Reproductive Strategies of Adult Female Weddell Seals (*Leptonychotes weddellii*) and Their Implications for Pup Survival

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Declaration of originality

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Kathryn E. Wheatley September 19th, 2007

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Thesis Abstract

Life history strategies reflect variation in the allocation of an individual's resources (i.e., time, effort and energy expenditure) to competing life functions such as growth, survival and reproduction. For mammals, producing milk is one of the most energetically expensive activities for females, so factors determining its delivery to offspring essentially define the reproductive strategy a species evolves. The efficiency with which energy is transferred via milk also determines the reproductive investment trade-off that exists between survival and future reproduction. The key objective of this study was to examine physiological aspects of lactation in Weddell seals (*Leptonychotes weddellii*), to gain a better understanding of reproductive strategies of an upper trophic level predator that must cope with unpredictable food availability in an extreme and highly variable environment.

Female body mass (and absolute body fat) at parturition differed between the two years of study and this difference appeared to drive the length of the lactation period, maternal energy expenditure, pup mass gain and weaning mass. Effects were more marked in smaller individuals that did not increase energy expenditure to reconcile this disparity.

Milk composition was independent of maternal post-partum mass (MPPM) and condition, but did change over lactation. Protein tripled from post-partum (PP) to end-lactation (EL) while lipid and energy increased to mid-lactation (ML) then slightly decreased. This pattern of changes may be related to the relatively long lactation period demonstrated by this species and the energetic and physiological needs of the mother and pup. There was evidence through both milk energy output and fatty acid transfer that feeding occurred in some individuals later in lactation.

A major source of energy during lactation is provided through the mobilisation of blubber fatty acids (FA). I investigated the extent to which FA were mobilised to support both maternal metabolic requirements and milk production, and how this was reflected in the FA composition of the pups at EL. Fatty acid composition at PP was similar in females from both years indicating similar diets. However, selective mobilisation and transfer did occur during lactation which not only affected the vertical stratification of FA within the blubber layer but also the composition of the pups at EL. This was related to total body lipid stores of females at PP. It appears that

selective mobilisation was most likely related to the physiological requirements of the developing pup. Highly mobilised fatty acids are underestimated in the blubber and affect diet predictions. Failing to account for mobilisation during periods of high turn-over may seriously bias FASA diet estimates. Results suggest that dietary predictions will be improved when samples are taken at parturition.

Differences in MPPM between years reflects environmental variability during the period of prey acquisition, and this manifests as differences in expenditure during lactation. These differences translate to changes in pup mass and condition at weaning with consequences for future survival and recruitment. My results confirm that differences in life history strategies exist within lactating Weddell seals and the trade-off between long-term survival in breeding females and the success of their offspring is contingent on individual size, which is further complicated by feeding to offset nutritional constraints imposed during poor-resource years.

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List of Abbreviations

Abbreviation	Measurement
%DE	per cent deviance explained by each model
%L	per cent lipid content of milk
%ME	per cent milk energy
%P	per cent protein content of milk
%W	per cent water content of milk
AIC_c	Akaike's Information Criterion corrected for small samples
DF	discriminant function
DFA	discriminant function analysis
dpp	days post-parturition
$\Delta w+$	predictor weight of evidence
EFA	essential fatty acid
EL	end-lactation
ER	information-theoretic evidence ratio
FA	fatty acid(s)
FASA	fatty acid signature analysis
GC	gas chromatographic
GLM	generalized linear model
GLMM	generalized linear mixed-effects model
HDO	deuterium oxide
HTO	tritiated water
IM	intramuscular
IV	intravenous
LC-MUFA	long-chain monounsaturated fatty acid
MI	milk intake
ML	mid-lactation
MPPM	maternal post-partum mass
PCA	principal component analysis
PP	post-parturition
PUFA	polyunsaturated fatty acid
SC-MUFA	short-chain monounsaturated fatty acid
SEM	standard error of the mean
SFA	saturated fatty acid
TAG	triacylglycerol
TBL_{MPPM}	total body lipid at post-partum
TBP_{MPPM}	total body protein at post-partum
TBW	total body water
TWI	total water influx

Chapter 1

General Introduction

1.1 Introduction

A trophic level is the nutritional position occupied by an organism in a food web and ranges from primary producers to tertiary consumers (top predators). Upper trophic level species, or predators, are downstream from energy flow variation within an ecosystem, and can therefore be reliable and cost-effective for examining the biological consequences of environmental change because variability in resource abundance may affect their diet, reproductive performance and population size (Erikstad *et al.*, 1998; Hindell *et al.*, 2003; Le Boeuf & Crocker, 2005; Orzack & Tuljapurkar, 2001; Reid *et al.*, 2005). Therefore, research on upper trophic level species may improve our understanding of how predators respond to predicted changes in the distribution of resources as the result of global climate change or commercial exploitation.

The evolution of reproductive strategies is driven by a balance between the costs incurred by the parents through investment in offspring (essentially, the energetic trade-off between successful reproduction and the maintenance of future reproductive opportunities), and the value they obtain from this investment in terms of their offspring's subsequent reproductive output (Shuster & Wade, 2003). To understand fully how mammals translate variability in resources to reproductive output, data on key demographic and physiological parameters such as the quantification of energy transfer, assimilation efficiency, resource allocation and survival are needed.

In this thesis, I investigate reproductive strategies of adult female Weddell seals at McMurdo Sound to examine the causes and consequences of intra-specific and inter-annual differences in the allocation of resources. I focus primarily on the quantification of mass and energy transfer during lactation, milk and fatty acid composition and transfer and how these are reflected in pup condition and future survival.

1.2 Life history theory

Life history theory describes and predicts variation in physiological and behavioural characteristics that reflect differing allocation of an individual's resources

(e.g., time, effort and energy expenditure) to competing life functions such as growth, body maintenance and reproduction. It suggests that organisms should invest in those aspects of their life histories that contribute most to fitness (Ricklefs & Wikelski, 2002). For example, long-lived organisms with low fecundity should attempt to maximize adult survival (and therefore future reproduction) at the expense of current reproduction (Stearns, 1992). Thus, the allocation of resources involves trade-offs, often exhibiting a negative functional interaction between traits. Given that energy-limited organisms cannot simultaneously maximize all components of fitness, these trade-offs act as constraints and determinants of life history evolution. Numerous studies have attempted to explain why species vary in life history traits (Ricklefs & Wikelski, 2002; Roff, 1992; Stearns, 1992), and the measurement and interpretation of energy trade-offs have played a prominent role in the development of the resultant life history theory (Roff & Fairbairn, 2007). A broader understanding of trade-offs and their mechanisms will ultimately improve our knowledge of the evolution of species diversity and how organisms cope with variable environments.

The demographic cost of reproduction, (i.e., decreased survival and future reproduction as a function of current reproduction) is a pivotal trade-off through which life histories are thought to evolve (Harshman & Zera, 2006; Stearns, 1992). Therefore, examining the proximate mechanisms that evolved to deal with environmental variability may assist in the evaluation of the functional explanations for both inter- and intra-specific differences in reproductive effort. A variety of selective pressures such as seasonality and unpredictability of the environment, food availability, predators and disease, mould the evolution of particular life histories. The strategic response of individuals to the environment is the foundation of life history theory (Stearns, 1989). Indeed, most of the variation in life histories reflects responses to environmental stresses, and physiology mediates an organism's sensitivity to its environment (Ricklefs & Wikelski, 2002). Therefore, physiological studies are ideal to identify functional interactions among various components of life history traits by focusing on differential allocation of limiting internal nutrients to reproduction, maintenance metabolism, growth and storage.

1.3 Reproductive effort

The term *reproductive effort* was selected to describe the measurement of the fraction of resources invested in reproduction and the costs of reproduction on somatic investments (Williams, 1966). A phenotype for a given level of reproductive effort must enhance the individual's fitness if it is to be selected. Therefore, the term implies a direct, fixed interaction between current and future reproductive success. Reproductive effort, and life history traits in general, are optimised by maximizing fitness under purely demographic forces of selection (Tuomi *et al.*, 1983).

Measuring differences in reproductive effort between individuals or species requires quantitative measures of effort as opposed to simple measurements of phenotypic characteristics (Hirshfield & Tinkle, 1975). Limited internal resources have typically been viewed as the constraint that causes allocation trade-offs (Zera & Harshman, 2001). Through detailed studies of energy intake, expenditure and storage, physiological studies can quantify trade-offs for individual and species comparisons.

Reproductive strategies are suites of co-evolved anatomical and physiological traits for optimal partitioning of the available energy to growth and reproduction according to a set of 'priorities' (Fig. 1.1) that ensure survival and optimise long-term reproductive success. The priorities for energy allocation can change according to intrinsic factors such as age and parity or extrinsic (environmental) factors such as decreased prey availability. However, cellular maintenance, thermoregulation and locomotor costs of obtaining food must be satisfied first (Bronson, 1985). To understand the evolution of reproductive strategies therefore requires measurements of behaviour and energy requirements of mothers and their offspring.

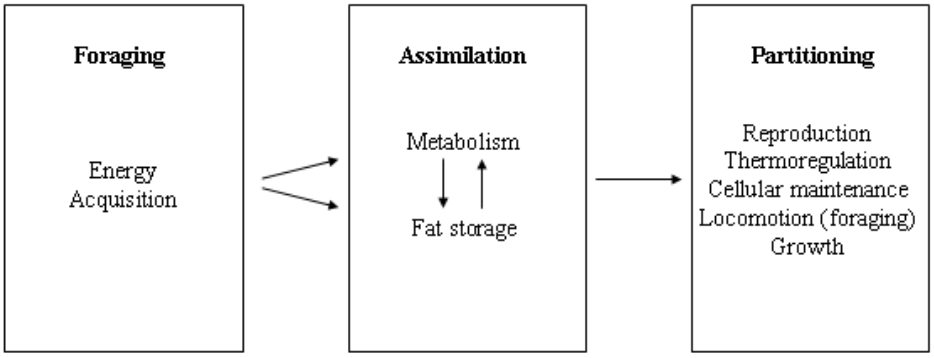

Figure 1.1 *A diagram representing the energy flow and partitioning among various life history traits.*

1.4 Lactation

Lactation is the period following birth during which milk is secreted to provide nourishment to the offspring. It is one of the principal features characterising mammals, and in many species, it represents a period of extreme demand on energy reserves (Gittleman & Thompson, 1988; Rogowitz, 1996). This high energy demand has a profound effect on a female's consumption, utilisation and partitioning of metabolic fuels, so that females can adjust more efficiently their provisioning effort to changes in environmental productivity (Dall & Boyd, 2004). Diverse energy provisioning strategies have evolved to form a continuum of energy utilisations ranging from immediate energy use (income breeding strategy) to long-term storage for later use (capital breeding strategy). Regional, seasonal and year-to-year variation in food availability and different aspects of physiology may also affect the expression of a particular strategy that a species adopts (Boyd, 2000).

The production of milk offers reliable nourishment to young and a great advantage in terms of success of a reproductive attempt, especially when the risk of an energetic shortfall while foraging (during offspring dependence) is large (Dall & Boyd, 2004). Furthermore, although lactation imposes energetic stress on mothers, it allows offspring to devote a higher proportion of energy (from milk) to growth rather than maintenance since almost no energy is required to obtain food (Pond, 1977). Ultimately, the strategy that a female adopts will influence the daily rates of milk energy output and offspring growth during lactation.

In some mammals, understanding lactation strategies is complicated by allomaternal care, multiple offspring litters and post-weaning maternal care (Schulz & Bowen, 2005). However, pinnipeds are excellent models for the study of lactation strategies; they are easily accessible during their terrestrial breeding season, they are highly philopatric to breeding site, there is no paternal care and maternal care ends at weaning. Furthermore, variation in maternal size, breeding habitat and lactation length make this an interesting group in which to examine factors that influence reproductive effort.

Within the Suborder Pinnipedia (Order Carnivora) most females from the Family Phocidae ('true seals') generally follow a capital- (fasting-) based strategy, while females from the Family Otariidae (fur seals and sea lions) generally follow a more income-based strategy (Boyd, 2000). Originally this division was thought to

arise phylogenetically; however, there is evidence that some phocids follow a mixed strategy, in that capital expenditure is supplemented by some feeding during some phases of the lactation period (Bowen *et al.*, 2001; Eisert *et al.*, 2005; Lydersen & Kovacs, 1999), suggesting that these species fall somewhere between the two extremes of the continuum. Therefore, rather than purely a phylogenetic basis, variation in lactation strategies may also be driven by physiological adaptations and local environmental pressures that unbalance strictly binary strategies. Studies of reproductive effort should therefore focus on examining lactation strategies and energy expenditure simultaneously with local fluctuations in the environment and prey abundance.

1.5 Weddell seals

Weddell seals (*Leptonychotes weddellii*) are long-lived, upper trophic level predators of the Southern Ocean. Like all large phocids, Weddell seals deposit subcutaneous fat during the non-breeding season and then use this stored energy for milk production and maintenance during lactation. This allows prior foraging success to fuel current provisioning, so that provisioning is limited by maternal reserves. Although some maternal feeding during lactation may complicate measurements (see below), the reproductive effort of Weddell seals can be quantified because a female's energy budget can be determined by measuring body mass and composition at the beginning and end of lactation. The difference will be a measure of the energy allocated to reproduction and infers that the rest was for growth and maintenance.

The temporal separation between acquisition and expenditure of resources is often associated with a spatial separation as well, and is an important dimension to life history variation. The physiology of lactation is influenced strongly by the constraints resulting from this division between foraging and reproduction (Crocker & Costa, 2001), and fluctuations in energy acquisition prior to breeding are expressed as variation in maternal mass and condition. These may impose energetic constraints that influence pup birth mass, growth or survival (Crocker *et al.*, 2001; Hall *et al.*, 2001; McMahon & Burton, 2005; McMahon *et al.*, 2000b; Pomeroy *et al.*, 1999), and ultimately affect the lifetime reproductive success of mothers (Trillmich, 1996). Thus, the constraints imposed by the capital breeding strategy are useful for examining the

consequences of environmental variability over a short time span (*c.* 1 year).

However, there is evidence that some females also forage during lactation (Eisert *et al.*, 2005; Hindell *et al.*, 2002; Sato *et al.*, 2002). This behaviour is unusual for a large phocid and may have evolved to compensate for the high energy expenditure required for the relatively long lactation period (6-7 weeks, Tedman & Bryden, 1979) compared to similar sized phocids (e.g., elephant seals, 21-23 days) , and/or the bouts of swimming and diving (starting 10-12 days) necessary to introduce the pup to the water. Nevertheless, not all individuals exhibit this behaviour, questioning its significance in supplementing the energetic costs of lactation. Environmental conditions may affect the magnitude of female energy stores, favouring flexibility of reproductive effort, so that occasionally lactational strategies may deviate from expectations, and females may compensate for poor pre-breeding foraging by feeding during lactation. Therefore, it appears that Weddell seals are ideal for studying flexibility in reproductive effort as they must cope with high interannual variability in resource abundance, are highly philopatric and they are easily accessible during the breeding periods.

1.6 Energy storage - fatty acids

In capital breeding mammals, blubber is the primary site for energy storage; since lipids are used as the principal metabolic energy source during fasting, the blubber reserves of capital breeders are highly dynamic

In response to the high physiological demands of lactation, a major source of energy is provided through the mobilisation of fatty acids (FA) from the breakdown of triacylglycerol. Fatty acids are stored primarily in the blubber and form an essential part of physiological regulation as precursors to the synthesis of other compounds, as fuels for energy production, and as building blocks for cell membranes (Dalsgaard *et al.*, 2003). Fatty acids in blubber may be accumulated directly from the diet, modified once ingested, or formed endogenously. Omega-3 and omega-6 fatty acids are termed essential fatty acids (EFA) because they cannot be made *de novo* by mammalian cells and must be provided through diet. These essential fatty acids are required for growth and normal cell development (Innis, 2005).

The omega-3 and omega-6 fatty acid composition of milk varies considerably

among species, probably reflecting differences in diet and lipid metabolism, and studies have shown that their secretion depends on the amount of fatty acids in the maternal diet (Innis, 2005). This will influence the EFA available to offspring, and may affect growth and development. In response, specific fatty acids may be differentially mobilised or sequestered to accommodate the physiological requirements of both mother and pup (Samuel & Worthy, 2004). Therefore, metabolism and deposition of fatty acids may not be predictable, depending on the physiological needs of the mother and pup at any given time. In addition, some fatty acids provide more chemical energy (per unit) than others (e.g., saturated fatty acids), so that diet composition, and its reflection within the blubber, may itself affect energy expenditure (Maillet & Weber, 2006; Pierce & McWilliams, 2005). As a result, diet, fatty acid mobilisation and transfer during lactation are important for the energetic requirements of the mother and the development of her offspring.

Fatty acids have been used as qualitative markers to trace or confirm predator-prey relationships in the marine environment for more than thirty years (Dalsgaard *et al.*, 2003). In particular, interest has focussed on using fatty acids to elucidate the diet of upper trophic level species, based on the premise that differences will aid in the assessment of abundance and demographic shifts in lower trophic level taxa (i.e., prey), and thus, advance broad-scale ecosystem-level information. However, to quantify relationships using fatty acids in marine mammals, specific aspects of fatty acid dynamics (e.g., incorporation and mobilisation) are required. Because lipids are used as the principal metabolic energy source during fasting, the blubber reserves of phocid seals are highly dynamic; therefore, detailed knowledge of deposition and turnover is required for species-specific dietary predictions.

1.7 Thesis outline

The aim of this research was to examine the behavioural and physiological patterns of maternal care and lactation of an upper trophic level predator with respect to pre-breeding foraging success. The results will facilitate a better understanding of the effects of physiological characteristics (as a response to the environment) on the partitioning of resources to reproduction and other life history traits.

Chapters 2 - 6 were written as separate scientific articles that have either been

published, or are currently in review. In all cases, I was the senior author, conducted laboratory work, analysis of data and writing of the papers. My co-authors contributed to data collection, advice on analysis and by critically reviewing and contributing to manuscripts in preparation for publication. These papers include research on a number of aspects of lactation in Weddell seals, but the thesis is tied together by the common theme of reproductive strategies of Weddell seals. Each chapter is essentially self-contained; therefore, there may be some repetition in content throughout the thesis.

Chapter 2: Chemical immobilisation

Historically, chemical immobilisation of Weddell seals has been problematic, often resulting in death. I investigated the use of an immobilising agent, Zoletil, which has been successful with a number of other species. The physiological status (age, condition and stage of lactation) of individual females was included in analyses to quantify the variation in sensitivity to induction and recovery times. I propose recommendations for safe and effective immobilisation of Weddell seals for future safe handling practices. This was an essential first step, as the remainder of this study relied on safe handling and immobilisation practices.

Chapter 3: Lactation energetics

In this chapter, maternal post-partum mass and body composition were measured for females at the beginning of lactation, as an indicator of overwinter foraging success for two consecutive years. Inter-annual differences in maternal expenditure (mass and composition) and offspring mass and condition were quantified in two years when individuals exhibited marked differences in these traits. I hypothesised that when maternal mass is low, females would transfer less mass and energy to the pup during lactation, and that this would have detectable consequences on pup growth rate, condition and survival. Furthermore, differences in expenditure may reflect environmental variability during the period of prey acquisition.

Chapter 4: Breeding strategies

This chapter examined the degree to which adult female Weddell seals have

evolved a mixed capital-income breeding strategy to cope with the energetic costs of lactation. Changes in milk composition and milk output were measured to determine the extent to which lactation was fuelled by food intake, and how this varied among individuals. I hypothesised that (1) smaller females or females with lower total body lipid stores would fuel lactation by lipid reserves (capital breeding strategy) at the beginning, but would later supplement reserves with income-based provisioning as reserves were depleted; (2) larger females would be able to sustain lactation entirely through capital reserves, and; (3) because larger animals need higher food intake to achieve a positive energy balance, foraging would make a relatively small contribution to the total energy budget of lactation. Differences in reproductive strategies will most likely have detectable consequences on pup growth rate, condition and survival.

Chapter 5: Fatty acid mobilisation

Chapter 5 details the analysis of fatty acid composition and mobilisation of adult female Weddell seals to support maternal metabolic requirements and milk production during lactation. The fatty acid composition of the pup's blubber at weaning was also examined to determine the proportion of fatty acids that were used for growth and maintenance and stored in the pup's blubber. My aims were to determine (1) if particular fatty acids were selectively mobilised and/or transferred during lactation; (2) if mobilisation was influenced by initial fatty acid composition, and; (3) if particular fatty acids were selectively deposited or used. Differential mobilisation may vary in response to the energetic demands of the pup over the course of its development.

Chapter 6: Fatty acids and dietary predictions

In this chapter I examined the amount of vertical stratification in fatty acid composition of female blubber, and how this changed over lactation. This was to collect detailed, species-specific information on fatty acid deposition, distribution and mobilisation to develop fatty acid signature analysis further for identifying energy-flow linkages within ecosystems. The specific aims were to determine (1) the extent of fatty acid stratification in the blubber of female Weddell seals; (2) if particular fatty

acids were selectively mobilised from the inner compared to the outer blubber layer during lactation, and; (3) how mobilization affected diet predictions. This information will aid in future dietary investigations, improving our understanding of the relationship between diet, condition and reproductive effort.

Chapter 7: General Discussion

This thesis concludes with a general discussion that synthesises inter-annual and intra-specific differences in reproductive effort of Weddell seals, and the possible consequences for pup survival. These results are discussed in relation to reproductive flexibility, life history trade-offs and long-term survival of long-lived mammals in highly variable and changing environments.

Chapter 2

Chemical immobilisation of adult female Weddell seals with tiletamine and zolazepam: effects of age, condition and stage of lactation

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Abstract

Chemical immobilisation of Weddell seals (*Leptonychotes weddellii*) has previously been, for the most part, problematic and this has been mainly attributed to the type of immobilising agent used. In addition to individual sensitivity, physiological status may play an important role. We investigated the use of the intravenous administration of a 1:1 mixture of tiletamine and zolazepam (Telazol®) to immobilise adult females at different points during a physiologically demanding 5-6 week lactation period. We also compared performance between IV and IM injection of the same mixture.

The tiletamine:zolazepam mixture administered intravenously was an effective method for immobilisation with no fatalities or pronounced apnoeas in 106 procedures; however, there was a 25 % (one animal in four) mortality rate with intramuscular administration. Induction time was slightly longer for females at the end of lactation (54.9 ± 2.3 seconds) than at post-parturition (48.2 ± 2.9 seconds). In addition, the number of previous captures had a positive effect on induction time. There was no evidence for effects due to age, condition (total body lipid), stage of lactation or number of captures on recovery time.

We suggest that intravenous administration of tiletamine and zolazepam is an effective and safe immobilising agent for female Weddell seals. Although individual traits could not explain variation in recovery time, we suggest careful monitoring of recovery times during longitudinal studies (≥ 3 captures). We show that physiological pressures do not substantially affect response to chemical immobilisation with this mixture; however, consideration must be taken for differences that may exist for immobilisation of adult males and juveniles. Nevertheless, we recommend a mass-specific dose of 0.50 – 0.65 mg/kg for future procedures with adult female Weddell seals and a starting dose of 0.50 mg/kg for other age classes and other phocid seals.

2.1 Introduction

Immobilisation of captive and free-ranging pinnipeds is often required for biological studies, translocation or the examination of sick or injured animals. However, pinnipeds present unique problems when using chemical immobilisation agents because they have evolved specific adaptations in their respiratory, cardiovascular and thermoregulatory systems enabling them to dive for extended periods. These adaptations can exacerbate problems associated with chemical immobilisation procedures (Gales, 1989; Haulena & Heath, 2001). This physiological "dive response" is characterised by profound bradycardia, shunting of blood away from peripheral tissues, and periods of prolonged apnoea (Kooyman *et al.*, 1981) that can be aggravated by the presence of immobilising agents in the blood and tissues. This may result in relatively high concentrations of drug being transported to central organs, particularly the brain, which also affects the level of immobilisation and recovery time (Bornemann & Plötz, 1993). The physiological status of an animal has also been shown to have a profound effect on sensitivity to immobilisation and on the ability to metabolize chemicals (Woods *et al.*, 1989). Furthermore, the number of previous captures and immobilisations can increase recovery time (Field *et al.*, 2002). Therefore, knowledge of the physiological (i.e., total body lipid) and anatomical characteristics (e.g., in some species the trachea is flat and has incomplete cartilaginous rings which may increase the risk of respiratory obstruction), methods of administration, and species-specific response to particular drugs are important for the effective, safe and optimal application of chemical immobilisation in free-ranging wildlife species.

