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------	-----

CHAPTER 6

Table 21.	Fatty acid (FA) composition (% contribution by weight of total FA) of New Zealand sea lion milk in early lactation for 1997, 2003, 2004 and 2005 and of major prey species collected at Campbell plateau summer and autumn 2005/2006. Saturated FA (SAFA), monounsaturated FA (MUFA), polyunsaturated FA (PUFA). Values are means \pm standard errors.	265
-----------	-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------	-----

APPENDIX

Table 22.	Milk composition of some mammalian species.	308
Table 23.	Descriptive statistics for New Zealand sea lion, <i>Phocarctos hookeri</i> , maternal traits and milk composition for each female age. Values indicate means (\pm SE) maternal weight (kg), maternal length (cm) and BCI (body conditions index, kg/cm) and percentage for each milk component.	314
Table 24.	Measures of statistical fitness to assess agreement between standard method (Folch <i>et al.</i>) and alternative method (ASE) to extract milk lipid of New Zealand sea lions	315

LIST OF FIGURES

CHAPTER 1

Figure 1.	Maternal foraging strategy of otariid seals (Bonner, 1984).	5
Figure 2.	Maternal foraging strategy of phocids seals (Bonner, 1984).	9
Figure 3.	Lactation strategy in northern elephant seals (Bonner, 1984).	12
Figure 4.	The relationship between foraging trip length and milk fat content in 13 species of otariids (Data from sources in Table 4 and 7). SL= sea lions, FS= fur seals.	33
Figure 5.	Northeast Auckland Islands showing the main breeding areas for New Zealand sea lion (NZSLs): Sandy Bay, Enderby Island (in grey, 50° 50'S, 166° 28' E) and Dundas Island, 8 km south (in grey). Inset: New Zealand's sub-Antarctic. Grey shaded area indicates NZSLs' current distribution. From Chilvers <i>et al.</i> (2007a).	62

CHAPTER 2

Figure 6.	New Zealand sea lion, <i>Phocarctos hookeri</i> , milk fat concentration, (a) determined with the Roesse-Gottlieb method versus the Milkoscan (FT 120) method, (b) determined with the Roesse-Gottlieb method versus the Accelerated solvent extraction method, with the regression equation and line of best fit (solid line) and line of equality $x=y$ (broken lines). CCC =Concordance Correlation Coefficient.	121
Figure 7.	The concentration in New Zealand sea lion, <i>Phocarctos hookeri</i> , milk of (a) protein (%), and (b) total milk solids (%), determined by the Milkoscan (FT 120) (y axis) versus by the standard methods, Kjeldahl for protein and a gravimetical method for total milk solids (x axis), with the regression equation and line of best fit (solid line) and line of equality (broken lines). CC=Concordance Correlation Coefficient.	124
Figure 8.	Results of replicated analyses of New Zealand sea lion, <i>Phocarctos hookeri</i> , milk for (a) fat concentration (%), (b) protein concentration (%), (c) total solids concentration (%), determined with the Milkoscan (FT 120), with the regression equation and line of best fit (solid line) and with the line of equality (broken lines). CCC=Concordance Correlation Coefficient.	126
Figure 9.	Difference between the results of replicated analyses of New Zealand sea lion, <i>Phocarctos hookeri</i> , milk for (a) fat concentration (%) against mean milk fat concentration (%), (b) protein concentration (%) against mean milk protein concentration (%), (c) total solids concentration (%) against mean milk total solids	

concentration; determined with Milkoscan (FT 120) method, with the 95% limits of agreement (broken lines).

127

CHAPTER 3

Figure 10. The correlation between a) lipid (g/kg), b) protein in skim milk (g/kg) and c) gross energy (kJ/g) and the water content (%) of milk from New Zealand sea lions, *Phocarctos hookeri*, in early lactation over 7 years (1997-2005) at Sandy Bay, Enderby Island, Auckland Islands. 150

Figure 11. The mean concentrations (\pm SE) for a) gross energy (triangle) content (kJ/g); b) lipid (square) in the whole milk and c) protein (circle) in the skim milk; b) of milk of New Zealand sea lions, *Phocarctos hookeri*, in early lactation over seven summers from 1997, 1999 to 2003, and 2005 at Sandy Bay, Enderby Island, Auckland Islands. 151