Intramuscular (IM) injection has been one of the most commonly used routes for administration of immobilising agents in pinnipeds (see Gales, 1989), and it is thought to be relatively safe and easy compared to other methods. Immobilisation by intravenous (IV) injection has recently become more common with some species (Field *et al.*, 2002; McMahon *et al.*, 2000a; Slip & Woods, 1996). Although physical restraint is required prior to the administration of drugs using IV methods, smaller doses and better control of the intensity and duration of immobilisation are generally achieved compared to IM injection methods. Pinnipeds that received the same drugs by IV and IM injection have been reported to have shorter induction and recovery times and less variable responses when IV methods were used (Engelhardt, 1977;

Sepulveda *et al.*, 1994; Slip & Woods, 1996).

Weddell seals (*Leptonychotes weddellii*) are deep-diving (> 500 m) predators that have been the subject of many studies requiring immobilisation. Many of these studies have reported varying responses to immobilising agents (Cline *et al.*, 1969; Flyger *et al.*, 1965; Gales & Burton, 1988; Hammond & Elsner, 1977; Phelan & Green, 1992), and most have reported mortality rates ranging from 10 to 31 %, indicating that Weddell seals may be particularly sensitive. More recently, a safe method of gas anaesthesia (zero mortality) of Weddell seals has been reported by Kusagaya & Sato (2001) and Bodley *et al.* (2005) (n = 9, n = 11, respectively); however, this procedure is not always practical for field situations due to the cumbersome equipment required. Therefore, a reliable, safe and direct technique of immobilisation is still required for this species.

A 1:1 mixture of tiletamine and zolazepam (available commercially as Telazol®, Fort Dodge, Castle Hill, Australia, or Zoletil®, Virbac, Peakhurst, Australia) has been characterised by rapid, smooth induction, good analgesia (unresponsive to painful stimuli), maintenance of pharyngeal and laryngeal reflexes, and a smooth recovery phase (Lynch *et al.*, 1999). The tiletamine:zolazepam mixture has been used successfully to immobilise a range of domestic and wild mammals, including some pinniped species (Baker *et al.*, 1990; Field *et al.*, 2002; Gage, 1993; Hammond & Elsner, 1977; Karesh *et al.*, 1997; Massolo *et al.*, 2003; McMahon *et al.*, 2000a; Samelius *et al.*, 2003; Stirling & Sjare, 1988; Tracy *et al.*, 1988). In its experimental stage (2:1 ratio mixture of tiletamine and zolazepam), Telazol was effective with Weddell seals (Hammond & Elsner, 1977); however, some complications (i.e., apnoea leading to death) were encountered in a later study by Phelan & Green (1992). This may have been due to the method of administration rather than the drug itself (see Discussion). The 1:1 mixture of tiletamine:zolazepam potentially offers a safe and effective method for immobilising Weddell seals in the field.

In this study we investigated the use of tiletamine:zolazepam (Telazol®) for Weddell seals. We compared performance between IV and IM injection, and examined the relationship between age, body condition (total body lipid, TBL) and stage of lactation on induction and recovery time. We hypothesised that variation in these parameters among individuals would influence drug sequestration and recovery time and that this may be more pronounced than at other stages in this species' life history due to the physiological pressures and energetic constraints of lactation

(Woods *et al.*, 1989). In addition, we examined differences in recovery time due to the number of previous immobilisations. We hypothesised that recovery time would decrease for individuals that had been chemically immobilised previously, as has been found in other species (Field *et al.*, 2002).

2.2 Methods

2.2.1 Field Procedures

A total of 110 chemical immobilisation procedures using Telazol (1:1 mixture of tiletamine and zolazepam) were done on adult female Weddell seals as part of a study on maternal energy expenditure and lactation energetics. One hundred and six of these were by IV injection and 4 were by IM injection. Some individuals were immobilised more than once during the course of their lactation period (5-6 weeks), but no individuals were immobilised more than three times. Females were caught on the sea ice at Erebus Bay, Antarctica (77° 51' S, 166° 45' E) during the breeding season (October to December) of 2002 and 2003. Individuals were identified by flipper tags attached in previous years as part of a long-term tagging study (Cameron & Siniff, 2004), and ages ranged from 6 to 22 years old.

Females were easily approached on the ice and pups were relocated several metres away to avoid potential injury. Subsequently, a canvas bag was placed over the female's head (McMahon *et al.*, 2000a), after which the majority of individuals remained in a prone position without struggle. The few that were slightly agitated would commence a 'rolling' behaviour and could not be restrained effectively on the ice. However, this behaviour typically ceased within 2-3 minutes. Females were then injected with Telazol intravenously via the extra-dural vein in the lumbar region (McMahon *et al.*, 2000a) using a 5 ml syringe and 15 cm (6") 18G spinal needle, or intramuscularly in the rear flank with a 10 ml syringe and 9 cm (3.5") 18G needle. We attempted to give dosages of 0.5 mg/kg (McMahon *et al.*, 2000a) and 0.75 mg/kg (Hammond & Elsner, 1977) IV and IM, respectively. Dosages at the first capture were calculated using an estimate of female body weight based on researcher's previous experience working with phocids. For additional captures, dosages were calculated by estimating mass loss rates through lactation. Drug induction and recovery times were

recorded and the respiratory rate and volume of air moving (as estimated by listening to breath sounds) was monitored throughout procedures. Induction time (seconds) was defined as the time from injection until the animal did not respond to a tap on the nose (McMahon *et al.*, 2000a). The recovery time (minutes) was defined as the time from immobilisation until the seal responded to a tap on the nose by moving and raising its head and maintaining its head in a raised position for ~ 10 seconds (Woods *et al.*, 1994). This was repeated several times to ensure complete recovery. An endotracheal tube, oxygen, doxapram hydrochloride (Dopram®, Wyeth, Baulkham Hills, Australia) and flumazenil (Anexate®, Roche, Castle Hill, Australia) were available in the event of respiratory arrest.

After immobilisation, females were weighed to the nearest 1 kg using electronic scales and standard body length and six girth measurements (G1-G6, Field *et al.*, 2002) were recorded. The precise dosages of tiletamine:zolazepam were calculated for each female based on measured weights. Body composition (i.e., proportion of lipid and lean tissues) was measured using an isotopically labelled water technique. A 10 ml blood sample was collected to measure background isotope levels followed by the IV injection of a pre-weighed dose (to the nearest 0.1 mg) of 222 MBq of tritiated water (HTO) into the extradural vein. The syringe was flushed with blood twice to ensure complete isotope delivery. A second blood sample (10 ml) was taken approximately 150 minutes after initial injection for the calculation of dilution space and body composition. Houser & Costa (2001) found that HTO equilibration occurs within 90 minutes of an intravenous injection of northern elephant seal (*Mirounga angustirostris*) pups. Equilibration occurs in southern elephant seal (*M. leonina*) pups within 120 minutes of administration (IV; K.E. Wheatley, unpublished data). Therefore, we considered 150 minutes to be sufficient time before collecting a second blood sample. All samples were stored at -20° C until analysis.

2.2.2 Laboratory analysis

Plasma samples were analysed for HTO activity using liquid scintillation spectrometry. Plasma samples (100 µl) were distilled in triplicate using the method of Ortiz *et al.* (1978). For each vial of water recovered, 4 ml of EcoLite scintillate (ICN, Costa Mesa, USA) was added and HTO activity was counted for 15 minutes using a

Beckman LS6500 scintillation counter. Correction for quenching was made by automatic external standardization. Calculations of body composition were done as described by Reilly & Fedak (1990).

2.2.3 Data analysis and calculations

We did not obtain body composition data for 37 captures, but for 11 of these animals we obtained composition data for captures before and after the capture in question. Body composition for this intermediate capture was estimated by interpolation, assuming the change in composition was linearly proportional to a change in mass.

A set of generalised linear models (GLM) and penalized quasi-likelihood (PQL, Mammen & Van de Geer, 1997) generalised linear mixed-effects models (GLMM) were constructed to examine the relationships between recovery and induction time and the various state variables. GLMs extend the standard regression model by (1) distributing the response y about its expected value μ according to a distribution F (e.g., normal, gamma, binomial, etc.), and (2) entering the predictors x_1, x_2,., x_m into the model through the linear predictor η, which is related to the expected response μ by a monotonic link function $\eta_i = \eta(\mu_i)$ (McCullagh & Nedler, 198̃9) GLMMs are linear models that include both fixed and random effects, where random effects are those associated with individual experimental units drawn at random from a population (e.g., individuals as in this study, Pinheiro & Bates, 2000). GLMMs offer the advantage of partitioning variances due to the effects under investigation (fixed) and those that do not contribute to the hypotheses being tested (random).

Model comparison used Kullback-Leibler information to assign relative strength of evidence (Akaike's Information Criterion corrected for small samples, AIC_c, Burnham & Anderson, 2002; Lebreton *et al.*, 1992) to each model in the set (Burnham & Anderson, 2002). To compare a more complex model *a* to a simpler model *b*, we employed the information-theoretic evidence ratio (*ER* = AIC_c weight of model *a* ÷ AIC_c weight of model *b*) to quantify the relative support of *a* versus *b*, and used the per cent deviance explained (%DE) to determine structural goodness-of-fit of model *a* (test for model adequacy). Higher *ER* values indicate higher likelihoods of the tested model relative to model *b* (e.g., the null model).

The weights of evidence ($w+_i$) for each predictor were calculated by summing the model AIC_c weights (w_i) over all models in which each term appeared. However, the $w+_i$ values are relative, not absolute because they will be > 0 even if the predictor has no contextual explanatory importance (Burnham & Anderson, 2002). Therefore, a baseline for comparing relative $w+_i$ across predictors is required to ascertain which predictors are relevant. We randomised the data for each predictor separately within the dataset, re-calculated $w+_i$, and repeated this procedure 100 times for each predictor. The median of this new randomised $w+_i$ distribution for each predictor was taken as the baseline (null) value ($w+_0$). For each term the relative weight of evidence ($\Delta w+$) was obtained by subtracting $w+_0$ from $w+_i$. Predictors with $\Delta w+$ of zero or less have essentially no explanatory power (Burnham & Anderson, 2002).

To account for repeated captures (measurements), a series of GLMMs were constructed to examine relationships between induction and weighted recovery times and the age, total body lipid, stage of lactation and total number of captures. Examination of the residuals for the GLMMs determined that the gamma error distribution family and an identity link function were the most appropriate for each analysis. All statistical analyses were done using the R Package (Ver. 2.0.1, Ihaka & Gentleman, 1996). Values are presented as mean ± one standard error (SE) unless otherwise stated.

2.3 Results

There was a strong linear relationship between dosage (mg/kg) and recovery time (GLM: information-theoretic evidence ratio, $ER = 3.3 \times 10^7$, per cent deviance explained, %DE = 21.0 %). Examination of two outliers revealed that there was nothing unusual about these individuals. Both were captured more than once and had average recovery times for the other captures. Exclusion of these outliers improved the relationship ($ER = 8.2 \times 10^7$, %DE = 31.0 %, Fig. 2.1). To control for the size of the seal and the level of immobilisation, recovery times were weighted by the reciprocal of the dosage (Field *et al.*, 2002), referred to as 'weighted recovery time'.

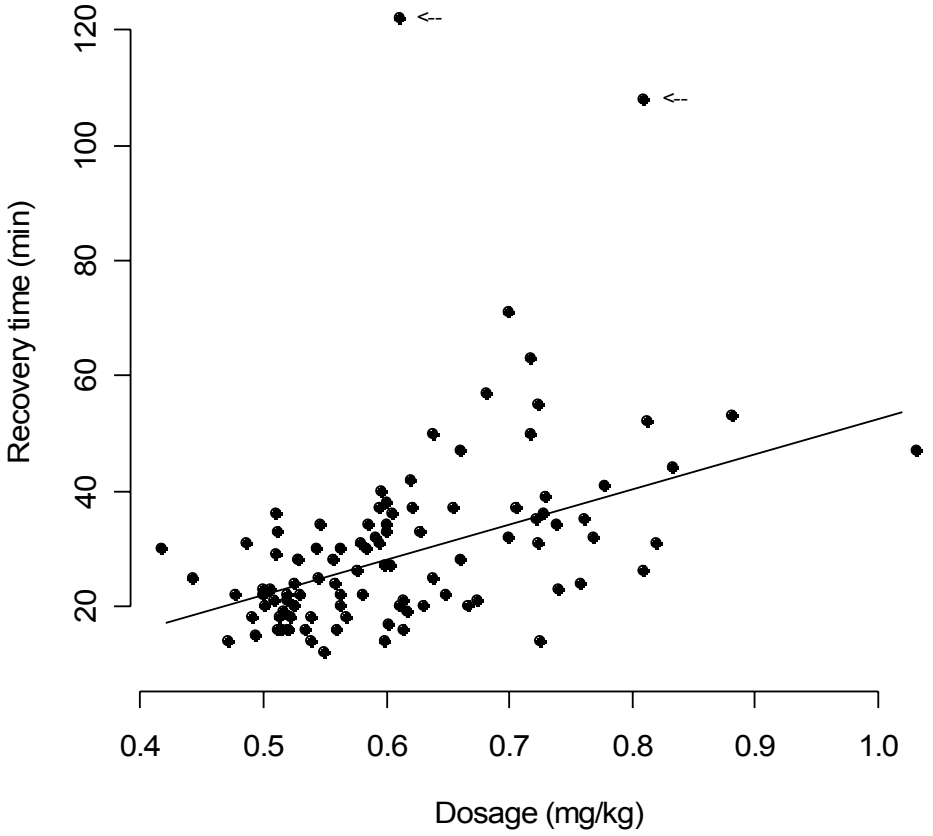

Figure 2.1 *Linear relationship between dosage (mg/kg) of tiletamine and zolazepam (1:1 mixture) and recovery time (min) in lactating Weddell seals. Arrows denote two animals (outliers) with extended recovery times.*

2.3.1 Intravenous injection

2.3.1.1 Induction

The mean dosage of tiletamine:zolazepam injected IV was 0.60 ± 0.01 mg/kg, with an average induction time of 54.8 ± 1.68 seconds (Fig. 2.2). Using information-theoretic weights of evidence (Burnham & Anderson, 2002) to examine the variation in induction time, there was no evidence that *TBL* or *age* affected induction time ($\Delta w+ \leq 0$ for both terms), but that *stage* (of lactation) had some effect ($\Delta w+ = 0.193$), with induction time being longer at the end of lactation (beginning: 48.2 ± 2.9 seconds; end: 54.9 ± 2.3 seconds).

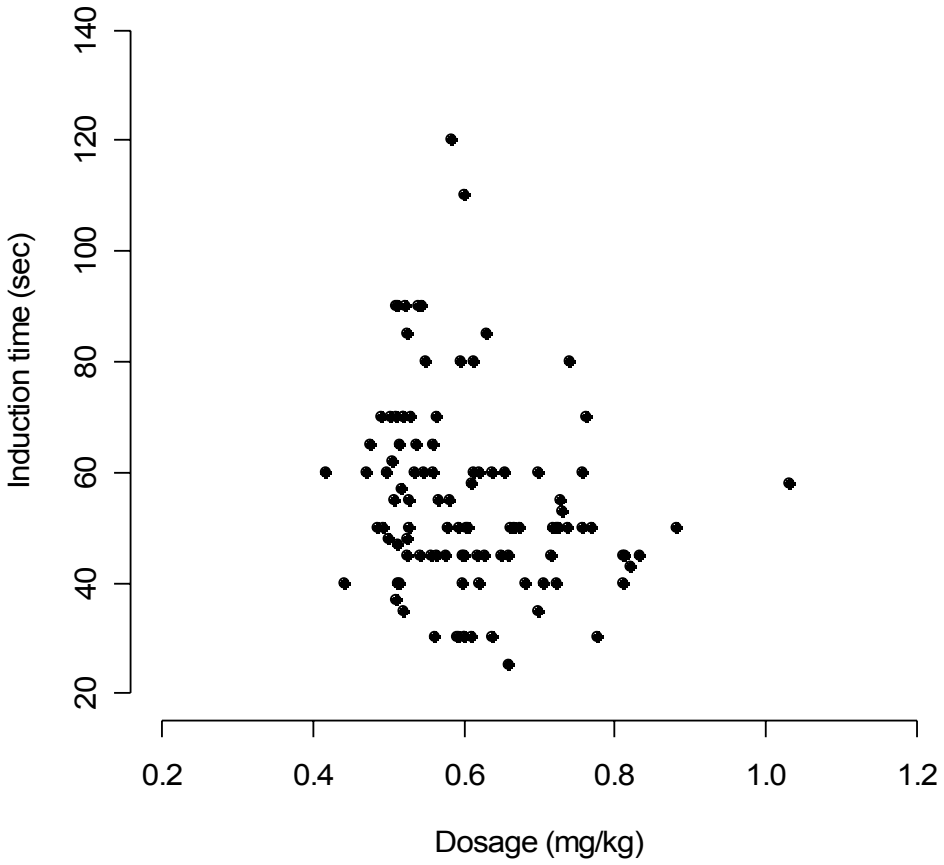

Figure 2.2 *Relationship between dosage (mg/kg) of tiletamine and zolazepam (1:1 mixture) and induction time (sec) in lactating Weddell seals.*

We examined if there was an effect of the number of previous captures on induction time using a generalised linear mixed-effects model (GLMM). Here, c*apture* was the total number of captures experienced by that female and *induction* was the induction time measured for the last capture. The term *stage* was also included as a random effect to account for variation due to stage of lactation (the terms *capture* and *stage* were uncorrelated). The results revealed that *capture* explained 64.4 % of the variation in *induction* time (Fig. 2.3), indicating (via the evidence ratio) that this model was 7.47 times more likely to explain variation in induction time than the null model (i.e., a model with no effect of capture). Table 2.1 shows average induction times for each of the model predictors at average dose rates.

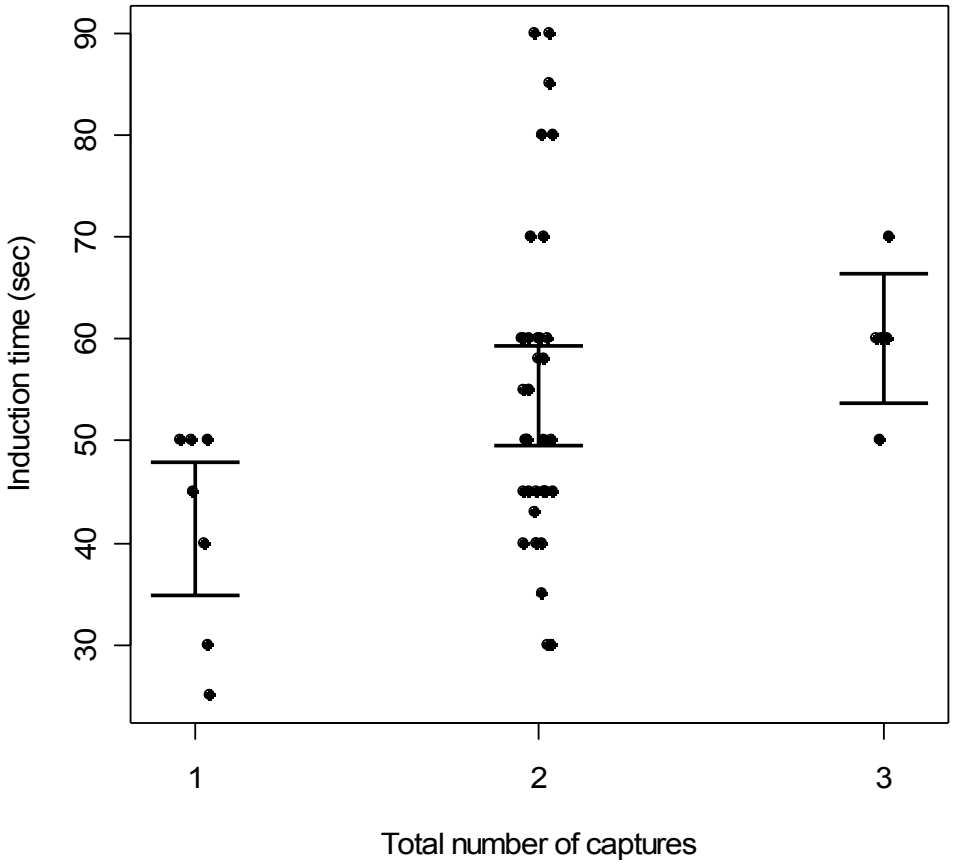

Figure 2.3 *Average induction time (± 2 SE) of adult lactating Weddell seals immobilised with tiletamine and zolazepam (1:1 mixture). Induction time increased with the number of previous captures.*

2.3.1.2 Recovery

Information-theoretic weights of evidence revealed that none of the terms considered explained the variation in weighted recovery times ($\Delta w+ \leq 0$ for all terms). The GLMM used to examine the influence of the number of previous captures on weighted recovery time revealed that *capture* only explained 21.0 % of the variance in weighted recovery time and the evidence ratio of this model to that of the null model was only 0.31, indicating no evidence of an effect of *capture* (see Table 2.1 for average recovery times for each of the model predictors).

Table 2.1 *Summary of induction and recovery times at average dose rates for different physiological conditions tested.*

		Dosage (mg/kg)		Induction (sec)		Recovery (min)	
	Range	Mean	SE	Mean	SE	Mean	SE
Capture	1	0.60	0.01	49.5	2.98	32.2	1.61
	2	0.52	0.00	64.4	2.88	20.4	0.97
	3	0.69	0.02	54.9	2.24	30.6	2.27
Age (yr)	6 - 9	0.62	0.02	48.4	1.95	29.1	1.91
	10 - 14	0.60	0.02	57.5	2.84	26.9	2.07
	15 +	0.57	0.02	61.3	6.07	29.7	3.20
% TBL	21 - 33	0.65	0.02	52.7	2.74	27.8	2.51
	34 - 40	0.62	0.02	57.9	3.20	27.6	1.78
	41 +	0.62	0.02	48.6	3.39	35.2	2.40

2.3.2 Intramuscular injection

Only 4 females were injected intramuscularly with the tiletamine:zolazepam mixture. Three of these were post-partum captures and one was an end-lactation capture. Average dosage was 0.86 ± 0.6 mg/kg, with an average induction time of 15.3 ± 1.5 minutes. One female was immobilised (IM) both post-parturition and at the end of lactation. No problems were associated with her first immobilisation procedure. During her second capture at the end of lactation, induction was fast (4 minutes), indicating possible accidental intravenous injection. Regular shallow breathing was maintained through most of the procedure. However, after approximately 60 minutes she experienced a prolonged apnoeic event, was unresponsive to resuscitation procedures and subsequently died.

2.4 Discussion

Telazol administered intravenously was an effective drug for the immobilisation of Weddell seals. However, intramuscular administration was less successful with a longer induction and recovery times and a 25 % (one animal in four)

mortality rate. The IM route of injection has been previously favoured because physical restraint is often not required so administration is easy and safe for personnel. Nonetheless, the IM route of administration has some disadvantages. Accidental injection into the blubber (which can be > 50 mm in adult Weddell seals) can lead to variable induction and recovery times. Furthermore, how quickly the drug is absorbed into the bloodstream depends, in part, on the blood supply to the muscle. Blood supply increases during physical activity, which could account for the deaths reported by Phelan & Green (1992) because they physically handled and restrained females before injection. In this study, females were only immobilised IM when IV injection was impossible (due to this species' tendency to 'roll' when restrained), so individuals were physically handled and potentially agitated similar to those in the Phelan & Green study. The single death in this study may have resulted from the accidental injection of a larger amount of drug into a vein. Other studies administering the tiletamine:zolazepam mixture IM have also shown variable results, especially with higher doses (Table 2.2), although these may have also resulted from accidental injection IV. Taken together, these observations suggest that the tiletamine:zolazepam combination may have a narrow margin of safety in some seal species when administered IM, and that IM administration increases the risks associated with immobilisation. By contrast, IV administration may be an acceptable alternative.

Weddell seals appear to be more sensitive, to some extent, than other species to drug type (Cline *et al.*, 1969; Flyger *et al.*, 1965; Gales & Burton, 1988; Hammond & Elsner, 1977) and method of administration. Some drugs (e.g., phencyclidine HCl and succinylcholine chloride) that have lead to fatalities in Weddell seals have also had variable and lethal results with other similar-sized phocids (Gales, 1989). Ketamine HCl, a rapid acting dissociative with a similar molecular structure to phencyclidine HCl, has also been lethal to Weddell seals, but not to other species at similar dosages (Gales, 1989). In general, Weddell seals appear to respond to tiletamine and zolazepam in a similar way to southern elephant seals, but we can only speculate as to why differences might exist for other drug types. Weddell seals live in an extreme environment year round and their energetic adaptations might influence their sensitivity and response.