Figure 12. Milk lipid and protein concentrations (g/kg) and relative contribution of lipid and protein to total milk energy (kJ/g) in some otariids (sea lions and fur seals) species. The energy content of the milk was calculated from standard caloric values of lipid (38.12 kJ/g) and protein (23.64 kJ/g) (Perrin, 1958). Lipid and protein values for each species were obtained from the literature as follow: NZSL, New Zealand sea lions, *Phocarctos hookeri* (this study); ASL, Australian sea lions, *Neophoca cinerea* (Gales *et al.*, 1996); SASL, South American sea lions, *Otaria byronia* (Werner *et al.*, 1996); CASL, California sea lions, *Zalophus californianus* (Oftedal *et al.*, 1987a); GSL, Galapagos sea lions, *Zalophus californianus wolfebaeki* (Trillmich and Lechner, 1986); AFS, Australian fur seals, *Arctocephalus pusillus doriferus* (Arnould and Hindell, 1999); SFS, Subantarctic fur seals, *A. tropicalis* (Georges *et al.*, 2001); GFS, Galapagos fur seals, *A. galapagoensis* (Trillmich and Lechner, 1986); CFS, Cape fur seals. *A. pussillus pussillus* (Gamel *et al.*, 2005). 154

CHAPTER 4

Figure 13. The relationship between maternal weight (kg) and maternal length (cm) for 402 adult lactating female New Zealand sea lions, *Phocarctos hookeri*, at Enderby Island, Auckland Islands. 185

Figure 14. The a) body weight (kg) (1997 n=5, 1999 n=62, 2000 n=70, 2001 n=98, 2002 n=32, 2003 n=61, 2005 n=30) and b) body condition index (kg/cm) (1997 n=5, 1999 n=62, 2000 n=70, 2001 n=98, 2002 n=31, 2003 n=61, 2005 n=30) of lactating New Zealand sea lions, *Phocarctos hookeri*, in early lactation during seven years at

CHAPTER 5

- Figure 15. Most abundant fatty acids and their baseline composition (mass %) of a cocktail of natural vegetable oil composed of 60% sunflower oil and 40% coconut oil administered to lactating New Zealand sea lion; lipid extracted from milk (n=16), serum (n=22) and blubber (n=18) from lactating New Zealand sea lions, *Phocarctos hookeri*, at Enderby Island, Auckland Islands. Values are means \pm SE. 227
- Figure 16. Temporal changes in the concentration of C10:0, C12:0 and C14:0 fatty acids (mass %) in a) milk, b) serum and c) blubber from 4 hrs to 72 hrs after a cocktail of natural vegetable oil was administered at 0 hrs to lactating New Zealand sea lions, *Phocarctos hookeri*, at Enderby Island, Auckland Islands. Values are means \pm SE. * P>0.05. 228
- Figure 17. Temporal changes in the concentration of selected fatty acids mass (%) in a) milk, b) serum and c) blubber from 4 hrs to 72 hrs after a cocktail of natural vegetable oil was administered at 0 hrs to lactating New Zealand sea lions, *Phocarctos hookeri*, at Enderby Island, Auckland Islands. Values are means \pm SE. * P<0.05. 230
- Figure 18. Temporal changes in the concentration (mass %) of saturated fatty acids (SAFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) in a) milk and b) serum 4 to 72 hrs after a cocktail of natural vegetable oil was administered at 0 hrs to lactating New Zealand sea lions, *Phocarctos hookeri*, at Enderby Island, Auckland Islands. Values are means \pm SE.* P<0.05 231
- Figure 19. The calculated equivalency of oil (g/100) that was needed to be secreted into the milk to change the concentration of (a) C10:0, (b) C12:0, (c) C14:0, (d) C16:0 and (e) C18:2n-6 fatty acids in the milk fat from that measured at 0 hrs to that observed at each of the intervals from 4 to 72 hrs after a cocktail of natural vegetable oil was administered at 0 hrs to lactating New Zealand sea lions, *Phocarctos hookeri*, at Enderby Island, Auckland Islands. Each point represents the value for an individual sea lion. 232
- Figure 20. Plot of canonical scores of fatty acid data showing the relationship between the fatty acid composition of milk samples collected from New Zealand sea lions, *Phocarctos hookeri*, at the Auckland Islands and a mixture of natural vegetable oils (oil) administered at time 0. Legends represent the pooled time intervals at which the