Table 2.2 *Summary of dosages of tiletamine:zolazepam (1:1) used for chemically immobilising phocid seals.*

Species	*n*	Dosage	Route	Mortality	Reference
Halichoerus grypus gray seal	44	1.0 mg/kg	IM dart	0 %	Baker *et al.*, 1990
Phoca vitulina harbour seal	7	0.75 – 2.0 mg/kg	IM	0 %	Hammond & Elsner, 1977
Hydrurga leptonyx leopard seal	1	2.0 mg/kg	IM	100 %	Mitchell & Burton, 1991
Leptonychotes weddellii weddell seal	30	0.3 – 1.1 mg/kg	IM	10 %	Phelan & Green, 1992
	106	0.42 – 1.03 mg/kg	IV	0 %	This study
	4	0.76 – 1.03 mg/kg	IM	25 %	This study
Mirounga leonina southern elephant seal	90	1.0 mg/kg	IM dart	0 %	Baker *et al.*, 1990
	5	1.6 – 2.4 mg/kg	IM	40 %	Mitchell & Burton, 1991
	4	0.6 – 1.7 mg/kg	IM	0 %	Karesh *et al.*, 1997
	597	0.46 mg/kg	IV	0 %	McMahon *et al.*, 2000a
	1033	0.3 – 0.7 mg/kg	IV	0 %	Field *et al.*, 2002

In this study, chemical immobilisation with tiletamine and zolazepam using the IV method was successful in all cases. There was a small effect of stage of lactation on induction time, with an increase of about six seconds (12.2 %) at the end of lactation. The number of previous captures appeared to increase induction time (although sample size was admittedly low for animals immobilised more than twice), suggesting a decrease in sensitivity to the chemical when first introduced into the bloodstream, even up to 2-3 weeks later. On the other hand, there were no detectable effects of age, condition, stage of lactation or number of previous captures on weighted recovery time. The animals immobilised were under varying degrees of physiological pressures associated with the negative energy balance of lactation (Woods *et al.*, 1989). It seems reasonable that if physiological state was to affect weighted recovery time it would be most evident in these individuals.

Previous studies on southern elephant seals have shown an effect of age and condition on recovery time (Field *et al.*, 2002; Woods *et al.*, 1989). However, Woods *et al.* (1989) found no significant difference in recovery time between post-parturition and end-of-lactation females, although they did find that pre-moult seals (i.e., in better condition) had shorter recovery times. The differences between individuals found in the Field *et al.* (2002) study were based on measurements of condition and recovery times at three different haul-out periods separated by months as opposed to weeks in our study. Therefore, our results in combination with the findings of Woods *et al.* (1989) suggest that recovery time does not differ between physiologically stressed animals within the same state (e.g., lactation), although differences resulting from changes in physiological status at other times (e.g., non-moult to moult) may affect recovery times enough to be measurable in field studies.

Although we did not find a difference in weighted recovery time and number of captures, our sample size was low. With a larger sample size and more repeated captures (up to 5), Field *et al.* (2002) found a positive relationship between weighted recovery time for southern elephant seals and the number of times an individual had been immobilised previously. However, this relationship was not as apparent up to 3 captures (as in this study), so it is possible that we did not have the statistical power to detect a relationship. Consequently, we suggest careful monitoring of immobilisation recovery times during longitudinal studies ($\geq$ 3 captures) on Weddell seals to examine this potential relationship further.

The 1:1 mixture of tiletamine and zolazepam appears to be a suitable and safe drug for intravenous immobilisation of Weddell seals. It appears that variability in recovery rates generally increases with higher doses (Fig. 2.1) likely due to individual differences in the rates of metabolism and elimination of tiletamine and zolazepam (Tracy *et al.*, 1988). Taking these differences into consideration, we recommend a mass-specific dose of 0.50 – 0.65 mg/kg which gives an average recovery time of 26 minutes that should be suitable for most procedures requiring immobilisation (e.g., deployment of dataloggers, tissue sampling, injection of isotopic compounds to examine body composition, etc.). This corresponds to the dosage recommended for southern elephant seals by McMahon *et al.* (2000a). Although immobilisation techniques will vary for species and situations, this suggests that a mass-specific dose of 0.50 mg/kg may be a good starting point for other age classes and other phocid seals.

Chapter 3

Influence of maternal mass and condition on energy transfer in Weddell seals

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Abstract

Environmental variation influences food abundance and availability, which is reflected in the reproductive success of top predators. We examined maternal expenditure, offspring mass and condition for Weddell seals in two years when individuals exhibited marked differences in these traits. For females weighing > 355 kg there was a positive relationship between maternal post-partum mass (MPPM) and lactation length, but below this there was no relationship, suggesting that heavier females were able to increase lactation length but lighter females were restricted to a minimum lactation period of 33 days.

Overall, females were heavier in 2002, but in 2003 shorter females were lighter than similar-sized females in 2002 suggesting that the effects of environmental variability on foraging success and condition are more pronounced in smaller individuals. There was no relationship between MPPM and pup birth mass, indicating pre-partum investment did not differ between years. However, there was a positive relationship between MPPM and pup mass gain. Mass and energy transfer efficiency were 10.2 and 5.4 % higher in 2002 than 2003, which suggests costs associated with a putatively poor-resource year were delayed until lactation. Heavier females lost a higher proportion of mass during lactation in both years, so smaller females may not have been able to provide more to their offspring to wean a pup of similar size to larger females. Maternal post-partum mass had only a small influence on total body lipid; therefore, regardless of mass, females had the same relative body composition. Females with male pups lost a higher percentage of lipid than those with female pups, but by the end of lactation female pups had 4.5 % higher lipid content than males.
It appears that for Weddell seals the consequences of environmentally induced variation in food availability are manifested in differences in maternal mass and expenditure during lactation. These differences translate to changes in pup mass and condition at weaning with potential consequences for future survival and recruitment.

3.1 Introduction

In many mammals lactation represents a period of extreme demand on energy reserves (Gittleman & Thompson, 1988; Rogowitz, 1996). Young mammals are born nutritionally dependent and therefore, exert high energetic demands on mothers during lactation. Mammals have developed two main strategies to support the energetic costs of lactation: 'capital breeding' relies largely on stored body reserves, while 'income breeding' relies on more-recently acquired energy. The costs and benefits of these alternative resource-use strategies differ depending on the timing and variability of resource availability (Boyd, 2000). Long-lived mammals must therefore have the ability to locate food in successive years and in environments where the temporal predictability of food patches can be low (Constable, 2000; McCafferty *et al.*, 1998; Planque & Taylor, 1998).

Annual variation in foraging success influences body reserves, which in turn influences current and future reproduction (Beauplet *et al.*, 2004; Croxall *et al.*, 1999; Lynnes *et al.*, 2004). The evolution of reproductive strategies is driven by a balance between the costs incurred by the parents through investment in offspring, and the value they obtain from this investment in terms of their offspring's subsequent reproductive output (Shuster & Wade, 2003). To understand fully how mammals translate variability in resources to reproductive output, data on key demographic and physiological parameters such as the quantification of energy transfer, assimilation efficiency, resource allocation and survival are needed.

In marine ecosystems, high inter-annual variability in resource abundance is the norm resulting from cyclic environmental patterns (e.g. El-Niño-Southern Oscillation) and unpredictable fluctuations in oceanographic conditions (Pinaud & Weimerskirch, 2002). This variability can affect all trophic levels, but upper-trophic-level species are sometimes regarded as 'ecosystem indicators' on the notion that such proximate (environmental) factors will affect their diet, reproductive performance and population size (Hindell *et al.*, 2003; Le Boeuf & Crocker, 2005; Reid *et al.*, 2005). Examining the biological consequences of environmental change (Barbraud & Weimerskirch, 2001; Beauplet *et al.*, 2004; Bradshaw *et al.*, 2000; Thompson & Ollason, 2001) may improve our understanding of how predators respond to changes in the distribution of marine resources as the result of global climate change or commercial exploitation.

Most marine ecosystem studies have focussed on environmental change and population dynamics of seabirds (e.g. Croxall *et al.*, 2002; Pinaud *et al.*, 2005). However, demographic variation between species suggests that we should not expect all marine predators to respond to environmental change in the same way (Croxall, 1992). Phocid seals are one of the few groups of mammals within which some species exhibit extreme capital breeding where females meet the energy requirements of lactation solely through stored body reserves. Although a temporal separation exists between energy acquisition and maternal expenditure, there is often a spatial separation as well, with females remaining on land or ice. The physiology of lactation is influenced strongly by the constraints resulting from this division between foraging and reproduction (Crocker & Costa, 2001). Fluctuations in energy acquisition prior to the breeding season are expressed as variation in maternal mass and condition. These may impose energetic constraints that influence both the duration and magnitude of maternal expenditure measured in terms of pup birth mass and growth (Crocker *et al.*, 2001; McMahon & Burton, 2005; Pomeroy *et al.*, 1999). In turn, pup growth will have consequences for survival (Hall *et al.*, 2001; McMahon *et al.*, 2000b) and ultimately, the lifetime reproductive success of mothers (Trillmich, 1996). Therefore, the amount of resources available and possibly some expectation of future resource availability may influence maternal expenditure. As a result, quantifying the ecological and physiological aspects of lactation offers an opportunity to measure immediate (short time lag) responses to environmental change.

Weddell seals (*Leptonychotes weddellii* Lesson) are long-lived, upper-trophic-level predators in the Southern Ocean. They are the only phocid to inhabit these high latitudes year-round and are easily accessible during the breeding and moulting periods when they haul out at predictable cracks in the fast-ice (Kooyman, 1981). Their ability to cope with polar conditions and their accessibility makes them excellent candidates to study constraints on lactation. Like all large phocids, Weddell seals deposit subcutaneous fat during the non-breeding season and then use this stored energy for milk production and maintenance during the lactation period. However, the 6- to 7-week lactation period of Weddell seals (Tedman & Bryden, 1979) is different from other phocids (see Trillmich, 1996 for a review) given that it is one of longest recorded, especially compared to that of similar-sized species (elephant seals, *Mirounga* spp., 22 to 28 days).

For most phocid species, pups are weaned abruptly and must learn to swim

and dive on their own. However, Weddell seal pups are introduced to the water during lactation (starting at 10 to 12 days) when they accompany mothers during short bouts of diving Stirling, 1969. Some females also forage during lactation (Eisert *et al.*, 2005; Hindell *et al.*, 2002; Sato *et al.*, 2002), but the reasons for this are still unclear. This behaviour is unique in that most other phocids fast for the entire duration of lactation, and those that do forage are considerably smaller (harbour seal, *Phoca vitulina* - Bowen *et al.*, 2001) with relatively limited body stores and higher relative energy expenditure (Bowen *et al.*, 2001). So the question remains as to why a larger phocid should need to forage during lactation.

In this study, we measured maternal post-partum mass (MPPM) and body composition of Weddell seals at the beginning of the breeding season as an indicator of over-winter foraging success for two consecutive years. We examined how MPPM and body condition related to a mother's energy allocation and the growth of her pup, and whether this relationship differed between years. We hypothesised that when maternal mass is relatively low these individuals should transfer less mass and energy to the pup during lactation. In turn, we expected that these differences in provisioning would have detectable consequences on pup growth rate, condition and survival.

3.2 Methods

This study was done at Hutton Cliffs, Antarctica (77° 51' S, 166° 45' E) during the 2002 and 2003 breeding seasons (October to December). Thirty mother-pup pairs in 2002 and twenty-five pairs in 2003 were captured 1 to 6 (mean 3.8 ± 0.22) days post-partum (dpp). Females were chosen randomly from the 60 to 100 females usually present at this site. Individual females were identified by flipper tags attached in previous years and pups born to study females were marked with hind flipper tags soon after birth as part of a long-term tagging study (Cameron & Siniff, 2004). Age was known for 19 females in 2002 and 21 females in 2003.

Weaning was defined as when the pup was seen alone for ≥ 4 days, with multiple surveys throughout the day. Due to restrictions of working on fast-ice, not all weaning dates could be determined accurately, and mother-pup pairs were captured 34 to 43 (37.4 ± 0.40) dpp. However, some females remained up to six days after capture. For statistical comparisons requiring absolute measurements (as opposed to rates),

parameters of both females and pups were extrapolated to weaning based on a linear relationship between MPPM and lactation length for the females with known weaning dates (n = 32; $y = 0.07x + 6.98$; see Results).

3.2.1 Maternal body mass and composition

We used isotopically labelled water to measure body composition of females and their pups at the post-parturition (PP) and end-lactation (EL) periods. Once captured, each female was immobilised by an intravenous injection of Telazol® (combination of 1:1 tiletamine:zolazepam, Fort Dodge, Castle Hill, NSW, Australia), at an average dose of 0.6 mg·kg^{-1} into the extradural vein, or intramuscularly at an average dose of 1.0 mg·kg^{-1} in the rear flank. Females were weighed to the nearest 1 kg. Standard body length and six girth measurements (Field *et al.*, 2002) were recorded for all females.

After immobilisation, a 10 ml blood sample was collected in heparinized vacutainers to measure background isotope levels. Immediately after, a pre-weighed dose (to the nearest 0.1 mg) of approximately 222 MBq of tritiated water (HTO) was administered to each female. Due to a shortage of HTO at the beginning of the 2002 field season, ten females were administered a dose of deuterium oxide (HDO, specific concentration: 99.8 %) at the PP capture while HTO was used for all other females and the EL captures. The syringe was flushed with blood twice to ensure complete isotope delivery. During equilibration, mothers were prevented from entering the water by blocking access to the nearest water holes. A second blood sample (10 ml) was taken 150 min on average after initial injection to determine enrichment level. Equilibration occurs in southern elephant seal (*M. leonina* Linnaeus) females within 90 min of administration (IV; M.A. Hindell, unpublished data), so we were confident equilibration was achieved in all cases. All procedures were repeated near the end of lactation.

Body mass and composition measured after the day of birth were interpolated to estimate MPPM and composition using calculated rates of daily loss for each individual. For females that were captured only at PP (n = 14), body composition was adjusted to birth values by taking the average change in total body water (TBW·g^{-1}·day^{-1}) of females with multiple captures.

3.2.2 Pup body mass and composition

Each pup was weighed to the nearest 0.5 kg and body length and axial girth were measured. Body composition was measured for all pups (n = 25) at PP in 2003 and for 25 and 22 pups at EL in 2002 and 2003, respectively. A 10 ml blood sample was collected to measure background isotope enrichment levels. A pre-weighed dose of approximately 74 MBq of HTO in 2002, and a 10 ml dose of HDO (specific concentration: 99.8 %) in 2003 was administered subsequently to each pup. Pups were prevented from suckling during the equilibration period (average = 150 mins), after which a second blood sample was taken.

Plasma was separated and stored at -20 ºC. Due to high TBW content (low total body lipid; TBL) and analytical errors, some measurements of body composition at PP were implausibly low (i.e., TBL < 3 %, n = 6). Therefore, all values greater than 3 % were averaged to give a mean body composition at birth, which was applied to all pups in 2002 and those pups in 2003 with values below 3 %. With such low TBL values at birth we believe this averaging would not introduce a large amount of error in further calculations. The TBL values greater than 3 % corresponded to a TBW of < 70 % and Tedman & Green (1987) found similar values at birth (mean = 72.5 %).

3.2.3 Sample Analysis

Plasma samples were analysed for HTO activity using liquid scintillation spectrometry. Plasma samples (100 µl) were distilled in triplicate using the method of Ortiz, Costa & Le Boeuf (1978). For each vial of water recovered, 4 ml of Eco-lite scintillate was added and HTO activity was counted for 15 min using a Beckman LS6500 scintillation counter. Mass spectrometric analysis of deuterium enrichment was done in triplicate using H_2 gas and a platinum-on-alumina catalyst according to the methodology described in Scrimgeour *et al.* (1993).

3.2.4 Data analysis

Measuring TBW by dilution space consistently overestimates water volume (Arnould *et al.*, 1996; Lydersen *et al.*, 1992; Reilly & Fedak, 1990); therefore, we

calculated total body water by multiplying the isotope dilution space by a correction factor (HTO:4.0 % and HDO: 2.8 %) derived for grey seals (*Halichoerus grypus* - Reilly & Fedak, 1990).

Per cent total body lipid (*%TBL*), per cent total body protein (*%TBP*) and total body gross energy (*TBGE*) were then estimated from body mass (M_b) and TBW according to equations of Reilly *et al.*(1990):

$$\%TBL = 105.1-(1.47 \times \%TBW) \quad (3.1)$$

$$\%TBP = (0.42 \times \%TBW) - 4.75 \quad (3.2)$$

$$TBGE \text{ (MJ)} = (40.8 \times M_b) - (48.5 \times TBW) - 0.4 \quad (3.3)$$

Four females in 2002 and 5 pups in 2003 were injected with HTO and HDO simultaneously to compare both techniques for measuring TBW. In each case the estimated value of TBW was higher from HDO than from HTO dilution (differences for females: 6.05 ± 0.02 %; pups: 5.69 ± 0.01 %). For between-year comparisons, all values derived from HDO analyses were corrected for direct comparison to HTO values.

A series of generalised linear models (GLM) were constructed to examine intraspecific and interannual differences of females and pups. Examination of the residuals for all models determined the statistical error distribution and link function most appropriate for each analysis. Model selection was based on Akaike's Information Criteria corrected for small samples (AIC_c, Burnham & Anderson, 2002). AIC_c values were ranked, with the most parsimonious model(s) having the lowest AIC_c values and highest model weights. From a set of *a priori* models we used predictive model-averaging to determine the magnitude of the effect of some terms, keeping all other dependent variables constant (Burnham & Anderson, 2002). Percentages were not arcsine transformed prior to analyses because most values fell between 30 – 70 %. The information-theoretic weight of evidence ($w+_i$) for each predictor was calculated by summing the model AIC_c weights (w_i) over all models in which each term appeared. However, the $w+_i$ values are relative, not absolute because they will be > 0 even if the predictor has no contextual explanatory importance (Burnham & Anderson, 2002). To judge which predictors were relevant to the data at hand, a baseline for comparing relative $w+_i$ across predictors was required, so we randomised the data for each predictor separately, re-calculated $w+_i$, and repeated this

procedure 100 times for each predictor. The median of this new randomised $w+_i$ distribution for each predictor was taken as the baseline (null) value ($w+_0$). For each term the absolute weight of evidence ($\Delta w+$) was obtained by subtracting $w+_0$ from $w+_i$, and predictors with $\Delta w+$ of zero or less have essentially no explanatory power (Burnham & Anderson, 2002). All statistical analyses were done using the R Package (Ver. 2.0.1, Ihaka & Gentleman, 1996). Values are presented as mean ± one standard error (SEM) unless otherwise stated.

3.3 Results

We obtained mass and body composition measurements at PP for 26 females and 29 pups in 2002, and for 25 females and 25 pups in 2003 (Table 3.1). End-lactation measurements were calculated for 24 females and 25 pups in 2002, and 11 females and 22 pups in 2003. In 2003, 14 females were not captured at EL because they ended lactation earlier than expected. However, the average MPPM of these females was not significantly different to that of the females that were re-captured (384.8 ± 12.98 kg and 403.6 ± 18.82 kg, respectively; $t_{18.6} = -0.82$, $P = 0.422$), so we considered that any potential bias that this may have posed for between-year comparisons was negligible.

Table 3.1 *Average mass and body composition of Weddell seal females and pups during lactation in 2002 and 2003.*

	2002						2003					
	Birth	*n* = 26	Wean	*n* = 24	Change	*n* = 24	Birth	*n* = 25	Wean	*n* = 11	Change	*n* = 11
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Females												
Body mass (kg)	450.9	6.13	294.0	6.32	158.9	4.07	393.1	10.94	267.6	10.92	135.9	9.29
Total body lipid (kg)	181.5	3.78	82.2	3.16	100.2	4.17	153.4	6.18	70.1	4.27	93.8	9.87
Total body protein (kg)	62.4	1.31	43.2	1.23	19	1.69	55.5	1.74	43.2	2.28	14.9	2.48
Body lipid %	40.2	0.67	31.2	1.09	9.3	1.32	38.9	0.93	28.2	1.55	11.4	2.30
Body protein %	13.8	0.19	16.4	0.31	2.6	0.38	14.2	0.27	17.2	0.44	3.2	0.66
Total body gross energy (MJ)	8757	150	4326	119	4466	145	7467	260	3842	179	4106	361

	2002						2003					
	Birth	*n* = 29	Wean	*n* = 25	Change	*n* = 25	Birth	*n* = 25	Wean	*n* = 22	Change	*n* = 22
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Pups												
Body mass (kg)	27.6	0.73	96.8	2.23	69.2	1.97	26.7	0.95	76.5	3.19	49.6	3.27
Total body lipid (kg)	2.4*	0.06	40.5	2.01	48.1	1.99	2.4	0.15	34.7	2.09	32.4	2.12
Total body protein (kg)	6.3*	0.16	12.1	0.32	5.8	0.35	6.1	0.22	11.6	0.44	5.8	0.49
Body lipid %	8.6*	-	47.8	1.07	39.2	1.07	8.6	0.49	40.1	1.00	31.3	1.20
Body protein %	22.8*	-	11.6	0.30	11.2	0.31	22.8	0.14	13.8	0.29	8.9	0.34
Total body gross energy (MJ)	246*	6	2307	80	2061	78	244	10	1666	91	1432	94

* Values averaged from pup TBW measurements in 2003.

3.3.1 Body Mass Changes

3.3.1.1 Females

The most-parsimonious GLM testing for the effect of *age*, *year* and *length* on MPPM included all terms and a *length*year* interaction. Using relative weights of evidence ($\Delta w+$), *year* and *length* were the only factors driving the relationship ($\Delta w+_{year} = 0.741$, $\Delta w+_{length} = 0.743$, $\Delta w+_{age} = 0.000$), demonstrating that females were heavier in 2002. In 2003, shorter females were considerably lighter compared to females of similar length in 2002 (Fig. 3.1). A plot of the body length to the mass/body length residuals further highlights the observation that shorter females were particularly lighter in 2003, with smaller differences found in larger females (Fig. 3.2). From predictive model averaging, using all *a priori* models and keeping *age* and *length* constant, overall MPPM was 12.7 % higher in 2002 than in 2003.

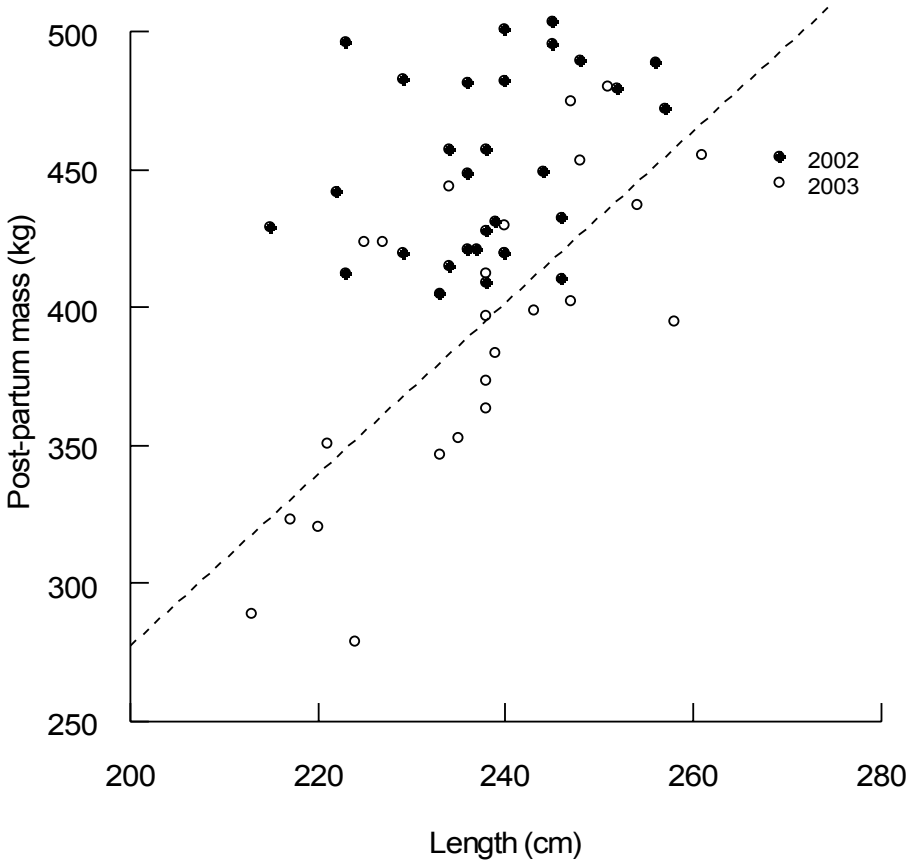

Figure 3.1 *Relationship between body length and post-partum mass. The dashed line represents the positive relationship for 2003 (y = 3.12x – 346.4).*

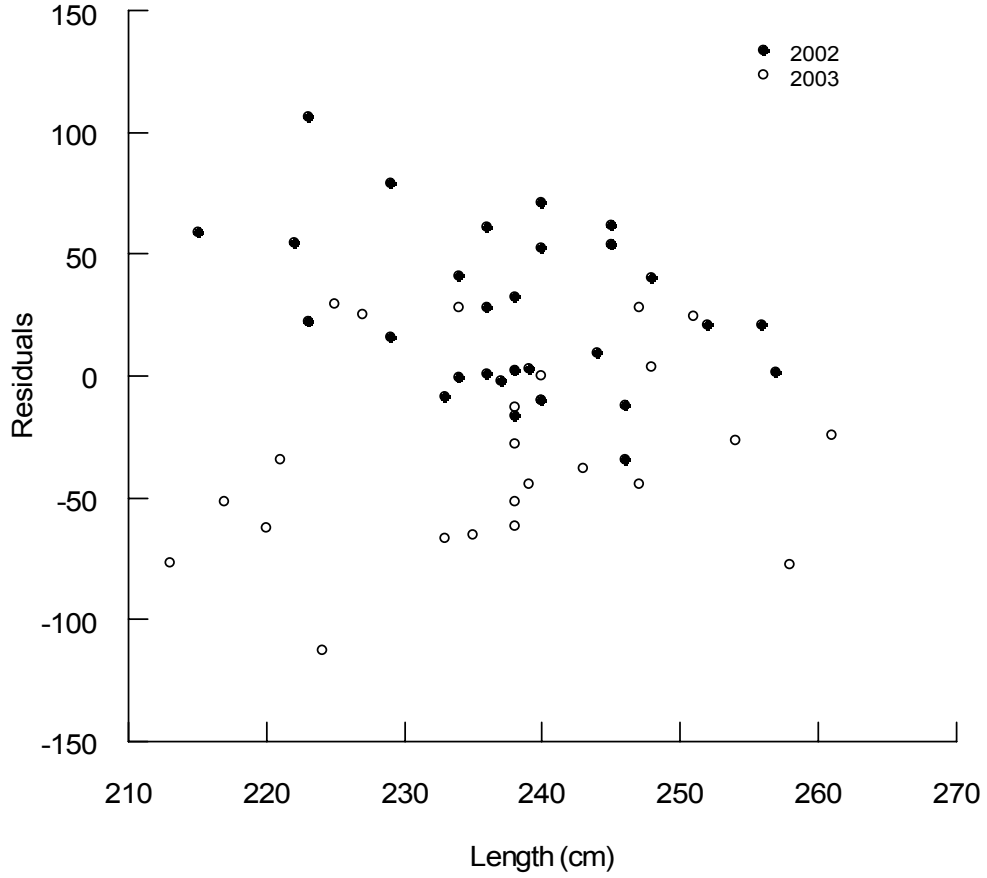

Figure 3.2 *Relationship between body length and residuals from the mass/body length relationship.*

Mass loss rates ranged from 3.9 – 5.9 kg·day^{-1} (4.7 ± 0.12) in 2002, and 2.1 – 5.1 kg·day^{-1} (4.0 ± 0.27) in 2003. MPPM was largely responsible for differences in mass loss rates, although *TBL* was also an important factor ($\Delta w+_{MPPM} = 0.769$, $\Delta w+_{TBL} = 0.320$, $\Delta w+_{year} < 0.0$, $\Delta w+_{age} = 0.003$). Therefore, heavier females had higher mass loss rates (Fig. 3.3). Mass-specific mass loss rates averaged 10.67 ± 0.24 g·kg^{-1}·day^{-1} and 10.38 ± 0.42 g·kg^{-1}·day^{-1} in 2002 and 2003, respectively. Over the entire lactation period, total mass loss ranged from 161 – 245 kg in 2002 and 78 – 206 kg in 2003. This represented 41.5 ± 0.01 % of MPPM in 2002 and 38.5 ± 0.02 % in 2003. *MPPM* had the most influence on per cent mass loss ($\Delta w+_{MPPM} = 0.639$) with no *year* or *TBL* effect ($\Delta w+ \leq 0.0$). Therefore, heavier females lost a higher proportion of body mass over lactation, with no differences between years.

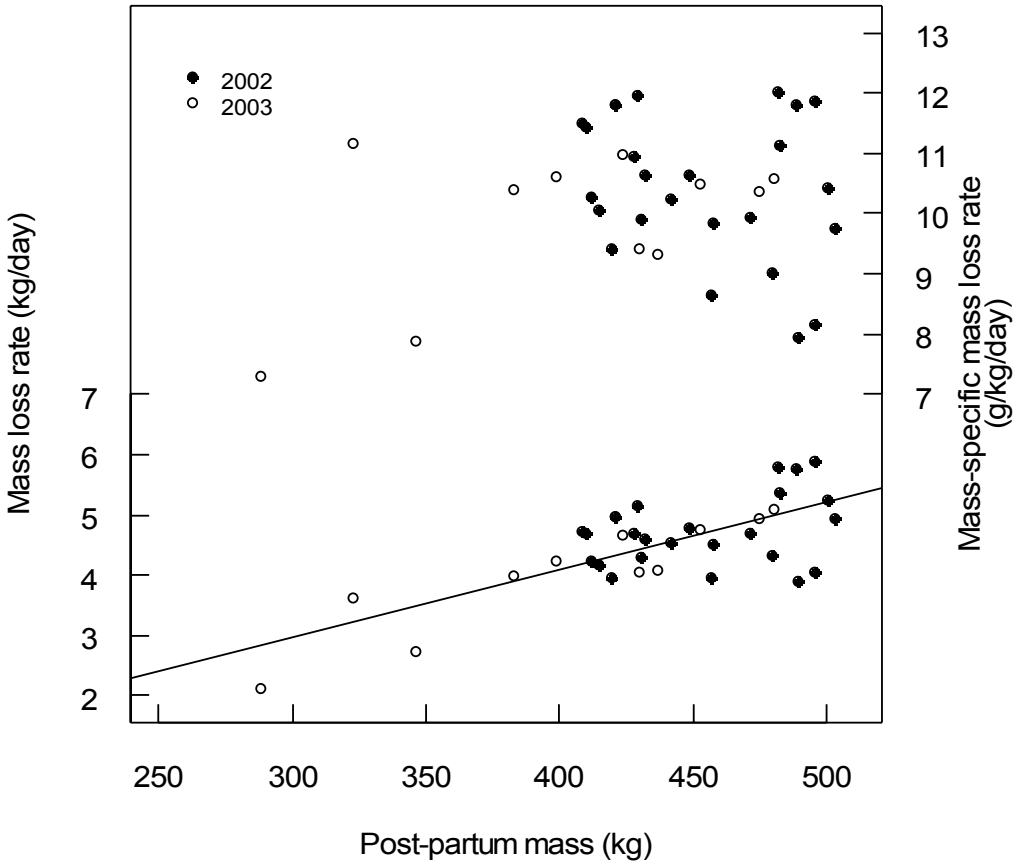

Figure 3.3 *Daily rate of mass loss and the mass-specific mass loss rate in relation to post-partum mass.*

3.3.1.2 Assimilation

In 2002, pups assimilated 41.9 ± 0.01 % (34.1 – 59.6 %) of the mass lost by their mother, and in 2003 pups assimilated 36.9 ± 0.01 % (25.8 – 46.1 %). The most-parsimonious model testing for the effect of *age*, *MPPM*, *birth mass* (*BM*) and *year* on mass transfer efficiency (pup mass gain/female mass loss) only included a *year* effect ($\Delta w+_{year} = 0.394$; $\Delta w+ < 0.0$ for all other terms). Model averaging revealed that mass transfer efficiency was 10.2 % higher in 2002 than in 2003.

Pups assimilated 45.7 ± 0.02 % (22.4 – 57.7 %) of the energy (MJ) lost by the mothers in 2002, and 40.4 ± 0.03 % (28.7 – 59.2 %) in 2003. The *year* term had a strong influence on this proportion ($\Delta w+_{year} = 0.396$), but *MPPM*, *age* and *TBL* had no effect ($\Delta w+ \leq 0.0$). Model averaging estimated that energy transfer efficiency was 5.4 % higher in 2002 than in 2003.

3.3.1.3 Pups

Average birth mass of pups was 27.6 ± 0.7 kg in 2002, and 26.7 ± 1.0 kg in 2003. The most-parsimonious model testing for the effect of *MPPM*, TBL_{MPPM}, *year* and *sex* on average birth mass revealed that *MPPM* was the most important term in the model ($\Delta w+_{MPPM} = 0.386$, $\Delta w+ \leq 0.0$ for all other terms). However, only 7.6 % of the deviance was explained by this model.

Pup mass gain ranged from 1.5 – 2.5 kg·day^{-1} (1.9 ± 0.04) in 2002, and 0.5 – 2.4 kg·day^{-1} (1.6 ± 0.10) in 2003. The most-parsimonious model testing for the effect of *MPPM*, TBL_{MPPM}, *sex* and *year* on total mass gain (to EL) included the *MPPM*, *year* and TBL_{MPPM} terms, with *MPPM* driving the relationship ($\Delta w+_{MPPM} = 0.720$, $\Delta w+_{year} = 0.002$, $\Delta w+_{TBL} = 0.000$). Thus, larger females produced larger pups (Fig. 3.4).

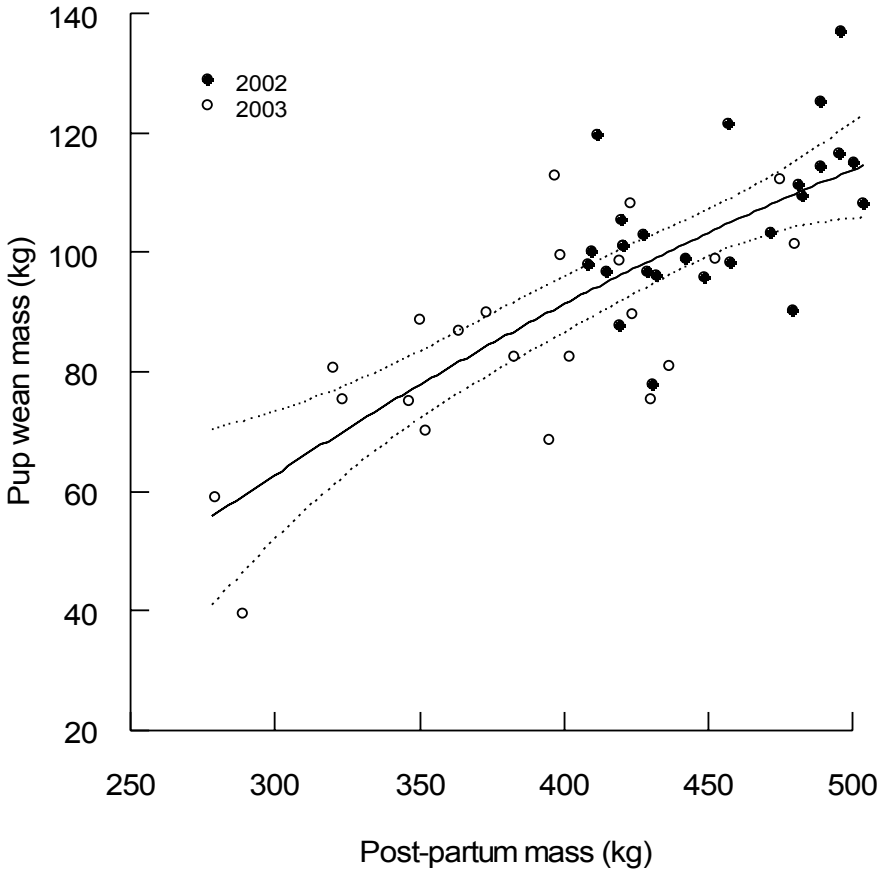

Figure 3.4 *Relationship between post-partum mass and pup mass at weaning. Broken lines represent the 95 % confidence interval of the fitted curve.*

3.3.2 Body composition and energy expenditure

3.3.2.1 Females

Post-partum lipid content ranged from 150.5 – 223.6 kg in 2002, representing 32.5 – 45.7 % of female body mass. In 2003, lipid content ranged from 105.3 – 199.3 kg, or 25.7 – 45.5 % of body mass. *MPPM* had only a moderate influence on this proportion ($\Delta w+_{MPPM} = 0.287$), but *year* and *age* had no effect ($\Delta w+ \leq 0.0$); therefore, proportionately all females had similar compositions. Protein content ranged from 54.0 – 77.0 kg, representing 12.2 – 16.0 % of MPPM in 2002. In 2003, TBP ranged from 40.4 – 77.2 kg or 12.2 – 17.9 % of MPPM. The total body gross energy (TBGE) varied from 7539 – 10384 MJ in 2002 and 5180 – 9292 MJ in 2003, representing an energy density of 16.9 – 21.2 $MJ \cdot kg^{-1}$ (19.4 ± 0.22) and 14.6 – 21.0 $MJ \cdot kg^{-1}$ (19.2 ± 0.60) in 2002 and 2003, respectively.

At EL, TBL ranged from 50.5 – 115.6 kg in 2002 and 49.5 – 91.7 kg in 2003, representing 22.9 – 42.0 % and 19.0 – 33.6 % of body mass, respectively. The most-parsimonious GLM testing for the effect of *MPPM*, TBL_{MPPM} , *year* and *pup sex* on total lipid loss indicated that *pup sex* was largely responsible for driving the relationship ($\Delta w+_{sex} = 0.385$, $\Delta w+ \leq 0.0$ for all other terms). Mothers with male pups lost 19.6 % more lipid on average than those with female pups (male lipid loss = 11.1 %; female lipid loss = 9.3 %).

Lipid constituted 36.1 – 92.4 % (54.5 ± 0.03 %), and protein 1.1 – 15.0 % (9.7 ± 0.01 %) of the mass lost over lactation in 2002, and mass lost comprised 23.1 – 70.1 % (57.0 ± 0.03 %) lipid and 5.1 – 18.7 % (9.3 ± 0.01 %) protein in 2003. TBL_{MPPM} was the only variable that influenced per cent lipid loss over lactation ($\Delta w+_{TBL} = 0.891$, $\Delta w+_{year} = 0.125$, $\Delta w+_{MPPM} < 0.0$).

3.3.2.2 Pups

Body composition at birth was measured for all pups ($n = 25$) in 2003 and average values applied to pups in 2002 (see Methods; Table 3.1). For pups in 2002, lipid and protein represented 61.9 ± 0.01 % (48.8 – 84.0 %) and 7.6 ± 0.005 % (1.3 –

12.1 %) of the mass gained, respectively, and lipid constituted 55.3 ± 0.01 % (46.5 – 69.9 %) and protein 9.8 ± 0.004 % (7.2 – 12.0 %) of the mass gained in 2003. The most-parsimonious model testing for the effect of *MPPM*, TBL_{MPPM}, *sex* and *year* on TBL_{pup} at EL included all variables; however, *MPPM* and *sex* were largely responsible for driving this relationship ($\Delta w+_{MPPM} = 0.735$; $\Delta w+_{sex} = 0.560$, $\Delta w+_{year} = 0.159$, $\Delta w+_{TBL} < 0.0$). Model averaging estimated that females had 4.5 % higher lipid content than males at the end of lactation.

3.3.3 Lactation length

Despite extrapolating lactation duration for 23 individuals, those females for which the entire duration of lactation was known demonstrated that duration was longer in 2002 (39.8 ± 1.40 days; range = 31 – 49 days; $n = 13$) than in 2003 (35.4 ± 0.93 days; range = 28 to 42 days; $n = 19$). However, *year* did not have a large effect ($\Delta w+_{year} = 0.086$), although *MPPM* did ($\Delta w+_{MPPM} = 0.667$). We fitted a smoothed spline function to the relationship between MPPM and lactation length (Fig. 3.5) to illustrate the non-linear trend in the data. There appeared to be a mass (355 kg) where the relationship changed, so we tested the importance of MPPM on lactation length for females above and below this threshold. MPPM was an important term for females ≥ 355 kg ($\Delta w+_{MPPM} = 0.757$) but not for females < 355 kg ($\Delta w+_{MPPM} < 0.0$) where lactation length averaged 33 ± 1.4 days. There were two noticeable outliers: “P130” had a longer lactation (41 days) than expected for her mass, while “Y4125” had a shorter one (28 days).

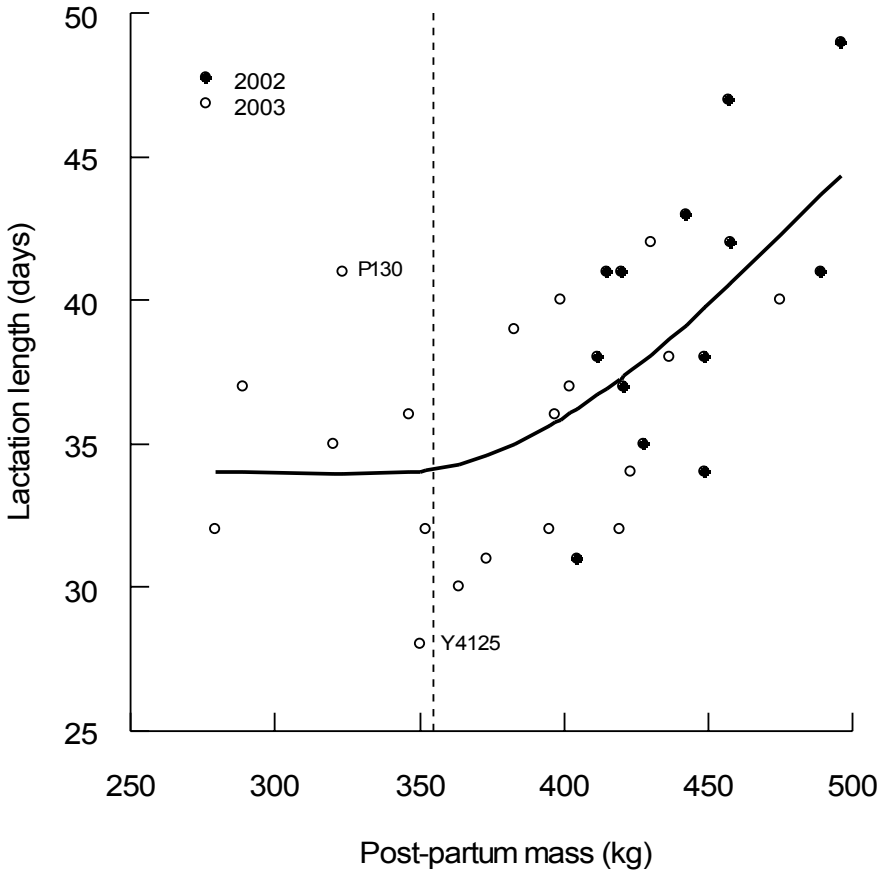

Figure 3.5 *Relationship between post-partum mass and lactation length. The curve is a smooth spline function fitted to illustrate the trend of the data. The vertical line denotes a suggested minimum mass range (< 355 kg) where a female will limit her lactation length. The linear function of the curve after 355 kg is* $y = 0.07x + 6.98$, $r^2 = 0.36$, $P = 0.002$.

3.4 Discussion

Reproductive performance responds to and reflects interactions with the environment, and it is thought to relate to current or recent phenomena (Croxall, 1992). The Southern Ocean is subject to a number of events that have measurable effects on sea ice extent and ecosystem structure (Nicol *et al.*, 2000). For instance, ice thickness was higher in 2003 than in 2002 (Lesser *et al.*, 2004), and this variation can strongly influence phytoplankton production (Seibel & Dierssen, 2003) and ultimately the performance of higher-trophic-level grazers and predators. Therefore, physiological or behavioural patterns in these species might reflect such environmental variation (Le Boeuf & Crocker, 2005). As capital breeders, phocid seals rely heavily on foraging success prior to parturition and their resultant body

reserves influence reproductive rate and the total amount and rate of energy expenditure to offspring. Thus, the constraints imposed by this strategy for lactation are useful for examining the consequences of environmental variability over a short time span (~ 1 year).

Life-history theory predicts that for species in which survival rates are higher for adults than juveniles, fitness is maximized by forgoing reproduction when conditions are unfavourable (Stearns, 1992). For a female in poor body condition, the optimal strategy is to allocate resources to self-maintenance rather than reproduction. Although previous studies of Weddell seals have shown variability in reproductive rate (Siniff *et al.*, 1977; Testa *et al.*, 1990) there is also evidence that females in poor condition will still produce and rear a pup (this study), although the long-term costs remain unknown.

We found that female body mass (and absolute body fat) at parturition differed between the two years of study and that this difference influenced lactation length, maternal expenditure, pup mass gain and weaning mass (and by inference, pup survival - Hall *et al.*, 2001; McMahon *et al.*, 2000b). Life history theory predicts a trade-off between mass and reproduction when resources are limited, and populations of upper-trophic-level predators are thought to be limited ultimately by food availability (Croxall, 1992; Testa *et al.*, 1991), so individuals that encounter different levels of resource availability during their lifetimes should select for a flexible strategy of maternal expenditure (Festa-Bianchet *et al.*, 1998). The variation in reproductive effort we observed may demonstrate the flexibility in expenditure as a function of female condition and environmental constraints, i.e. in 'good' years females were able to expend more because the potential cost (loss of future breeding) was lower.

We suggest that the lactation length for Weddell seals at McMurdo Sound is 5 to 6 weeks, compared to the generally accepted estimation of 6 to 7 weeks by Tedman *et al.* (1987). Our estimate agrees with two previous accounts (Kaufman *et al.*, 1975; Stirling, 1969). Although lactation is generally longer for Weddell seals than in other phocids, the duration appears to be highly variable. There was a positive relationship between MPPM and lactation length for larger females (≥ 355 kg), but not for smaller females (< 355 kg). This suggests that there was a minimum lactation time (~ 35 days) regardless of how small a female was, but as mass increased (≥ 355 kg) more energy was available for maintenance and allocation resulting in a longer lactation.

Therefore, lactation length for smaller seals would be shorter than expected (like in 2003) according to previous estimates.

Newly arriving females were heavier in 2002 than in 2003; however, there were inter-annual differences in the relationship between length and mass. In 2003, shorter females were lighter compared to their counterparts of the same length in 2002, regardless of age. On the other hand, longer females were the same mass in both years, indicating that the foraging success of shorter seals is more susceptible to environmental variation. If true, quantifying these patterns in smaller females may provide the best 'ecosystem indicator' of change within this marine ecosystem.

Eisert *et al.* (2005) found that females fasted within the first 3 to 4 weeks of lactation but as weaning approached, feeding occurred in 70 % of the sample population. However, it is still unclear which individuals (smaller versus larger) invoke this behaviour during lactation, how much feeding (net gain) occurs, and whether feeding depends on the success of over-winter foraging. Our results appear to indicate that at-sea differences prior to lactation are evident in MPPM, but differences in expenditure throughout lactation could be still influenced by foraging. This may be particularly important for smaller individuals if some can achieve higher mass and energy transfer and longer lactation periods than expected. Consequently, records of longer lactations (53 ± 5 days, Thomas & DeMaster, 1983) may be the result of females foraging and being able to remain with their pups longer. Nevertheless, females must have a considerable net gain from foraging before they can deliver any extra energy to their pup (Boyd, 1998). Presumably foraging would have to occur in the immediate vicinity where the prey source was abundant; otherwise, increased searching may influence available suckling time. High prey abundance is perhaps unlikely in McMurdo Sound where seal densities are high (Stirling, 1969) and the available foraging area (per individual) is small (Hindell *et al.*, 2002). Furthermore, Testa *et al.* (1989) inferred feeding from exceptionally high mass transfer efficiency rates, and although there was a negative relationship between efficiency and MPPM, the variation explained by MPPM was small ($r^2 = 0.099$). We found only a narrow range of efficiency (36.9 – 59.6 %), so we were unable to predict female foraging in either year. However, recent fatty acid composition analysis has indicated that some feeding did occur within smaller individuals (Chapter 4). Furthermore, the outliers in the MPPM-lactation length relationship (Fig. 3.5) may provide more insight into probable feeding. Seal 'P130' was small in 2003 yet had a relatively long lactation

period (41 days) for her size, and 'Y4125' was larger but departed earlier than expected (Fig. 3.4). Unfortunately, we were unable to collect EL data for Y4125; however, P130 had higher-than-average per cent mass loss (45.5 versus 37.8 %), and a higher-than-average energy transfer efficiency (45.0 versus 40.4 %). It may be that P130 foraged during lactation, but also invested more in her pup by staying longer at a possible cost to her own condition and survival.