- samples were collected before (0 hrs) and from 4 hrs to 72 hrs after the oil was administered. 233
- Figure 21. Plot of canonical scores of fatty acid data showing the relationship between the fatty acid composition of serum samples collected from New Zealand sea lions, *Phocarctos hookeri*, at the Auckland Islands and a mixture of natural vegetable oils (oil) administered at time 0 hr. Legends represent the pooled time intervals at which the samples were collected before (0 hrs) and 4 to 72 hr after the oil was administered. 234
- Figure 22. Canonical scores plot of the fatty acids data showing the relationship between blubber samples of New Zealand sea lions, *Phocarctos hookeri*, collected at the Auckland Islands. Legends represent the time intervals at which sea lion were captured before (0 hrs) and after (4 hrs to 72 hrs) a mixture of vegetable was administered. 235
- Figure 23. Classification tree, determined by CART ($R^2=0.65$), of milk samples classified by the pooled time interval (0, 12, 24, 48, 72 hrs) that they were collected after a cocktail of natural vegetable oil was administered to lactating New Zealand sea lions, *Phocarctos hookeri*, at the Auckland Islands. Each node is labelled with the fatty acid used by CART algorithm to create the split. Values on the branches (arrows) show the concentration (% mass) of the fatty acid at which the split was made. Bracketed numbers at the terminal nodes indicate the numbers of samples from each time interval (0, 12, 24, 48, 72 hrs). 236
- Figure 24. Classification tree, determined by CART ($R^2=0.48$), of serum samples classified by the pooled time interval (0, 12, 24, 48, 72 hrs) that they were collected after a cocktail of natural vegetable oil (CoNVO) was administered to lactating New Zealand sea lions, *Phocarctos hookeri*, at the Auckland Islands. Each node is labelled with the fatty acid used by CART algorithm to create the split. Values on the branches (arrows) show the concentration (% mass) of the fatty acid at which the split was made. Bracketed numbers at the terminal nodes indicate the numbers of samples from each time interval (0, 12, 24, 48 and 72 hrs). 237

CHAPTER 6

- Figure 25. Fatty acid (FA) composition (weight percent of major (FA)), sum of Omega 3 fatty acids, Omega 6 fatty acids (and their ratio) and saturated fatty acids (SAFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) in the milk fat of New

- Zealand sea lions, *Phocarctos hookeri*, during early lactation in 1997 (n= 12), 2003 (n= 14), 2004 (n= 9) and 2005 (n= 17) summer seasons. Values are given as means \pm SEM.*p<0.05. 267
- Figure 26. Means \pm SE of the weight percent of major fatty acids in milk of the New Zealand sea lion, *Phocarctos hookeri*, collected in summer ('97:1997, n=12; '03:2003, n=14; '04:2004, n=9; and '05:2005, n=17) at the Auckland Islands and prey species collected at Campbell plateau in the summer and the autumn of 2005/2006 (Meynier unpublished data). Prey species: RC: Red cod (*Pseudophycis bachus*); JL: Javelin (*Lepidorhynchus denticulatus*); OF: Opalfish (*Hemerocoetes spp.*); AS: Arrow squid (*Nototodarus sloanii*); HK: Hoki (*Macruronus novaezelandiae*). 268
- Figure 27. The weight percent of selected fatty acids and the ratio of C20:1n-11 : C20:1n-9 in milk (sampled in four summer seasons) of New Zealand sea lion, *Phocarctos hookeri*, and in prey species collected at Campbell plateau summer and autumn 2005/2006 (Meyner et al. unpublished data). Values are means \pm standard errors. See (Iverson, 1993; Iverson *et al.*, 1997b). *Significantly different p<0.05. 269
- Figure 28. Classification tree, determined by CART, of the fatty acid composition of milk from New Zealand sea lions, *Phocarctos hookeri*, collected at their breeding site on either Dundas Island or Sandy Bay at the Auckland Islands. Each node is labelled with the fatty acid used by CART algorithm to create the split. Values on the branches (arrows) show the level (% mass) of the fatty acid at which the split was made. Bracketed numbers at the terminal nodes indicate the numbers of samples from each breeding site. 270
- Figure 29. Relationship between monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) in the austral summers of 1997, 2003, 2004 and 2005 (PUFA = -0.65*MUFA + 55.38, R² = 0.76) milk samples collected from New Zealand sea lions, *Phocarctos hookeri*, in early lactation at the Auckland Islands. 271
- Figure 30. Plot of the canonical scores of the milk fatty acids in milk of New Zealand sea lion, *Phocarctos hookeri* milk fatty acids and prey species in milk samples collected in 1997, 2003, 2004, 2005 (lines are illustrative and delimit the samples by years).at the Auckland Islands and prey species collected at the Campbell plateau in the summer and autumn of 2005/2006 (Meynier unpublished data). Prey species were as follow. Prey species: RC: Red cod (*Pseudophycis bachus*); JL: Javelin (*Lepidorhynchus denticulatus*);