Testa *et al.* 1989 found a weak negative relationship with MPPM and mass transfer efficiency from a pooled three-year dataset. Our longitudinal data revealed that mass and energy transfer efficiency differed between years but were not related to MPPM or TBL. In 2003 versus 2002, females had a 10.2 and 5.4 % lower rate of mass and energy transfer, respectively. If females foraged successfully during lactation in this poorer-resource year, one might predict a higher rate of transfer efficiency between mother and pup. However, our data revealed the opposite - in 2003 expenditure was lower. This suggests that females actively searched for food and expended more energy, but were unsuccessful in prey acquisition.

Although MPPM had a moderate effect on total body lipid (TBL) at post-parturition, only 2.6 % of the deviance was explained by this model. Therefore, although heavier females had more lipid and more lean tissue than lighter ones, the proportion of lipid to body mass was similar in all individuals, even at the end of lactation. Females with male pups lost proportionately more lipid than those with female pups; however, only 15.0 % of the deviance was explained by this model. Contrary to this, TBL of female pups at weaning was higher than that of males. Biuw (2003) found that post-weaned male elephant seal pups relied more on lipid metabolism for their energetic needs than females. Because Weddell seal pups swim and dive during the lactation period, their energetic demands are higher than those of most other species. Our data suggest that differential resource use by pups may occur during lactation. We suggest that males may be predisposed to use more lipid, so although they receive more lipid during lactation, they have less at the end of lactation relative to females.

We found no differences in pup birth mass between the sexes or between years, and only weak evidence for a relationship with MPPM. This indicates that there was no differential expenditure during gestation between differently sized females. This contrasts with other studies where birth mass was correlated to maternal mass (Bowen *et al.*, 1994; Costa *et al.*, 1986). Total mass loss as a percentage of initial

MPPM was similar between years for all females with known weaning dates (2002: 39.9 %, 2003: 37.8 %). This is comparable to that previously reported for Weddell seals (37 %, Hill, 1987), and to the 34 to 42 % mass loss of elephant seals (Arnbom *et al.*, 1997; Carlini *et al.*, 1997; Crocker *et al.*, 2001), suggesting that there is a relatively fixed proportion of MPPM (~ 60 %) to which an individual female can deplete her reserves before terminating lactation. As a result, pups of larger females acquired relatively more from their mother's energy stores and for a longer period of time.

Our results demonstrate differences in maternal mass and expenditure during lactation for a capital breeder that probably reflect environmental variability during the period of prey acquisition, and that these effects were more marked in smaller individuals. Smaller individuals did not increase energy expenditure to reconcile this disparity, resulting in reduced pup mass and condition at weaning. This is an important finding for general life history theory in long-lived mammals in terms of providing evidence for reproductive flexibility in a highly variable and challenging environment. We suggest that the trade-off between long-term survival in breeding females and the success of their offspring is contingent on individual size, and this is complicated by the possibility of feeding during lactation to offset nutritional constraints imposed during poor-resource years.

Chapter 4

Feast or Famine: evidence for mixed capital-income breeding strategies in Weddell seals

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Abstract

Evolved patterns of resource expenditure for reproduction have resulted in a life history continuum across species. A strictly capital-breeding strategy relies extensively on stored energy for reproduction whereas income breeding uses energy acquired throughout the reproductive period. However, some classically capital-breeding mammals may acquire food during their period of fasting to compensate for when conditions are not ideal. We examined milk composition and milk output for the Weddell seal to determine to what degree lactation was aided by food intake, and what physiological and environmental factors contributed to its manifestation.

Milk composition was independent of maternal post-partum mass and condition, but did change over lactation. Protein tripled between post-partum and end-lactation while lipid and energy increased to mid-lactation and then decreased slightly. This pattern of changes may be related to the relatively long lactation period demonstrated by this species. Feeding did not occur during early lactation and females devoted 54.9 % of energy loss to milk production. Differences in milk energy (%ME) were used to calculate a feeding index with which to rank the amount of feeding that occurred between individuals. Increased %ME coincided with a 59.7 % decrease in pup energy storage efficiency, suggesting that pups had higher energetic demands later in lactation.

Energy transfer efficiency and %ME were strongly correlated. This was used to estimate %ME for females captured in 2002. In 2002, females had 4.1 % higher %ME than females in 2003, and this was not due to increased feeding overall. Results indicate that, larger females fed more during lactation than smaller females. Our study confirms that Weddell seals use a mixed capital–income breeding strategy, and that considerable intra-specific variation exists. Questions remain as to the amount of energy gain derived from the income strategy, and the consequences of supplemental feeding for pup condition and survival.

4.1 Introduction

Life history strategies reflect variation in the allocation of an individual's resources (i.e., time, effort and energy expenditure) to competing life functions such as growth, survival and reproduction. The expenditure for reproduction can be broadly classified according to the temporal distribution of energy acquisition and expenditure. At one end of the continuum, capital breeding relies extensively on stored energy for reproduction, while at the other, income breeding uses energy acquired throughout the course of the reproductive period (Jönsson, 1997; Stearns, 1992). In mammals, larger species tend to employ the capital-breeding strategy, while smaller species generally rely on income breeding to fuel reproductive costs (Boyd, 2000; Trillmich & Weissing, 2006). However, various other life history characteristics of a species will determine the degree to which an animal is likely to rely on stored "capital" for breeding (Bonnet *et al.*, 1998), and the position of an organism along the capital – income gradient will in turn be influenced by the particular evolutionary context in which life history traits such as lactation length and size of offspring develop.

Lactation is one of the major defining characteristics of mammals and is a critical part of their reproductive strategies. The physiology of lactation is intertwined with that of reproduction itself, and the 'lactational capacity' of an individual, or species, relies on the interaction between several physiological and environmental factors (e.g., food availability, prey acquisition efficiency) that determine maternal body condition during foetal development and after parturition (Jenness, 1986). Although lactation imposes energetic stress on mothers, it allows offspring to devote a higher proportion of energy (from milk) to growth rather than maintenance (Pond, 1977). The proportions of nutritive constituents in milk differ greatly among species and vary during lactation according to the particular reproductive strategy (e.g., rapid development to offspring dependence versus extended parental care) and growth patterns of the offspring (Jenness, 1986).

Species within the Suborder Pinnipedia (order Carnivora) typify the extremes of the capital–income breeding continuum, with females of the Family Phocidae ('true seals') generally following the capital strategy, while females of the Family Otariidae (fur seals and sea lions) follow the income strategy (Boyd, 2000). Typically, phocids have a spatio-temporal separation between foraging and reproduction, resulting in

short, intense lactation periods where milk is derived from maternal reserves and has high lipid content (e.g., at times exceeding 50 % in some species – Hindell *et al.*, 1994; Iverson *et al.*, 1995a; Iverson *et al.*, 1995b). Thus, lactation allows prior maternal foraging success to fuel current provisioning. Pinniped provisioning is limited by these maternal reserves, and fluctuation in energy acquisition prior to the breeding season can influence both the duration and magnitude of maternal expenditure, so that females that forage successfully prior to birth will be able to transfer more energy to offspring during lactation. This in turn affects pup condition and survival (deLittle *et al.*, 2007; Hall *et al.*, 2001; McMahon *et al.*, 2000b).

Despite their categorization as capital breeders, some phocids engage in feeding during the lactation period (Bowen *et al.*, 2001; Eisert *et al.*, 2005; Lydersen & Kovacs, 1999), suggesting that these species fall somewhere between the two extremes of the continuum, and leading to the hypothesis that that food intake during this time provides an optional supplementation to body reserves for nutritionally challenged individuals. If true, the hypothesis predicts that income-like foraging should occur when the physiological state of the largely capital-investing mother is insufficient to see her pup through to independence. There also remain questions regarding the magnitude of this predicted contribution, its benefits for pup growth, body condition and survival, and the environmental contexts in which income supplementation becomes more probable.

Weddell seals (*Leptonychotes weddellii* Lesson) offer a unique opportunity to test predictions of a capital-breeding species' ability to adopt income-like reproductive behaviour through measures of lactation behaviour and energetic expenditure. They are one of the largest phocid species, and given their extensive maternal reserves, it is generally assumed that females should be able to sustain lactation entirely from body reserves. However, the 5 – 6 week lactation period (Wheatley *et al.*, 2006a) is one of the longest recorded for a phocid and increases the energetic demands on the fasting female relative to other phocids that have shorter lactation periods (see Trillmich, 1996 for a review). There is evidence that some females feed during lactation (Eisert *et al.*, 2005; Hindell *et al.*, 2002), so we can directly test hypotheses related to the magnitude and form of the capital–income breeding gradient among individuals. Previous work has hypothesised that a mother's particular combination of strategies will influence the delivery of energy to her pup and its subsequent wean mass, condition and survival probability (Wheatley *et al.*,

2006a).

To measure the energetics of lactation effectively, the mother and pup must be measured simultaneously to separate energy used for maternal maintenance from that used for production and transfer of milk. The measurement of milk energy output over the course of lactation is the most direct assessment of total energy transfer from mother to pup (Iverson *et al.*, 1993). This, combined with body composition changes, also reveal metabolic requirements of the female during lactation. These measurements should therefore contribute to predicting which females feed during lactation and identifying the causes and consequences of this behaviour on pup condition and survival. We therefore examined an individual's milk composition through time, milk output and the extent to which lactation was fuelled by income (food intake) in Weddell seals.

Larger animals need a higher food density in the marine ecosystem to achieve a positive energy balance on short foraging trips (Boyd, 1998; Trillmich & Weissing, 2006), and travel time between breeding and feeding areas influences most maternal behaviour (Trillmich & Weissing, 2006). Consequently, we predict that foraging by Weddell seal females makes a relatively small contribution to the total energy budget of lactation. We hypothesised that there were two alternatives to account for feeding in females: (1) similar to some small phocids (Bowen *et al.*, 2001), smaller or 'lipid-poor' female Weddell seals will follow a capital breeding strategy at the beginning of lactation and later supplement maternal reserves with income-based provisioning as body reserves are depleted, while larger females sustain lactation entirely through capital reserves; or (2) large-bodied females will be able to engage in foraging because they have attained a certain size allowing them to exploit resources (through increased dive durations) that might not be available to smaller females. We also predict these differences in mean reproductive strategy will have detectable consequences on pup growth rate and condition: (1) if foraging is energetically expensive than small females will only engage in it when critical, and pups will be in relatively poor condition relative to pups of larger females who have enough energy to support the costs of lactation; and (2) if large females forage than their pups should be in better condition than predicted by maternal reserves alone.

4.2 Methods

4.2.1 Field procedures

We studied breeding Weddell seals at Hutton Cliffs, Antarctica (77° 51' S, 166° 45' E) during the austral summer (October to December) of 2002 and 2003. Thirty mother-pup pairs in 2002 and 25 pairs in 2003 were captured 1 to 6 (mean ± standard error = 3.8 ± 0.22) days post-parturition (dpp). Individual females were identified by flipper tags attached in previous years and pups born to study females were marked with hind flipper tags soon after birth as part of a long-term tagging study (Hadley *et al.*, 2006). Age was known for 19 females in 2002 and 21 females in 2003 (range 6 – 21 years). Once captured, each female was restrained and chemically immobilised as described in Wheatley *et al.* (2006b). Females were weighed to the nearest 1 kg, and body length and axial girth measurements recorded. Each pup was weighed to the nearest 0.5 kg and length and girth measured.

4.2.2 Sample collection

Body composition, water flux rates and milk intake were determined using hydrogen isotope dilution at post-parturition (PP) and end-lactation (EL; 36 to 38 dpp; mean 36.9 ± 0.26). After immobilisation of females, a 10 ml blood sample was collected to measure background isotope levels. Immediately after, a pre-weighed dose (to the nearest 0.1 mg) of approximately 222 MBq of tritiated water (HTO) was administered to each female. Due to a shortage of HTO at the beginning of the 2002 field season, ten females were administered a dose of deuterium oxide (HDO, specific concentration: 99.8 %) at the PP capture while HTO was used for all other females and the EL captures (Wheatley *et al.*, 2006a). In 2003, mother-pup pairs were recaptured at mid-lactation (ML; 21 to 22 dpp), weighed and measured with blood and milk samples taken (n = 25). At EL, following an initial blood sample, isotope was re-administered to the mother (n = 11) and she was measured and sampled as with the PP capture. Body composition was measured for all pups (n = 25) at PP in 2003 and for 25 and 22 pups at EL in 2002 and 2003, respectively. Approximately 74

MBq of HTO was administered in 2002, and a 10 ml dose of HDO (specific concentration: 99.8 %) in 2003 to each pup. A second blood sample (10 ml) was taken from all animals 150 min on average after initial injection to determine enrichment level.

Milk samples were collected using a modified 50-ml syringe approximately 10-15 minutes after an intravenous injection of oxytocin (1 ml, 10 IU/ml), which was administered during immobilisation. All samples were stored at -20 ºC until laboratory analysis.

4.2.3 Laboratory and data analyses

Plasma samples were analysed for HTO activity using liquid scintillation spectrometry as described in Wheatley et al. (2006a). Mass spectrometric analysis of deuterium enrichment was done in triplicate using H_2 gas and a platinum-on-alumina catalyst according to the methodology described in Scrimgeour *et al.* (1993). Milk samples (n = 59) were thawed, homogenised and analysed for proximate composition (water, protein, and lipid content). Carbohydrate content has been found to be negligible in Weddell seals (Tedman & Green, 1987), so its consideration and analysis were regarded as unnecessary. Water content was analysed in duplicate by drying a weighed subsample (0.5 – 1.0 g) in an 80 °C oven for 96 hours. Protein content was determined by spectrophotometry according to the method of Clayton et al. (1988), modified for biological fluid with bovine serum albumin as a standard. Milk lipids were quantitatively extracted using a modified (overnight extraction) Bligh and Dyer 1959 one-phase methanol/chloroform/water extraction (2:1:0.8, v/v/v). Iverson et al. (2001) found a significant difference in lipid extraction between the Bligh & Dyer and Folch methods; therefore, we repeated extractions on some samples from PP (n = 4) and ML (n = 4) captures using a modified version of the Folch et al. (1957) method for comparison.

Isotope dilution overestimates total body water (TBW) by 4.0% for HTO and 2.8% for HDO in grey seals (*Halichoerus grypus* - Reilly & Fedak, 1990). Therefore, isotope dilution space was corrected for using these values. Body composition was estimated for mothers and pups from body mass and TBW according to the equations of Reilly and Fedak (1990). Total body water at ML was estimated by interpolation,

assuming that the decrease in water pool mass of the female was linearly proportional to her mass loss and that the increase in each pup was proportional to mass gain (Iverson *et al.*, 1993; Mellish *et al.*, 1999). Specifically, TBW at mid-lactation was estimated from the relationship between mass and TBW between post-partum and end-lactation. Therefore, TBW values at mid-lactation were restricted by the number of animals captured at end-lactation.

Total water influx (TWI) was calculated using equation 6 in Nagy and Costa (1980) based on TBW changing linearly with time. Milk intake (MI) was estimated according to the equation of Oftedal and Iverson (1987):

$$MI = 100 \times \frac{TWI + (1.07 \times L_d) + (0.42 \times P_d)}{W_m + (1.07 \times L_m) + (0.42 \times P_m)} \quad (4.1)$$

where L_d and P_d are the daily fat and protein deposition rates ($g \cdot day^{-1}$), respectively, over the lactation period and W_m, L_m and P_m are the water, lipid and protein content (%), of milk, respectively. The gross energy content of tissues and milk were calculated using values of 39.3 $MJ \cdot kg^{-1}$ and 23.6 $MJ \cdot kg^{-1}$ for lipid and protein, respectively (Blaxter, 1989). Although we captured 22 pups at the end of lactation, milk intake estimation was restricted by milk samples (n = 10).

A series of standard generalised linear and mixed-effects models (GLM; GLMM) were constructed to examine intra-specific differences of females and pups. Examination of the residuals for all models determined the statistical error distribution and link function most appropriate. Model selection was based on Akaike's Information Criterion corrected for small samples (AIC_c, Burnham & Anderson, 2002). The information-theoretic weight of evidence ($w+_i$) for each predictor was calculated by summing the model AIC_c weights (w_i) over all models in which each term appeared. However, the $w+_i$ values are relative, not absolute because they will be > 0 even if the predictor has no contextual explanatory importance (Burnham & Anderson, 2002). To judge which predictors were relevant to the data at hand, a baseline for comparing relative $w+_i$ across predictors was required, so we randomised the data for each predictor separately, re-calculated $w+_i$, and repeated this procedure 100 times for each predictor. The median of this new randomised $w+_i$ distribution for each predictor was taken as the baseline (null) value ($w+_0$). For each term the absolute

weight of evidence ($\Delta w+$) was obtained by subtracting $w+_0$ from $w+_i$, and predictors with $\Delta w+$ of zero or less have essentially no explanatory power (Burnham & Anderson, 2002). Specific model comparisons were based on the information-theoretic evidence ratio (*ER*) which is equivalent to the AIC_c weight (w) of the full model divided by the w of the null model (Burnham & Anderson, 2002). Higher *ER* values indicate higher likelihoods of the tested model relative to the null. We also calculated the per cent deviance explained (%DE) by each model constructed as a measure of model goodness-of-fit. All statistical analyses were done using the *R* Package (Ver. 2.3.1; *R* Development Core Team 2004). Values are presented as mean ± one standard error of the mean (SEM) unless otherwise stated.

4.3 Results

4.3.1 Milk Composition

Milk samples (n = 8, from PP and ML) had an average lipid content of 48.7 ± 3.0 % with the modified Folch *et al.* (1957) method, and 48.1 ± 3.0 % with the (overnight extraction) Bligh and Dyer (1959) method. Comparison between the two revealed no evidence for a difference (*ER* = 0.37).

Milk composition changed over the course of lactation (Table 4.1) and individual variability in composition can be seen at each stage of lactation (Fig. 4.1). Protein (*%P*) increased throughout lactation while lipid (*%L*) and energy content increased slightly to ML and then decreased again (Fig. 4.1). The number of days elapsed since parturition (days post-partum [dpp]) explained 63.2 % of the variation in *%P*. For *%L*, water (*%W*) and *energy*, *dpp* explained more of the variation than the null model (*ER*: $\%L = 5.06 \times 10^{13}$; $\%W = 2.55 \times 10^{115}$; $energy = 1.63 \times 10^{21}$); however, the goodness-of-fit of these models was lower (%DE: 1.19, 9.79 and 9.92, respectively). Milk composition at EL from one female (Pu194) was excluded from averages and analyses because there was an indication that weaning may have occurred (*%L* = 8.2, *%P* = 18.8, *%W* = 72.1).

There was no evidence that maternal post-partum mass (MPPM), total body lipid stores (TBL_{MPPM}) or total body protein stores(TBP_{MPPM}) affected milk

composition (*%L*, *%P*, *%W* and *energy* content) at post-partum ($\Delta w+ \leq 0$ for all terms).

Table 4.1 *Composition of Weddell seal milk during lactation.*

	Post-parturition		Mid-lactation		End-lactation	
	(n = 25; 4.8 ± 0.23)[a]		(n = 24; 21.2 ± 0.10)[a]		(n = 9; 37.2 ± 0.40)[a,b]	
Component	Mean	SEM	Mean	SEM	Mean	SEM
Lipid (%)	39.9	1.29	50.0	1.64	41.7	2.34
Protein (%)	5.4	0.24	12.3	0.67	14.6	1.39
Water (%)	46.0	1.16	35.7	1.61	40.7	2.47
Energy (MJ/kg)	17.0	0.52	22.5	0.69	19.8	0.92

[a] Average number of days post-partum that samples were collected

[b] Milk composition of female Pu194 was excluded from averages

4.3.2 Milk output and energy flux

For ten females with body and milk composition data at post-parturition, and milk and estimated body composition data at mid-lactation, we calculated total energy loss and total milk energy output to determine the proportion used for metabolism versus milk energy (%ME) to the pup. This was repeated for eight of the females from mid-lactation to end-lactation (Table 4.2). If the decrease in water pool mass of the female was not linearly proportionally to her mass loss (as assumed), milk intake may be underestimated, affecting pup energy gain calculations.

In early lactation, average %ME was 54.9 ± 0.04 % of the total energy lost by the female. Between mid-lactation and end-lactation, %ME exceeded that of total energy lost by the female in almost all cases (Table 4.2). From evidence that females at this location do not feed during the first three weeks of lactation (Eisert *et al.*, 2005), and that %ME (PP to ML) was similar to that of non-feeding southern elephant seals (57.2 %; *Mirounga leonina*, Hindell & Slip, 1997) and northern elephant seals (59 %; *Mirounga angustirostris*, Costa *et al.*, 1986), we considered the %ME from post-partum to mid-lactation to represent that of a non-feeding individual.

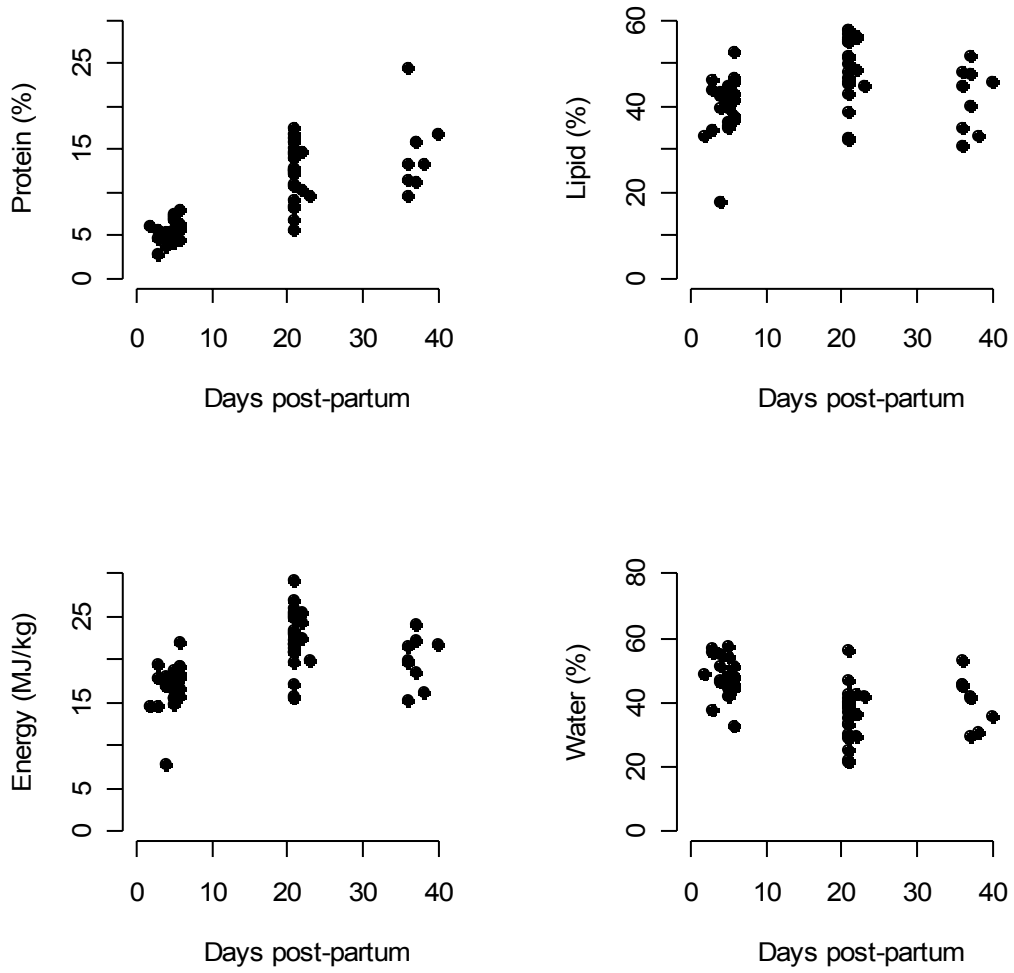

Figure 4.1 *Changes in milk composition of Weddell seals sampled at post-parturition (n = 25), mid-lactation (n = 24) and end-lactation (n = 10).*

Table 4.2 *Energy losses and transfer of Weddell seals during lactation in 2003.*

	Female	Total energy loss (MJ)	Total milk energy output (MJ)	Milk energy (%)	Energy transfer efficiency (%)*	Feeding index
PP to ML	Pu761	2892.9	1151.1	39.8	34.6	-
	W636	3508.2	1511.4	43.1	34.8	-
	Pu194	2578.6	1264.4	49.0	32.3	-
	Y4295	2146.5	1058.9	49.3	33.9	-
	Pu114	2821.4	1466.6	52.0	34.7	-
	P871	2259.9	1184.9	52.4	47.7	-
	P130	1594.7	939.7	58.9	42.9	-
	Y965	3149.3	1914.3	60.8	41.1	-
	Y536	1684.6	1048.9	62.3	43.9	-
	Pu517	1381.5	1127.2	81.6	64.2	-
ML to EL	Y965	1567.2	1531.9	97.7	33.1	0.61
	Y536	993.2	1183.9	119.2	35.7	0.91
	Pu114	1758.9	1784.9	101.5	30.1	0.95
	P130	1452.8	1936.3	133.3	47.4	1.26
	Pu517	911.4	1735.4	190.4	51.7	1.33
	W636	2280.6	2455.9	107.7	36.0	1.50
	P871	1849.8	2640.0	142.7	55.9	1.72
	Pu761	2258.8	2867.9	127.0	37.2	2.19

* pup energy gain / female energy loss

We assumed that per cent milk energy values above this (between ML and EL) represented an external energy source (i.e., mother feeding). We were unable to calculate metabolic rates and therefore could not quantify energy acquired from feeding. However, we calculated a 'feeding index' for the second half of lactation as:

$$\frac{\%ME_{ML-EL} - \%ME_{PP-ML}}{\%ME_{PP-ML}} \tag{4.2}$$

which represents the number of times that %ME exceeded that of non-feeding individuals (Table 4.3). This allowed us to rank individuals by the relative amount of feeding. Total energy loss (MJ) was 69.1 ± 0.05 % lower in the second half of lactation (n = 8), while total milk energy output (MJ) was 30.5 ± 0.10 % higher (n = 8). Therefore, although female energy loss was lower in the second half of lactation, more energy was transferred to the pups.

There was a strong correlation between %ME and energy transfer efficiency

(pup energy gain/female energy loss; Spearman's r = 0.93, $P < 0.01$). This relationship was used to estimate %ME for females captured in 2002, for which there were no milk data (Wheatley *et al.*, 2006a, Table 4.3). A feeding index for the last half of lactation was also calculated using the average $\%ME_{PP\text{-}ML}$ for non-feeding animals from 2003 (54.9 %, Table 4.3). Milk intake by pups was also a measure of milk output of mothers. The most-parsimonious GLM testing for the effect of *age*, *MPPM* and TBL_{MPPM} on maternal milk output ($kg \cdot day^{-1}$) from post-partum to mid-lactation included *MPPM* and *age*, although only *MPPM* explained an important component of the variation in this model ($\Delta w+_{MPPM} = 0.216$, $\Delta w+_{TBL} = 0.026$, $\Delta w+_{age} = 0.000$), with milk output increasing with MPPM. Daily milk output increased 34.7 % from 3.2 ± 0.19 $kg{\cdot}day^{-1}$ in early lactation to 4.9 ± 0.37 $kg \cdot day^{-1}$ in late lactation (Table 4.4). This coincided with an increase in daily milk energy output of 37.5 %, or 64.3 ± 4.22 $MJ{\cdot}day^{-1}$ in early lactation and 102.9 ± 7.77 $MJ{\cdot}day^{-1}$ in late lactation. However, the energy stored per day (as body tissue) by pups decreased by an average of 3.9 % between early and late lactation; this equated to a total decrease in energy storage efficiency of 59.7 %.

The most parsimonious model testing for the effect of *MPPM*, TBL_{MPPM} and *year* on total %ME included TBL_{MPPM} and *year*; however, *year* was the only variable with information-theoretic support ($\Delta w+_{year} = 0.183$; $\Delta w+ \leq 0.000$ for all others). Model averaging estimated that females in 2002 had 4.1 % higher %ME than females in 2003. The most parsimonious model testing for the effect of *age*, *MPPM* and *year* on feeding index included *age* and *MPPM*; however, *MPPM* was largely responsible

Table 4.3 *Per cent energy to pup in 2002, estimated from correlation with energy transfer efficiency in 2003.*

Female	Post-partum mass (kg)[a]	Energy transfer efficiency (%)	Milk energy (%)[b]	Feeding index[c]
Y3434	430.7	22.4	49.6	0.44
Pu514	410.1	36.6	72.8	0.91
Y4664	420.9	37.4	74.2	0.93
W464	503.6	39.3	77.2	1.00
Pu894	427.7	40.4	79.0	1.03
Y1463	442.1	40.8	79.8	1.05
Y4524	457.5	40.9	79.9	1.05
Pu526	420.9	41.3	80.6	1.06
Y2149	479.7	41.6	81.1	1.07
Y4876	471.7	43.2	83.6	1.12
Y1433	495.8	43.6	84.3	1.14
R957	429.3	43.6	84.4	1.14
Y1933	448.5	46.8	89.6	1.24
P282	432.2	48.7	92.7	1.30
R552	488.8	52.9	99.6	1.44
Y3298	481.6	55.0	103.0	1.51
Y481	482.8	55.6	104.0	1.53
Pu661	408.7	55.7	104.2	1.53
P244	419.7	55.8	104.3	1.54
Y2310	489.3	57.7	107.5	1.60
Y3243	456.9	60.3	111.7	1.69

[a] body mass measured after the day of birth was interpolated to estimate post-partum mass using calculated rates of daily loss for each individual.

[b] average for the entire lactation period

[c] estimate for the second half of lactation

Table 4.4 *Water flux and milk intake of Weddell seal females and pups.*

		Post-parturition to Mid-lactation		Mid-lactation to End-lactation	
		(n = 10)		(n = 10)	
		Mean	SEM	Mean	SEM
Females	Mass loss (kg·d^{-1})	4.7	0.25	3.7	0.23
	Water efflux (ml kg^{-1}·d^{-1})	13.4	0.62	23.8	1.87
	Milk water output (kg·d^{-1})	1.3	0.08	1.9	0.17
	Energy expenditure (MJ·d^{-1})	153.3	14.18	104.1	11.82
		(n = 21)		(n = 9)[a,b]	
Pups	Mass gain (kg·d^{-1})	2.0	0.11	1.0	0.09
	Milk intake (kg·d^{-1})	3.2	0.19	4.9	0.37
	Milk lipid intake (kg·d^{-1})	1.5	0.10	2.2	0.17
	Milk protein intake (kg·d^{-1})	0.3	0.02	0.7	0.06
	Milk energy intake (MJ·d^{-1})	64.4	4.24	100.3	7.57
	Energy stored (MJ·d^{-1})	40.6	2.72	39.0	2.97
	Storage efficiency (%)	63.6	2.30	39.0	0.89

[a] Milk composition at end-lactation from female Pu194 was excluded from averages

[b] Sample size dependent on the number of milk samples collected.

for driving the relationship ($\Delta w+_{MPPM}$ = 0.721, $\Delta w+_{age}$ = -0.022, $\Delta w+_{year}$ = -0.047), indicating that larger females fed more during lactation (Fig.4.2). There was also a strong positive relationship between feeding index and daily mass gain ($kg \cdot day^{-1}$) of pups (*feeding index* = 0.79 × *mass gain* + 0.85, $r^2 = 0.44$).

Due to the increase in milk protein throughout lactation, we measured whether there was a relationship between TBP depletion (kg) from the female's body stores and the feeding index (for both years). We found a positive linear relationship (*ER* = 10.25, %DE = 20.3 %), indicating that females who lost more protein had a higher rate of feeding.

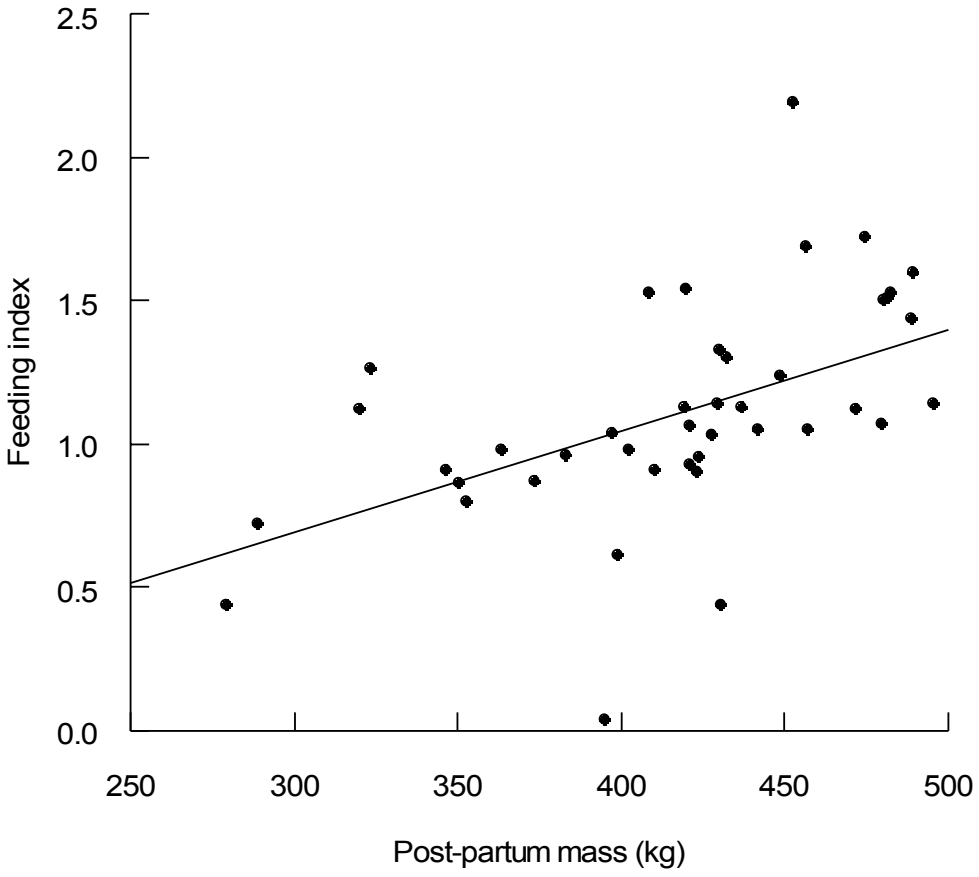

Figure 4.2 *Positive relationship between post-partum mass (x) and feeding index (y); $y = 0.003x - 0.37$, $r^2 = 0.26$.*

4.4 Discussion

Although we could not estimate the absolute amount of feeding that occurred (total MJ), we were able to rank females according to their relative feeding frequencies during the latter part of lactation. We found that there was up to a five-fold difference between individuals in the feeding index (see Tables 4.2 & 4.3), and contrary to our expectation, heavier females fed more, but there was no difference between years. Dive duration increases with body mass (Kooyman, 1989; Le Boeuf, 1994), so a plausible explanation for the observed trend is heavier females are able to exploit resources in McMurdo Sound more efficiently than their lighter counterparts, especially where population density (Stirling, 1969) and intra-specific competition is high (Hindell *et al.*, 2002). In other words, only after attaining a certain threshold body size and condition will the costs of extra foraging during late-lactation be outweighed by the additional energy supplied to the pup. The positive relationship we observed between the maternal feeding index and daily mass gain of pups may support the hypothesis that heavier females can deliver relatively more energy to their pup than predicted by their body reserves alone (i.e., the rich getting richer), or be confounded by the fact that heavy females (with a higher feeding index) have the capacity to deliver more energy to their pup (Wheatley *et al.*, 2006a), regardless of feeding. Therefore, lighter females not only had less capital available to pups at the onset of lactation, they were also less capable of supplementing that disadvantage with late-lactation feeding (i.e., the poor remaining poor). We conclude then that the lactation-feeding phenomenon in this mainly capital-breeding mammal does not provide an effective nutritional refuge for lighter females incapable of securing sufficient reserves during their pre-parturition foraging trips; rather, it appears to be a flexible strategy employed by those individuals with the physiological capacity to supplement their capital reserves with income feeding.

Although most (20 – 69 %) variation in pinniped lactation patterns can be explained by phylogenetic history and body size, the remaining variation has likely resulted from adaptations to local environment conditions (Ferguson, 2006). It appears that differences in life history strategies exist within lactating Weddell seals and these have resulted from several interrelated features: (1) relatively precocial pups that are able to swim and dive during lactation, (2) one of the longest lactation periods of any phocid, and (3) milk protein increases throughout lactation, depleting maternal

body stores. These factors may influence the extent to which each individual female relies on stored energy to fuel lactation and the physiological capacity to forage later in lactation. In some respects, the existence of flexible foraging strategies in Weddell seals questions the true nature of the phocid lactation continuum, and it also suggests that other species may also break out of their physiological pigeon-hole to engage in 'anomalous' lactation behaviours.

The dichotomy between capital and income breeders is a scheme that is increasingly used to understand the life history strategies of birds (Gauthier *et al.*, 2003; Meijer & Drent, 1999), reptiles (Bonnet *et al.*, 1998), parasites (Casas *et al.*, 2005) and pinnipeds (Boyd, 1998; Trillmich & Weissing, 2006). The demographic cost of reproductive investment (i.e., decreased survival and future reproduction as a function of current reproduction) is a pivotal trade-off around which life histories are thought to evolve (Harshman & Zera, 2006; Stearns, 1992). Therefore, examining the proximate mechanisms that species evolve to deal with their physiological limitations and the environmental conditions in which they live assist in the evaluation of the functional explanations for both inter- and intra-specific differences in reproductive effort. Energy requirements for reproduction in mammals can be satisfied through complete reliance on body reserves, reliance on regularly ingested energy, or on a combination of both. This capital–income breeding continuum is complex and varies among and even within species, but has most likely resulted from physiological limitations and adaptations to specific environmental conditions.

The results of our study therefore assist in our understanding of the trade-offs associated with reproductive strategies, the intra-specific variation that exists within Weddell seals, and life history theory in general. The pattern of milk composition in Weddell seals that we described is different to that reported by Tedman (1980), Tedman and Green (1987) and to that of other phocid seals. Tedman (1980) found no evidence for milk composition changes throughout lactation; however, his analysis was based on cross-sectional data, and low sample sizes (total $n = 11$) and high variance. Our study was more detailed and longitudinal, revealing overall that milk composition does indeed change over the course of lactation. Lipid content at PP (39.9 %) was higher than that reported for southern elephant seals (16.1 %; Hindell *et al.*, 1994) but similar to that of harbour seals (40.8 %; *Phoca vitulina*, Lang *et al.*, 2005) and grey seals (34.5 %; Mellish *et al.*, 1999). However, rather than a gradual increase throughout lactation, lipid increased to mid-lactation and then declined

toward post-parturition levels. Protein changes (low post-parturition then tripling) were the most dissimilar to any other seal species. Although there was individual variability in milk composition (at parturition, see Fig. 4.1), it was not related to maternal post-partum mass or body composition. However, maternal post-partum mass did influence milk output ($kg \cdot day^{-1}$), with heavier females producing more milk. This was consistent with the previously reported positive relationship between maternal post-partum mass and pup mass again (Wheatley *et al.*, 2006a).

The observed pattern of milk composition changes in Weddell seals helps explain why they have a longer lactation period than similar-sized phocids. For instance at parturition, mother's must devote resources to producing lipid-rich milk for the pup's thermoregulatory needs, followed by a later increase in protein required for lean tissue growth. Protein values at mid- and end-lactation were similar to those reported for the income-breeding Antarctic fur seal (*Arctocephalus gazella* – Arnould & Boyd, 1995) late in lactation. Therefore, this need for protein later in lactation may require females to feed rather than deplete their own stores (i.e., muscle), as well as influence total lactation length. Our measures of milk output and storage efficiency were similar to those measured by Tedman and Green (1987) averaged over the entire lactation period; however, we have demonstrated that changes occur during lactation. Milk output increased over lactation, similar to other species (Lydersen & Kovacs, 1996; Mellish *et al.*, 1999); in contrast, the rate of pup mass gain decreased. This may be the result of reduced storage efficiency and higher energy expenditure of pups that engage in swimming and diving, a phenomenon not normally observed in pre-weaned pups of most other phocids.

Milk energy represented the largest proportion of female energy expenditure during lactation, and fatter females were able to devote a higher per centage of their energy loss to milk than metabolism. This may result from lower lean body mass, culminating in a lower metabolic overhead that allows for more energy to be devoted to milk production. The *year* term was the only variable found to influence milk energy delivery for the entire duration of lactation, with more energy (4.1 %) delivered in 2002. This was not related to increased foraging success in one year (2002), but based on energy transfer efficiency rates (Wheatley *et al.*, 2006a), may be related to more time devoted to (largely unsuccessful) foraging in 2003 when maintenance (i.e., diving costs) expenditure dominated milk production.

We conclude that the income acquired during lactation in this mainly capital-

breeding mammal is relatively nominal because milk lipid values late in lactation were similar to those at parturition, mass loss rates of the females were not different between early and late lactation, and pup mass gain was lower than at the beginning of lactation. Although differences did exist in the amount of feeding occurring among individuals, effects on 'lactational capacity' were small and support the hypothesis that large Weddell seals mothers are more opportunistic feeders during lactation when they can add energetic value to their expenditure. This strategy favours fast delivery rates of energy over a short period of time, but providing the behavioural and physiological plasticity to maximize offspring survival via supplementary (income-fuelled) expenditure when conditions are suitable.

Chapter 5

Differential mobilisation of blubber fatty acids in lactating Weddell seals: evidence for selective use

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Abstract

A major source of energy during lactation in mammals is provided through the mobilisation of blubber fatty acids (FA). We investigated the extent to which FA were mobilised to support both maternal metabolic requirements and milk production in the Weddell seal, and how this was reflected in the FA composition of the pup's blubber at the end of lactation (EL). FA composition of post-partum (PP) female blubber was similar in the two years of study (2002 & 2003), but differed markedly by EL. Pup blubber FA (at EL) were also different between years and did not match that of the mother's milk or blubber. Milk FA composition changed during lactation, which may have been a reflection of an increase in pup energy demands at different stages of development. In addition, there was evidence of feeding by some females during lactation, with higher levels of some FA in the milk than in the blubber. Our results indicate that differential mobilisation of FA occurred in lactating Weddell seals and that this was related to total body lipid stores at PP. Furthermore, growing pups did not store FA unmodified, providing evidence that selective use does occur, and also that using FA composition to elucidate dietary sources may be problematic in growing individuals. We suggest that dietary predictions will be most reliable when full blubber core samples are taken at parturition.

5.1 Introduction

A major characteristic of mammalian reproductive strategies is the evolution of lactation modalities to deal with extreme energetic costs associated with offspring growth. Producing milk is one of the most energetically expensive activities for female mammals, so its delivery to offspring essentially defines the reproductive strategy a species evolves (Bonnet *et al.*, 1998). The storage of energy represents an important component of life history variation and its delivery to the offspring helps define the trade-off between survival and future reproduction (Ferguson, 2006). Within reproductive strategies, there has been recognized a capital–income breeding continuum that represents differing tactics of energy utilisation (Chapter 4; Houston *et al.*, 2007; Jönsson, 1997). This ranges from capital breeding that relies extensively on stored energy for reproduction, to income breeding where energy used in reproduction is acquired throughout the course of the reproductive period (Stearns, 1992; Jönsson, 1997). Lactation strategies of pinnipeds show high diversity from extreme capital breeding, with offspring provisioned entirely from stored reserves over just a few days, to income breeding with prolonged lactation over months or years (Boyd, 1998; Houston *et al.*, 2007; Trillmich & Weissing, 2006). Females of the Family Phocidae ('true seals') generally follow the capital strategy (Boness & Bowen, 1996; Oftedal & Iverson, 1987) which is typically associated with mobilisation of fat stores and higher milk-lipid content, reducing the time the pup is dependent upon the mother. However, some phocids feed during lactation (Bowen *et al.*, 2001; Eisert *et al.*, 2005; Lydersen & Kovacs, 1999) suggesting that late-lactation food intake may at times help offset the energetic costs of lactation.

In response to the high physiological demands of lactation, a major source of energy is provided through the mobilisation of fatty acids (FA) from the breakdown of triacylglycerol (TAG). Fatty acids are stored primarily in the blubber and form an essential part of physiological regulation as precursors to the synthesis of other compounds, as fuels for energy production, and as building blocks for cell membranes (Dalsgaard *et al.*, 2003). Fatty acids may be accumulated directly from the diet, modified once ingested, or formed endogenously. The omega-3 and omega-6 polyunsaturated fatty acids (PUFA) are essential fatty acids (EFA) required for structural growth, brain development, and normal cell development (Innis, 2005). These EFA cannot be formed *de novo* by mammalian cells and so must be obtained

from the diet. Therefore, FA mobilisation and transfer is not only important for the energetic requirements of the fasting mother, but also for development of her offspring.

During lactation, lipid metabolism is intensified and the FA composition of the blubber changes as a consequence of utilisation of depot FA (Grahl-Nielsen *et al.*, 2000; Iverson *et al.*, 1995a). Specific FA may be mobilised or sequestered to accommodate the physiological requirements of both mother and pup (Samuel & Worthy, 2004). In addition, as fatty acids can be differentially mobilised according to their molecular structure (Connor *et al.*, 1996; Herzberg & Farrell, 2003; Raclot, 2003), loss of FA from adipose tissue is not merely a function of the relative abundance of individual FA. Metabolism and deposition of FA may not therefore be predictable depending on the time of year or physiological state of the individual at the time of measurement. Furthermore, as FA composition of stored fat is primarily a product of diet, FA composition may itself affect energy expenditure (Maillet & Weber, 2006; Pierce & McWilliams, 2005), and so variation in diet composition too may have an influential role in FA mobilisation and transfer patterns during lactation.

Fatty acids have been of interest from both nutritional and trophodynamic perspectives, with FA used as qualitative markers to trace or confirm predator-prey relationships in the marine environment for more than thirty years (see Dalsgaard *et al.*, 2003). At higher trophic levels, markers become obscured as FA originate from a variety of dietary sources. However, research has focused on applying fatty acid signature analysis (FASA) to elucidate the dietary source of lipid reserves in upper-trophic-level predators such as pinnipeds (Iverson, 1993) and cetaceans (Hooker *et al.*, 2001). FASA has been used to provide support to traditional diet analyses (e.g., stomach content and faecal analyses) in determining temporal, physiological and spatial scales of diet variation. Fatty acids have also been used to identify species and group interactions in food webs, thereby defining trophic exchanges (Bradshaw *et al.*, 2003; Dalsgaard *et al.*, 2003; Iverson *et al.*, 1997b; Walton *et al.*, 2000).

The use of FASA in constructing linkages requires that FA be deposited and mobilised in a predictable way with little modification throughout the chain of ingestion (Iverson, 1993). The temporal dynamics (i.e., turnover rate of individual FA) can be species-specific and are often linked to metabolic condition or reproductive status (i.e., lactation). Consequently, FA have been used mostly as qualitative food web or trophic markers (Dalsgaard *et al.*, 2003). To quantify

relationships using FA in marine mammals, specific aspects of FA dynamics, including time scales for incorporation of new FA and differential utilisation are required. Although quantitative methods have been developed recently (Iverson *et al.*, 2004), an improved understanding of FA turnover and deposition is an essential precursor to their application in quantified FASA studies.

Nursing phocid mother-pup pairs offer a good opportunity to study FA mobilisation, use and deposition given that the extent of lipid depletion is a key factor in the selectivity of FA mobilisation (Raclot & Groscolas, 1995). We examined the blubber FA composition of adult female Weddell seals at the beginning and end of lactation in relation to the FA composition of their milk to determine the extent to which FA were used to fuel maternal energy requirements. Our approach also permitted the identification of FA that were selectively mobilised and transferred to the pup. We compared this to the FA composition of the pups at weaning to determine the proportion of FA that were used for growth and maintenance and stored in the pup's blubber. Our specific aims were to determine (1) if particular FA were selectively mobilised and/or transferred during lactation, (2) if mobilisation was influenced by initial FA composition, and (3) if particular FA were selectively deposited or used.

5.2 Methods

5.2.1 Data collection

This study was done at Hutton Cliffs, Antarctica (77° 51' S, 166° 45' E) during the austral summers (October to December) of 2002 and 2003. Thirty mother-pup pairs in 2002 and twenty-five pairs in 2003 were captured 1 to 6 ($\bar{x}$ ± SEM = 3.8 ± 0.22) days post-partum. Individual females were identified by flipper tags attached in previous years and pups were marked with hind flipper tags soon after birth as part of a long-term tagging study (Hadley *et al.*, 2006). Once captured, each female was immobilised with Telazol® (Wheatley *et al.*, 2006b), weighed to the nearest 1 kg, and body length and axial girth measurements recorded. Each pup was weighed to the nearest 0.5 kg and length and girth measured.