- OF: Opalfish (*Hemerocoetes* spp.); AS: Arrow squid (*Nototodarus sloanii*); HK: Hoki (*Macruronus novaezelandiae*). 273
- Figure 31. Plot of canonical scores of the fatty acids (FA) in milk of Zealand sea lions, *Phocarctos hookeri* milk fatty acids and FA in potential prey species. Milk samples collected in 1997, 2003, 2004, 2005 at the Auckland Islands and prey species collected at the Campbell plateau in the summer and autumn of 2005/2006 (Meynier unpublished data). a) only dietary “indicator FAs” and b) only main FAs in prey species were used. Prey species: RC: Red cod (*Pseudophycis bachus*); JL: Javelin (*Lepidorhynchus denticulatus*); OF: Opalfish (*Hemerocoetes* spp.); AS: Arrow squid (*Nototodarus sloanii*); HK: Hoki (*Macruronus novaezelandiae*). 275
- Figure 32. Classification tree, determined by CART, of the fatty acid (FA) composition of milk collected from the New Zealand sea lion, *Phocarctos hookeri*, in 1997, 2003, 2004 and 2005 at the Auckland Islands. Each node is labelled with the FA used by CART algorithm to create the split. Values on the branches (arrows) show the level (% mass) of the FA at which the split was made. Bracketed numbers at the terminal nodes indicate the numbers of samples from each year (1997, 2003, and 2004 2005) and letters at each the terminal indicate prey species (AS= arrow squid; HK=Hoki; JL=Javelin; OF=Opalfish; RC= Red cod). 277
- CHAPTER 7**
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- Figure 33. Fisheries annual catches (tonnes) of arrow squid (*Nototodarus sloanii*) at the Auckland Islands. (Data provided from Ministry of Fisheries, New Zealand). 297

LIST OF ABBREVIATIONS

• AMF	Anhydrous milk fat
• AOAC	Association of Official Analytical Chemists
• BCF	Bias Correction Factor
• BCI	Body condition index
• CART	Classification and regression tree
• CCC	Concordance correlation coefficient
• CDA	Canonical Discriminant analysis
• CLO	Cod liver oil
• CoNVO	Cocktail of Natural Vegetable Oils
• DFA	Discriminant Function Analysis
• ENSO	El Niño Southern Oscillation
• FA	Fatty acid
• FAME	Fatty acid methyl esters
• FASA	Fatty Acid Signature Analysis
• ICC	Intraclass correlation coefficient
• MUFA	Monounsaturated fatty acids
• NEFA	Non-esterified fatty acids
• NPN	Non-protein nitrogen
• NZSL	New Zealand sea lion
• PUFA	Polyunsaturated fatty acids
• QFASA	Quantitative Fatty Acid Signature Analysis
• r	Pearson correlation coefficient
• r^2	Coefficient of determination
• RPE	Relative prediction error
• R-G	Roese Gottlieb method for fat determination
• SAFA	Saturated fatty acids
• SDA	Stepwise Discriminant analysis
• TAG	Triacylglycerol