In 2003, milk was collected using a modified 50 ml syringe following an intravenous injection of oxytocin (1 ml, 10 IU·ml^{-1}). In both years, blubber biopsies were taken at post-partum (PP) and end-lactation (EL) captures (5 – 6 weeks later) for females and at the EL capture for pups. First, a small area on the posterior flank of each animal was shaved and disinfected. A small (~ 1 cm) incision was made with a scalpel blade in an anterior–posterior direction, a 6-mm biopsy punch was inserted through the incision, and a core was taken from the whole blubber layer (i.e., through until the muscle layer was reached; Bradshaw *et al.*, 2003). Each sample was stored in a pre-weighed glass vial (with a Teflon coated lid), containing a solution of 2:1 v/v chloroform and methanol, and 0.05 % (by weight) butylated hydroxytoluene (BHT; Sigma, St. Louis, USA). Vials were reweighed and all samples were stored at -20 ˚C until laboratory analysis. Data were collected under permits from the University of Tasmania Animal Ethics Committee (A6790 & A6711) and the Department of Conservation of New Zealand (Per/22/2002/149 & Per/17/2003/188).

5.2.2 Laboratory and data analyses

Body composition, water flux rates and milk intake were determined using hydrogen isotope dilution (Chapter 4; Wheatley *et al.*, 2006a). Blubber and milk lipids were quantitatively extracted using a modified overnight Bligh and Dyer (1959) one-phase methanol/chloroform/water extraction (2:1:0.8, v/v/v). Following extraction, chloroform and water (0.9 % NaCl) were added to make a biphasic system (final solvent ratio, 1:1:0.9, v/v/v, methanol/chloroform/water). Total lipid was concentrated from the lower chloroform phase by rotary evaporation at 40 ˚C. A subsample of lipid was trans-methylated to produce fatty acid methyl esters (FAME) using a methanol/chloroform/hydrochloric acid reagent (10:1:1, v/v/v; 80 ˚C; 2 h). After the addition of water, FAME were extracted into hexane/dichloromethane (4:1, v/v, 3 x 1.5 ml). Gas chromatographic (GC) analyses were done with an Agilent 6890N GC (Avondale, Pennsylvania, USA) equipped with a HP-5 cross-linked methyl silicone-fused silica capillary column (50 m x 0.32 mm i.d.), a flame ionisation detector, a split/splitless injector, and an Agilent 7683 auto-sampler. Helium was the carrier gas. Samples were injected in splitless mode at an oven temperature of 50 ˚C. After 1 min, the oven temperature was raised to 150 ˚C at 30 ˚C min^{-1}, then to 250 ˚C at 2 ˚C min^{-1},

and finally to 300 ˚C at 5 ˚C min^{-1}. FA peaks were quantified by Agilent Technologies GC ChemStation software (Palo Alto, California, USA). Individual components were identified by mass spectral data and by comparing retention time data with those obtained for authentic and laboratory standards. GC results are typically subject to an error of ± 5 % of individual component area. GC-mass spectrometric (GC-MS) analyses were performed on representative samples on a Finnigan Thermoquest GCQ GC-mass spectrometer fitted with an on-column injector with Thermoquest Xcalibur software (Austin, Texas, USA). The GC was fitted with a capillary column similar to that described above.

The concentration of individual FA were converted to a mass per cent of total FA, and FA present in trace amounts (< 0.5 %) were excluded from analyses. FA expressed as a percentage of mass composition did not accurately reflect the changes in FA over lactation because the overall lipid content of the blubber and milk changed over lactation. Therefore, proportional FA were converted to absolute (mg/g) values to compare changes over time. To do this, representative samples (n = 24) were analysed for lipid class composition by Iatroscan MK V TH10 thin layer chromatography-flame ionisation detection (Phillips *et al.*, 2002). Results indicated that extracted blubber and milk samples were composed virtually entirely of TAG (99.9 %). TAG stored in the tissue consists of glycerol esterified with three FA molecules, and the FA moieties represent about 95 % of the mass of TAG (Groscolas, 1990). Therefore, the mass (kg) of lipid in each animal, as determined from hydrogen isotope dilution techniques (see Wheatley *et al.*, 2006a), was multiplied by 95 % to obtain the mass (kg) of FA in each individual, at each capture. All proportional values were arcsine-square-root transformed before analysis.

From evidence that females do not feed during the first 3 weeks of lactation (Eisert *et al.*, 2005), we calculated a 'feeding index' for the second half of lactation based on differences in milk energy output values, which represented the number of times that milk energy (%) exceeded that of non-feeding individuals. This allowed us to rank the relative amount of feeding that occurred among individuals (Chapter 4) and these values were used as a covariate in some analyses.

Principal Components Analysis (PCA) was used on proportional (% of total FA) and absolute (mg/g) values to investigate patterns of FA in blubber, milk and over time. Principal component scores were used in a series of generalised linear and mixed-effects models (GLM, GLMM) to examine differences in FA composition and

mobilisation. Examination of the residuals for all models determined the statistical error distribution (Gaussian) and link function (identity). Model selection was based on Akaike's Information Criteria corrected for small samples (AIC_c, Burnham & Anderson, 2002). Models were ranked according to relative AIC_c weights ($wAIC_c$). Specific model comparisons were based on the information-theoretic evidence ratio (*ER*) which is equivalent to the AIC_c weight (w) of one model divided by the w of the null (or other) model (Burnham & Anderson, 2002). Model goodness-of-fit was assessed by calculating the per cent deviance explained (%DE). The FA most responsible for the multivariate patterns were identified in SIMPER (similarity percentages) analysis (Clarke, 1993). The SIMPER procedure compares the average abundances and examines the contribution of each FA to the average Bray-Curtis dissimilarity between two defined groups of samples (e.g., blubber or milk). All statistical analyses were done using PRIMER (Ver. 5.2.9) or the *R* Package (Ver. 2.4.1, R Development Core Team, 2004). Values are presented as mean ± one standard error (SEM) unless otherwise stated.

5.3 Results

Twenty-four FA (comprising 94 – 98 % of total FA) were found in greater-than-trace amounts (> 0.5 %; see Table 5.1a & 5.1b for absolute values). Short-chain monounsaturated fatty acids (≤ 18 carbons; SC-MUFA) dominated both the blubber and milk, with saturated fatty acids (SFA) the next highest in proportion, followed by PUFA and long-chain monounsaturated fatty acids (> 18 carbons; LC-MUFA; see Appendix I for a detailed description of fatty acid groups). There was a strong positive linear relationship between FA loss (kg) during lactation and total body lipid (TBL, kg) at post-parturition (TBL; $ER = 2.28 \times 10^3$, %DE = 88.5 %), but no evidence for a relationship with maternal post-partum mass (MPPM; *ER* = 1.23, %DE = 25.7 %). Therefore, TBL was used as a covariate in our analyses.

Table 5.1a *Absolute fatty acid composition (kg) of female and pup blubber in 2002.*

FA	2002					
	Female blubber				Pup blubber	
	PP	N = 18	EL	N = 25	EL	N = 26
	Mean	SEM	Mean	SEM	Mean	SEM
14:1ω5c	3.0	0.17	1.7	0.10	0.5	0.02
14:0	16.1	0.36	8.3	0.37	3.9	0.13
i15:0	0.7	0.02	0.4	0.02	0.1	0.00
16:1ω9c	0.6	0.02	0.3	0.01	0.2	0.01
16:1ω7c	23.2	0.84	10.7	0.54	6.5	0.25
16:1ω5c	0.7	0.02	0.3	0.01	0.2	0.01
16:0	14.8	0.33	6.6	0.32	4.8	0.15
i17:0	0.3	0.01	0.2	0.01	0.1	0.00
18:4ω3	1.7	0.04	0.8	0.03	0.4	0.01
18:2ω6	3.0	0.10	1.7	0.07	0.8	0.03
18:1ω9c	52.7	1.81	28.5	1.23	14.8	0.55
18:1ω7c	11.7	0.36	6.2	0.25	3.1	0.10
18:1ω5	0.9	0.03	0.5	0.02	0.2	0.01
18:0	1.7	0.06	0.9	0.04	0.5	0.02
20:4ω6	0.7	0.02	0.3	0.02	0.2	0.01
20:5ω3 EPA	5.8	0.15	2.1	0.12	1.5	0.05
20:4ω3	0.4	0.02	0.2	0.01	0.1	0.01
20:2ω6	1.0	0.05	0.7	0.03	0.1	0.01
20:1ω9c	8.0	0.30	5.2	0.25	1.3	0.06
20:1ω7c	0.8	0.03	0.5	0.02	0.1	0.01
22:6ω3 DHA	7.5	0.26	4.5	0.18	1.6	0.06
22:5ω3 DPA	2.0	0.10	1.4	0.07	0.5	0.02
22:1ω11c*	1.3	0.06	0.8	0.05	0.1	0.00
22:1ω9c	1.1	0.04	0.7	0.04	0.1	0.01
24:1	0.3	0.01	0.2	0.01	0.0	0.00

* includes 22:1ω13c

Table 5.1b *Absolute fatty acid composition (kg) female blubber, milk and pup blubber in 2003.*

FA	2003									
	Female blubber				Pup blubber		Milk			
	PP	N = 20	EL	N = 10	EL	N = 22	PP-ML	N = 8	ML-EL	N = 8
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
14:1ω5c	2.4	0.18	1.3	0.12	0.4	0.03	0.2	0.02	0.4	0.04
14:0	11.9	0.86	5.8	0.41	2.6	0.21	2.5	0.27	3.5	0.42
i15:0	0.5	0.03	0.3	0.02	0.1	0.01	0.1	0.01	0.1	0.02
16:1ω9c	0.5	0.02	0.3	0.02	0.2	0.01	0.2	0.01	0.2	0.02
16:1ω7c	19.2	0.75	8.2	0.49	4.7	0.31	3.3	0.31	4.6	0.51
16:1ω5c	0.5	0.02	0.2	0.01	0.1	0.01	0.1	0.01	0.2	0.02
16:0	11.5	0.63	4.9	0.32	3.5	0.25	3.9	0.39	4.7	0.55
i17:0	0.3	0.02	0.2	0.01	0.1	0.01	0.1	0.00	0.1	0.01
18:4ω3	1.4	0.06	0.6	0.04	0.3	0.02	0.2	0.02	0.3	0.04
18:2ω6	2.6	0.11	1.5	0.09	0.7	0.04	0.5	0.05	0.8	0.08
18:1ω9c	44.7	1.73	23.8	1.47	11.8	0.68	9.0	0.76	13.7	1.44
18:1ω7c	10.3	0.41	5.2	0.34	2.5	0.16	2.1	0.16	3.1	0.32
18:1ω5	0.8	0.03	0.4	0.03	0.2	0.01	0.2	0.01	0.2	0.03
18:0	1.4	0.06	0.8	0.05	0.4	0.03	0.5	0.04	0.8	0.08
20:4ω6	0.6	0.02	0.3	0.02	0.2	0.01	0.1	0.01	0.2	0.02
20:5ω3 EPA	4.9	0.25	1.6	0.13	1.0	0.08	1.1	0.11	1.2	0.15
20:4ω3	0.5	0.05	0.2	0.04	0.1	0.01	0.1	0.01	0.1	0.02
20:2ω6	0.9	0.05	0.6	0.05	0.2	0.01	0.1	0.02	0.2	0.02
20:1ω9c	6.4	0.32	4.4	0.36	1.1	0.07	0.8	0.07	1.5	0.17
20:1ω7c	0.7	0.03	0.5	0.03	0.1	0.01	0.1	0.01	0.2	0.02
22:6ω3 DHA	6.8	0.34	3.9	0.25	1.3	0.09	1.1	0.08	1.9	0.23
22:5ω3 DPA	2.2	0.22	1.4	0.20	0.5	0.05	0.4	0.04	0.6	0.10
22:1ω11c*	0.9	0.08	0.6	0.06	0.0	0.00	0.1	0.01	0.1	0.02
22:1ω9c	0.8	0.06	0.6	0.06	0.1	0.01	0.1	0.01	0.2	0.03
24:1	0.2	0.02	0.2	0.02	0.0	0.00	0.0	0.00	0.1	0.01

* includes 22:1ω13c

5.3.1 Female blubber fatty acids

There was little evidence for any difference between years in the proportional FA in female blubber samples at post-partum according to PCA (Fig. 5.1a). The terms *age* and *TBL* were the only important correlates of FA composition (GLM $wAIC_c$ = 0.596), although the %DE by this model was relatively low (%DE = 14.6). There was evidence for a difference between years in proportional FA at end-lactation along the PC2 axis (Fig. 5.1b). The GLM with the highest weight ($wAIC_c$ = 0.986) included all terms (*year*, *age* and *TBL*) and explained a relatively high proportion of the deviance in the first principal component for FA composition at end-lactation (%DE = 57.5 %). The top five FA that contributed to 38.9 % of the dissimilarity between years were SFA 14:0 and 16:0 (2002 > 2003), and MUFA 16:1ω7c (2002 > 2003), 18:1ω9c and 20:1ω9c (2003 > 2002). In absolute terms of FA mobilised, 73.5 % of the difference between years resulted in more SFA 14:0 and 16:0, and MUFA 18:1ω9c, 16:1ω7c and 18:1ω7c being mobilised in 2002 than in 2003.

The most parsimonious model testing for the effect of *TBL, year* and *feeding* index on fractional mobilisation of FA (i.e., the fraction of initial mass of the FA that was lost from the blubber during lactation) included both *TBL* and *feeding* ($wAIC_c$ = 0.533, %DE = 38.5 %). There was a strong negative linear relationship between feeding and fractional mobilisation ($ER = 1.49 \times 10^4$, %DE = 61.7 %). In all but three females, the EFA 20:5ω3 had the highest fractional mobilisation from the blubber (range: 36.9 – 81.6 %).

5.3.2 Milk fatty acids

A plot of the first two principal components divided the milk samples into three distinct groups (PP, ML, EL; Fig. 5.2). The first component (PC1) accounted for 40.8 % of the variation in FA composition among samples, while the second component (PC2) accounted for 26.5 %. The GLMM used to examine the influence of stage of lactation only on PC1 scores revealed that *stage* explained 87.6 % of the variation in samples. Between post-partum and mid-lactation there was an increase in 20:1ω9c, 22:6ω3 and 22:1ω9c and a decrease in 20:5ω3 and 16:0. Between mid-lactation and end-lactation, 18:1ω9c and 22:5ω3 increased while 16:0, 14:0 and

20:5ω3 decreased. Overall, there was a gradual decrease in SFA, a slight decrease in PUFA between post-partum and mid-lactation, and an increase in SC-MUFA and LC-MUFA.

The total mass of FA (kg) lost by some females during lactation in 2003 differed from the FA mass transferred in milk (Table 5.2a,b,c). As some of the shorter chain FA can be synthesised *de novo*, these differences were clear in the PUFA which for mammals can only be acquired through

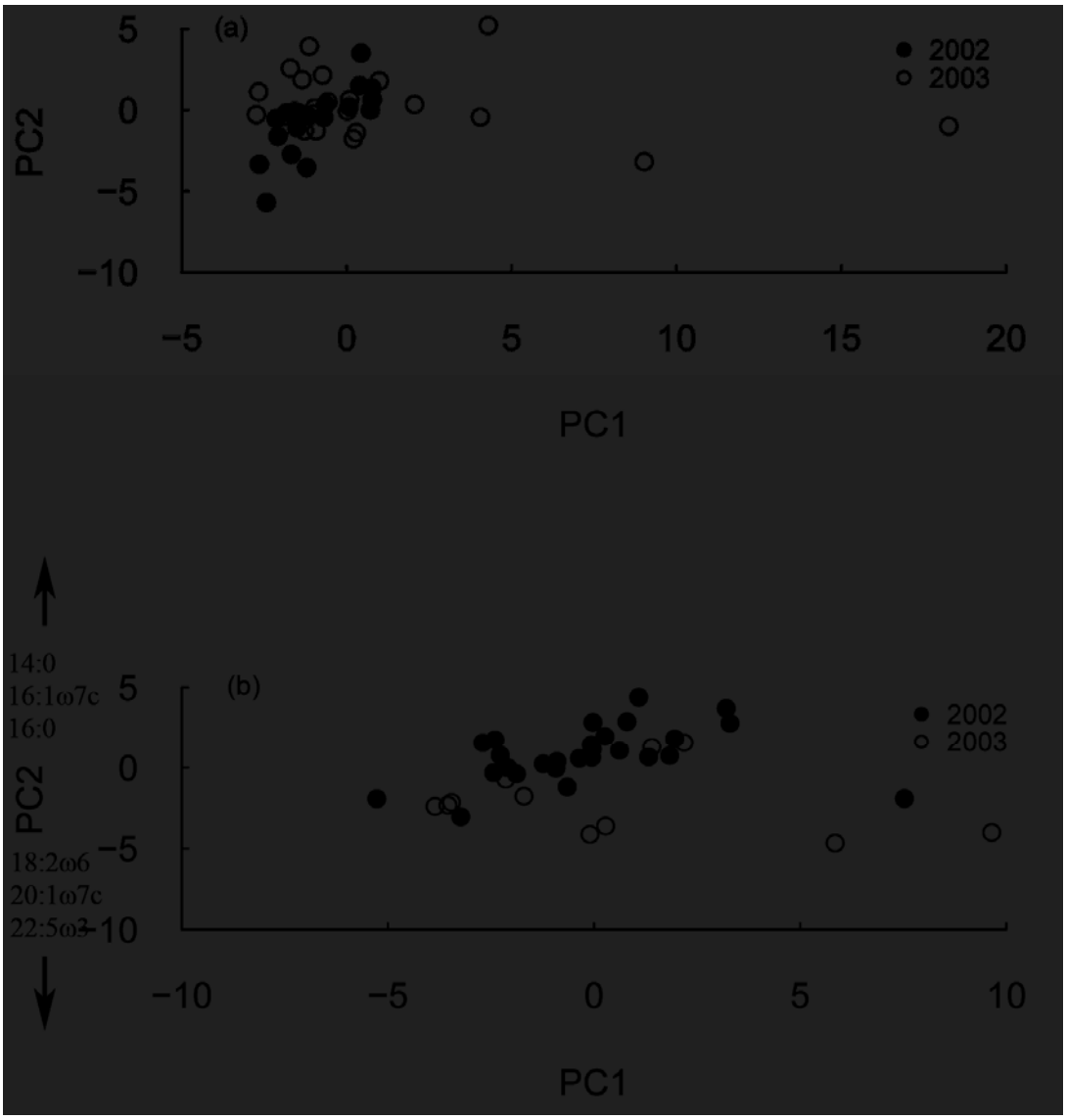

Figure 5.1 *Principal component analysis of female blubber fatty acids at (a) post-partum and (b) end-lactation in 2002 and 2003. The main determinants of the second principal component (PC2; eigen values) for end-lactation are shown along the axis in figure (b).*

dietary intake. Female Pu517 was a relatively large animal (430 kg), but had the lowest proportional total body lipid of the sampled females (25.7 %). The discrepancy between FA loss and transfer was the highest for Pu517, indicating that she appeared to feed more than any other female during lactation. This was further supported by data describing milk energy transfer to her pup, where milk energy output exceeded that of total energy lost by the female (Chapter 4). Using this disparity in FA mass loss and transfer as an index of feeding activity, some other females (50 %) also showed signs of feeding, while others showed virtually none (Table 5.2c).

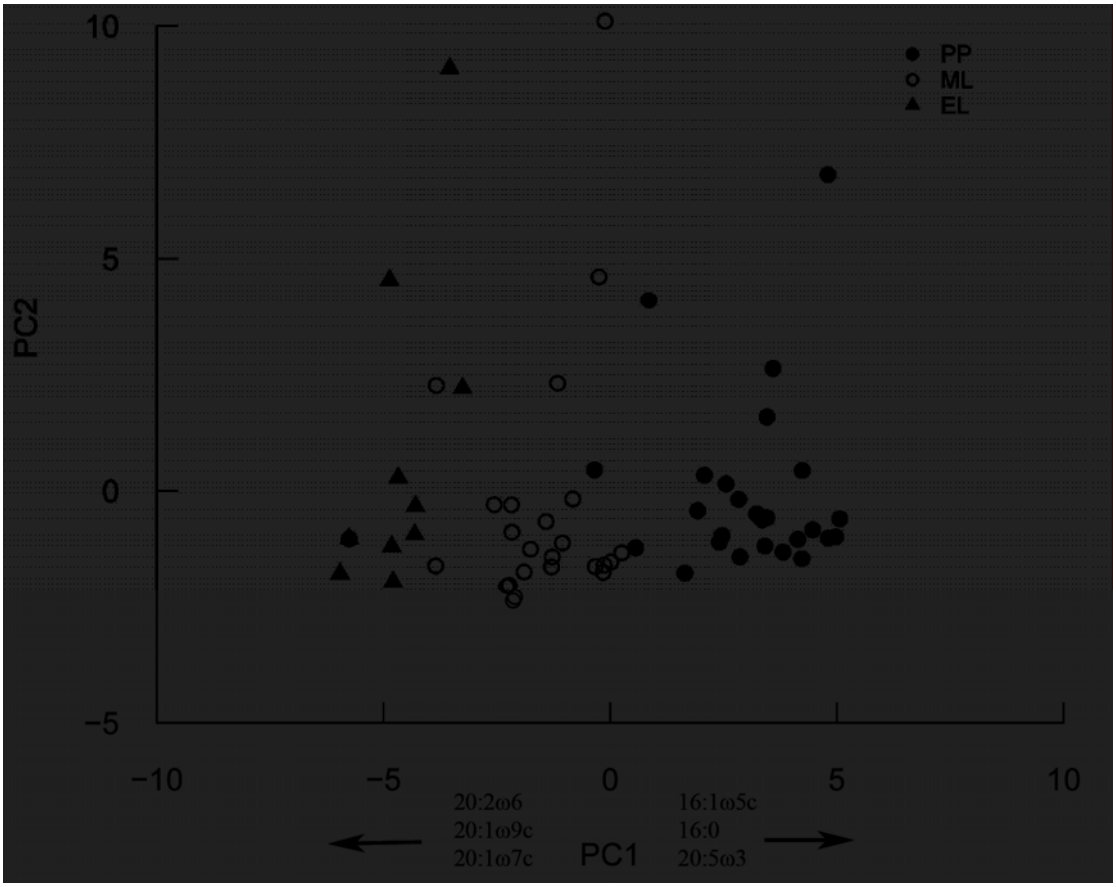

Figure 5.2 *Principal component analysis of milk fatty acids at post-partum (PP), mid-lactation (ML) and end-lactation (EL) in 2003. The three fatty acids with the most extreme positive and negative loadings (eigen values) for the first principal component (PC1) are shown along the axis.*

Table 5.2a *Total fatty acid loss (kg) during lactation for eight females.*

FA	Total FA lost (kg)							
	P130	P871	Pu114	Pu517	Pu761	W636	Y536	Y965
14:1ω5c	1.2	2.0	1.7	0.4	1.7	1.1	0.4	1.4
14:0	6.1	8.2	10.0	2.3	9.5	8.9	3.7	7.4
i15:0	0.2	0.3	0.3	0.1	0.3	0.3	0.1	0.3
16:1ω9c	0.2	0.2	0.3	0.1	0.3	0.3	0.2	0.3
16:1ω7c	8.2	11.8	11.9	5.2	17.0	15.1	8.6	13.5
16:1ω5c	0.2	0.3	0.3	0.2	0.5	0.4	0.3	0.4
16:0	4.9	6.4	7.7	2.7	10.3	10.4	5.3	8.9
i17:0	0.1	0.1	0.2	0.1	0.2	0.2	0.2	0.2
18:4ω3	0.5	0.7	0.8	0.3	1.1	1.1	0.7	1.0
18:2ω6	0.6	1.0	1.3	0.4	1.5	1.7	0.8	1.6
18:1ω9c	12.4	19.4	25.3	10.3	29.2	31.6	14.4	27.5
18:1ω7c	2.9	4.4	5.9	2.6	6.8	7.3	4.7	6.6
18:1ω5	0.3	0.4	0.4	0.2	0.5	0.6	0.3	0.5
18:0	0.4	0.5	0.7	0.3	0.8	0.9	0.6	0.9
20:4ω6	0.2	0.2	0.3	0.2	0.4	0.4	0.3	0.4
20:5ω3 EPA	2.4	2.7	3.0	1.5	5.6	4.2	3.1	4.3
20:4ω3	0.2	0.1	0.2	0.2	0.3	0.3	0.3	0.3
20:2ω6	0.1	0.1	0.5	0.1	0.3	0.3	0.3	0.4
20:1ω9c	0.8	1.5	3.7	0.5	2.1	3.8	1.7	3.3
20:1ω7c	0.1	0.1	0.4	0.1	0.2	0.4	0.2	0.4
22:6ω3 DHA	1.8	2.4	3.4	1.4	3.5	3.6	2.4	4.0
22:5ω3 DPA	0.4	0.5	1.0	0.4	0.7	0.9	1.1	1.1
22:1ω11c*	0.1	0.2	0.6	0.0	0.3	0.6	0.2	0.4
22:1ω9c	-0.1	0.0	0.4	0.0	0.2	0.6	0.1	0.3
24:1	0.0	0.0	0.1	0.0	0.0	0.1	-0.1	0.1
SUM	44.1	63.7	80.3	29.5	93.4	95.0	50.2	85.5

* includes 22:1ω13c

Table 5.2b *Total fatty acids in milk (kg) during lactation for eight females.*

FA	FA in Milk (kg)							
	P130	P871	Pu114	Pu517	Pu761	W636	Y536	Y965
14:1ω5c	0.4	0.6	0.6	0.6	0.5	0.5	0.3	0.5
14:0	4.0	6.2	5.6	3.8	5.7	6.0	3.1	5.8
i15:0	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.2
16:1ω9c	0.3	0.3	0.3	0.3	0.4	0.3	0.2	0.3
16:1ω7c	5.4	7.9	7.2	5.6	7.5	7.5	4.7	6.9
16:1ω5c	0.2	0.3	0.2	0.2	0.3	0.2	0.2	0.2
16:0	5.8	8.6	7.6	4.9	8.2	8.4	5.2	7.9
i17:0	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1
18:4ω3	0.4	0.5	0.5	0.4	0.6	0.5	0.4	0.5
18:2ω6	0.9	1.2	1.1	1.1	1.4	1.2	0.7	1.2
18:1ω9c	15.8	21.8	19.3	18.7	22.2	21.0	12.2	19.5
18:1ω7c	3.3	4.8	4.5	4.0	5.8	4.7	3.5	4.4
18:1ω5	0.3	0.4	0.3	0.3	0.4	0.4	0.3	0.3
18:0	0.9	1.2	1.0	1.0	1.5	1.2	1.0	1.1
20:4ω6	0.2	0.3	0.3	0.3	0.5	0.3	0.3	0.3
20:5ω3 EPA	1.6	2.0	2.0	1.6	2.8	2.1	1.6	2.2
20:4ω3	0.1	0.2	0.2	0.2	0.3	0.2	0.2	0.1
20:2ω6	0.3	0.4	0.3	0.3	0.4	0.3	0.2	0.3
20:1ω9c	1.7	2.3	1.9	1.6	2.3	2.3	1.3	2.0
20:1ω7c	0.2	0.3	0.2	0.2	0.3	0.3	0.2	0.2
22:6ω3 DHA	2.1	2.4	2.1	2.8	3.9	2.5	1.9	2.4
22:5ω3 DPA	0.6	0.7	0.6	1.1	1.5	0.6	0.8	0.6
22:1ω11c*	0.2	0.2	0.2	0.1	0.2	0.3	0.1	0.2
22:1ω9c	0.3	0.4	0.2	0.2	0.2	0.4	0.2	0.3
24:1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
SUM	45.1	63.4	56.4	49.7	67.6	61.7	38.8	57.7

* includes 22:1ω13c

Table 5.2c *The difference in fatty acids (kg) lost from the female blubber and those present in the milk. Negative values indicate that there was more FA (kg) in the milk than was lost from the blubber, indicating a possibility of feeding.*

FA	Difference (kg)							
	P130	P871	Pu114	Pu517	Pu761	W636	Y536	Y965
14:1ω5c	0.8	1.4	1.1	-0.2	1.2	0.6	0.2	0.9
14:0	2.1	2.0	4.3	-1.5	3.8	2.8	0.6	1.6
i15:0	0.0	0.0	0.1	-0.1	0.1	0.1	0.0	0.1
16:1ω9c	-0.1	-0.1	0.0	-0.2	-0.1	0.0	0.0	0.0
16:1ω7c	2.9	3.9	4.7	-0.3	9.5	7.6	3.9	6.7
16:1ω5c	0.1	0.1	0.1	0.0	0.2	0.2	0.1	0.2
16:0	-0.9	-2.1	0.1	-2.2	2.1	2.0	0.1	0.9
i17:0	0.0	0.0	0.1	-0.1	0.0	0.1	0.1	0.1
18:4ω3	0.1	0.1	0.4	-0.1	0.5	0.5	0.3	0.5
18:2ω6	-0.3	-0.3	0.2	-0.6	0.2	0.5	0.1	0.4
18:1ω9c	-3.4	-2.4	6.0	-8.3	6.9	10.6	2.2	8.1
18:1ω7c	-0.5	-0.4	1.4	-1.4	1.1	2.6	1.2	2.2
18:1ω5	0.0	0.0	0.1	-0.1	0.1	0.2	0.1	0.2
18:0	-0.5	-0.7	-0.2	-0.7	-0.7	-0.3	-0.3	-0.2
20:4ω6	0.0	-0.1	0.0	-0.1	-0.1	0.1	0.0	0.1
20:5ω3 EPA	0.9	0.7	1.0	-0.1	2.7	2.1	1.5	2.1
20:4ω3	0.0	0.0	0.0	-0.1	-0.1	0.1	0.1	0.1
20:2ω6	-0.2	-0.2	0.1	-0.2	-0.1	-0.1	0.1	0.1
20:1ω9c	-0.9	-0.8	1.8	-1.1	-0.2	1.5	0.4	1.3
20:1ω7c	-0.1	-0.1	0.1	-0.2	-0.1	0.1	0.0	0.1
22:6ω3 DHA	-0.3	-0.1	1.3	-1.4	-0.3	1.2	0.6	1.6
22:5ω3 DPA	-0.2	-0.1	0.4	-0.8	-0.8	0.2	0.3	0.4
22:1ω11c*	0.0	0.0	0.4	-0.1	0.1	0.4	0.1	0.2
22:1ω9c	-0.3	-0.4	0.2	-0.2	0.0	0.1	-0.1	0.1
24:1	-0.1	-0.1	0.0	0.0	-0.1	0.0	-0.1	0.0
SUM	-1.0	0.3	23.9	-20.2	25.8	33.3	11.4	27.7

* includes 22:1ω13c

5.3.3 Pup blubber fatty acids

There was evidence for a difference between years in the FA composition of pup blubber at end-lactation according to the PCA (Fig. 5.3a). This was supported by the top-ranked GLM including both *year* and *TBL* terms ($wAIC_c$ = 0.739, %DE = 34.4 %) from original models of PC1 versus *year*, *TBL* and *sex*. Forty-three per cent of the difference between years resulted from a dissimilarity in SFA 14:0 and 16:0 (2002 > 2003), PUFA 20:5ω3 (2002 > 2003) and MUFA 16:1ω7c (2002 > 2003) and 18:1ω9c (2003 > 2002). The FA composition of the pup blubber was different to that of the milk at all stages of lactation (PP, ML and EL), but did appear to fall somewhere between post-partum and mid-lactation composition (PCA; Fig. 5.3b). There was also a clear separation between the maternal blubber (PP) and the pup's blubber (PCA; Fig. 5.4). Of the FA received in the milk, 55.9 ± 1.03 % (range: 48.6 – 61.9 %) on average were stored in the pup's blubber (Fig. 5.5). Although LC-MUFA appeared to be utilised more by the pup (i.e., less stored), there was no evidence that FA group affected storage rate (GLMM, %DE = 4.3 %).

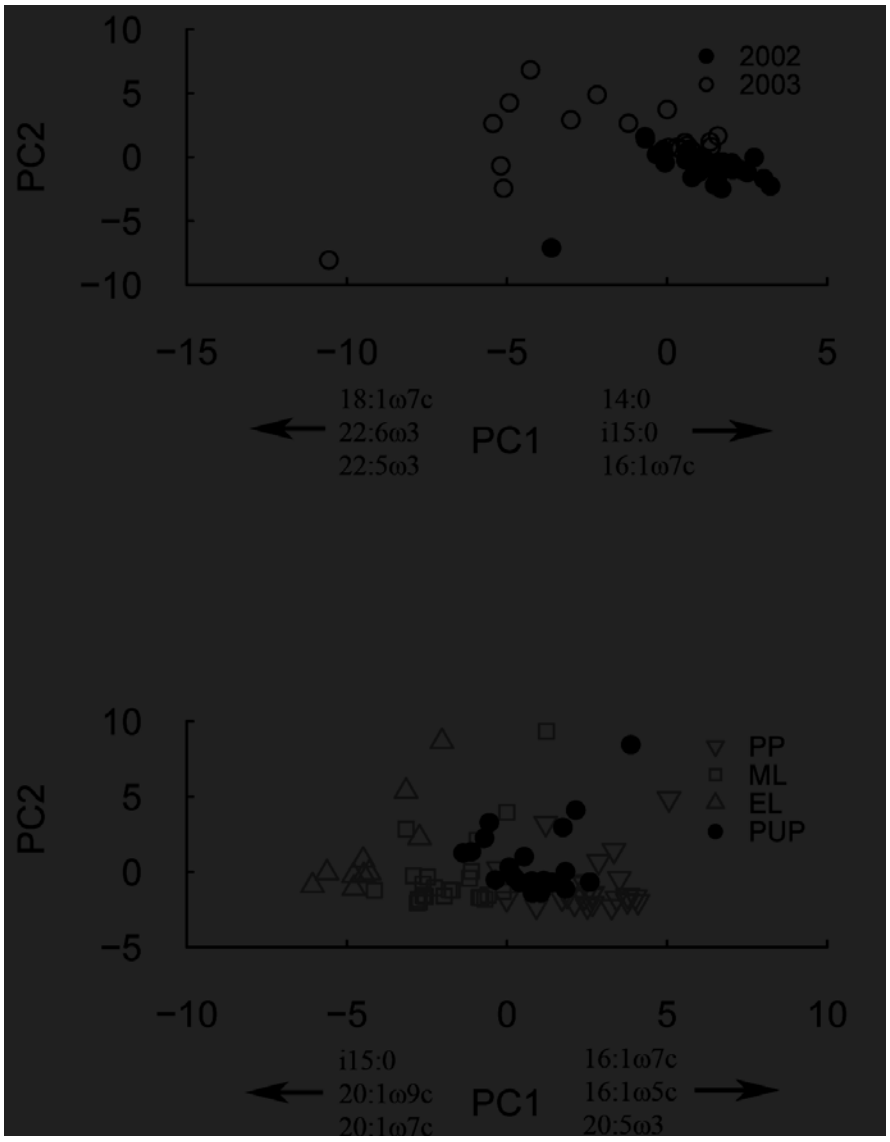

Figure 5.3 *Principal component plot of (a) fatty acid composition for pup blubber at end-lactation in 2002 and 2003 and (b) fatty acid composition of pups in 2003 compared to milk FA in 2003. The three FA with the most extreme positive and negative eigen values for the first principal component (PC1) are shown on each axis.*

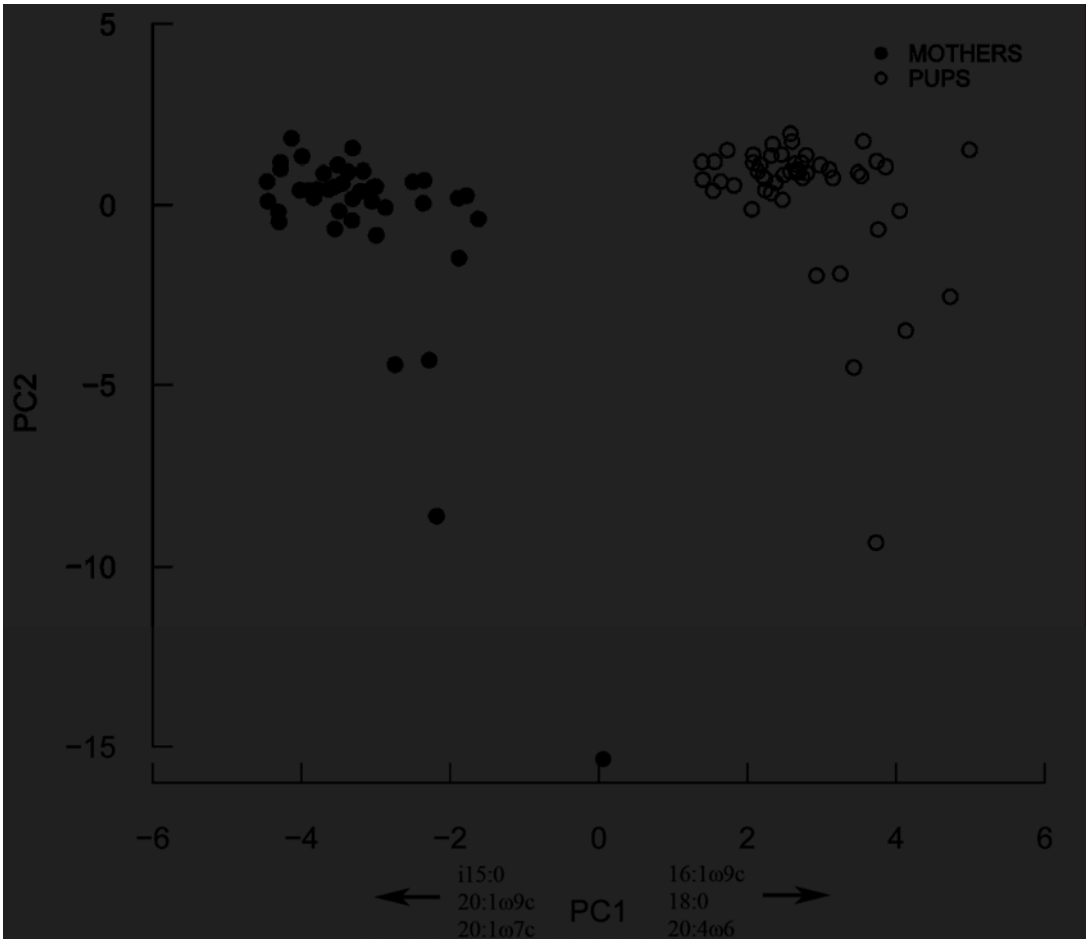

Figure 5.4 *Principal component analysis of female blubber at post-parturition and pup blubber at end-lactation.*

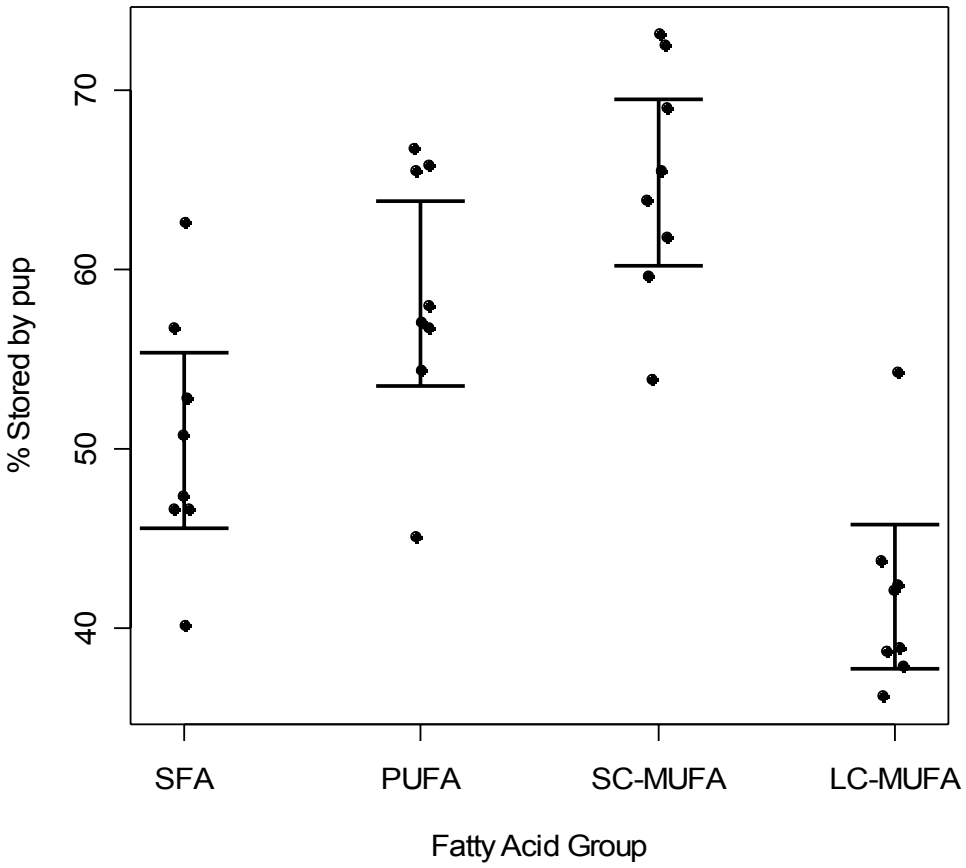

Figure 5.5 *Per cent fatty acid storage by pups in 2003 (± SEM). This was determined by dividing the amount of FA (kg) in the pup blubber by the amount of FA (kg) received in the milk, then averaging this for each FA group, for each pup. Groups are: SFA, saturated fatty acids; PUFA, polyunsaturated fatty acids; SC-MUFA, short-chain (≤ C18) monounsaturated fatty acids; LC-MUFA, long-chain (> C18 monounsaturated fatty acids).*

5.4 Discussion

In situations of negative energy balance such as fasting, mobilisation of adipose tissue is enhanced to provide fatty acids as metabolic fuel (Raclot, 2003). During lactation, maternal FA are mobilised to support maintenance metabolism and milk production, and females may differentially mobilize or modify FA to suit their particular physiological needs. For capital breeders, the diet composition prior to parturition will influence FA dynamics, so understanding the influence of diet and FA mobilisation on milk production and transfer is important for interpreting the foraging ecology, trophic dynamics and life history strategies of mammals (Iverson, 1993). We

found no evidence for a difference between the post-partum blubber FA composition of females in 2002 and 2003, suggesting that, overall, the diet of study females did not differ substantially between those years. However by the end of lactation, the FA composition of female blubber was notably different between years. In addition, the FA composition of the pup blubber (at EL) was also different, suggesting that there were differences in milk FA transfer. Unfortunately, we could not examine variation in milk FA transfer between years because milk was only collected during the second season (2003). However, we did determine that more SFA (14:0 and 16:0) and SC-MUFA (16:1ω7c, 18:1ω9c and 18:1ω7c) were mobilised from the female blubber in 2002, which corresponded to more 14:0, 16:0 and 16:1ω7c in the pup blubber of that year. Differences in FA mobilisation appear to be related to the dissimilarity in overall condition (TBL kg) of females between years. Females in 2002 had higher TBL stores than those in 2003. This not only affected FA mobilisation, but also lactation length, maternal expenditure and pup mass gain – larger females had higher transfer efficiency rates and weaned larger pups (Wheatley *et al.*, 2006a).

Most studies of milk FA transfer in marine mammals have collected one sample or have averaged values over the course of lactation. Our results demonstrate that this approach may not give a representative description of FA mobilisation and transfer. We found that milk FA differed markedly between each stage of lactation (post-partum, mid-lactation and end-lactation), with the proportions of each FA group either decreasing or increasing over lactation. These changes in proportions may be related to the individual properties and/or use of each FA. For example, SFA may have been higher in milk at PP because they store more chemical energy useful to blubber-poor neonates (Wheatley *et al.*, 2006a). Increasing the amounts of SFA delivered via milk early in the lactation period may therefore maximise the catalyzable energy the pup receives. Later in lactation, the proportion of MUFA increased in milk because the latter offer optimal characteristics for energy storage by providing higher energy density than PUFA, and higher mobilisation and oxidation rates than SFA (Maillet & Weber, 2006). There was also evidence of selective mobilisation of particular FA during lactation.

Of all fatty acids, the essential FA 20:5ω3 had the highest fractional mobilisation from the blubber during lactation. The proportion of 20:5ω3 was also the highest in the milk immediately post-parturition. Both 20:5ω3 and 22:6ω3 are associated with phospholipids of biomembranes and hormone precursors, and thus are

involved in many physiological processes including neurological function (Innis, 2005). Higher proportions of 20:5ω3 delivered immediately post-parturition may be required for early development the offspring's neurological and other functions. As 20:5ω3 decreased in milk, 22:6ω3 increased, further demonstrating selective mobilisation that was most likely related to the physiological requirements of the developing pup. Although the pre-partum diet did not differ substantially between years, this differential mobilisation of FA occurred. Thus, it appears likely that diet may also play an important role with selectivity of FA mobilisation at least during lactation.

There was evidence that some females fed during lactation as indicated by the presence of higher quantities of PUFA in the milk than were mobilised from the blubber. In addition, less fractional mobilisation of blubber FA occurred in response to higher feeding rates. Of all the females that appeared to feed, all but one (P130) had a higher MPPM than the average for that year (393 kg, Wheatley *et al.*, 2006a). This is consistent with the finding that larger females tended to feed more during lactation (Chapter 4). Female P130 also had a longer lactation and lost a higher proportion of her body mass than the average (see Wheatley *et al.*, 2006a) indicating that although she was feeding, it did not seem to facilitate higher energy delivery to her pup.

Feeding in Weddell seals appears to occur late in lactation (Eisert *et al.*, 2005), so we believe that our milk FA values between post-partum and mid-lactation represent those of non-feeding individuals. Although feeding generally occurred later, it was not apparent for all females, and there were no apparent outliers in milk fatty acid profiles at any stage of lactation (Fig. 5.2). This suggests that food intake was either insufficient to change overall milk FA composition and fuelled the females' energy requirements, or that it was from a similar source to that of the blubber. Therefore, we conclude that changes in the FA composition represented differential mobilisation of fat reserves, a mechanism which likely evolved in response to variation in the specific nutrient demands of the pup over the course of its development.

Of the FA received from the milk, an average of 55.9 % was stored in the pup's blubber, while the rest was used for the pup's growth and maintenance. Overall, the pup's blubber FA composition did not match that of the milk at any stage of lactation or that of the female's blubber at post-partum. Although some feeding has been observed by pups from the breeding colony (K. E. Wheatley, pers. observation),

post-weaning weights indicate that minimal feeding is occurring at that time (i.e., mass loss; K. E. Wheatley, unpubl. data). Therefore, it is likely that negligible feeding is occurring during lactation as well, and FA values would be representative of a single food source (i.e., milk). Given that milk was the only energy source for suckling pups, its FA composition was not well replicated in the blubber. This is most likely a reflection of the differential use of FA for growth and storage by the pup, as well as changes in milk FA that occurred during lactation. Furthermore, although some mothers likely fed during the latter stages of lactation, its influence on the mobilisation characteristics of FA during lactation was minimal. That the pup's blubber did not match the mother's further illustrates that unmodified FA transfer did not occur. It is unlikely that blubber would ever exactly match diet signatures, especially in an animal that is still growing. Therefore, using FASA as a biomarker of dietary intake should be used cautiously because individual FA are mobilised and stored with differential selectivity depending on the physiological state of the measured individual. Nevertheless, we have demonstrated that the analysis of FA can provide other insights into the functions of living systems besides the assessment of diet composition and trophodynamics.

Chapter 6

Temporal variation in the vertical stratification of blubber fatty acids alters diet predictions in lactating Weddell seals

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Abstract

Fatty acid signature analysis of blubber has been used to study the foraging ecology of some marine mammals. However, species-specific information on fatty acid (FA) deposition, distribution and mobilisation is required to develop further the application of FA as trophic markers within the marine environment. Blubber samples were collected from adult female Weddell seals post-parturition and end of lactation, and were divided into inner and outer half sections. We determined the degree to which there was vertical stratification in FA composition, and how this changed over the lactation period. Inner and outer layers of post-parturition blubber cores separated into two distinct groups. Sixty-two per cent of the dissimilarity between the two layers was accounted for by a higher abundance of monounsaturated fatty acids (18:1ω9c and 16:1ω7c) in the outer blubber layer, and more saturated fatty acids (16:0 and 14:0) in the inner layer. By end of lactation, the FA composition of the inner layer was different to post-parturition samples, and 20:5ω3 had the highest fractional mobilisation of all FA. In contrast, the proportion of FA in the outer layer did not change, and there was more variability in the fractional mobilisation of FA indicating mobilisation was not uniform across the blubber layer. Dietary predictions changed considerably when highly mobilised FA were removed from analyses, and predictions were more consistent with previous dietary studies. The lack of uniformity in FA mobilisation adds problems to the future use of FASA in dietary predictions, highlighting the need for more detailed information on FA mobilisation.

6.1 Introduction

Marine birds and mammals have been of increasing interest in ecosystem studies because of the premise that temporal shifts in their behaviour and physiology reflect the amplitude and timing of climate variability and change (Croxall, 1992; Hindell *et al.*, 2003). In particular, variation in diet composition is expected to aid in the assessment of abundance and demographic shifts in lower trophic level taxa (i.e., prey). A necessary precursor to this aim is an assessment of the accuracy and reliability of methods to measure diet variation (e.g., Bradshaw *et al.*, 2003) so that they can be applied across different taxa and ecosystems. The diet of marine birds and mammals has been determined traditionally through the analysis of stomach contents and prey remains in faeces (Coria *et al.*, 1995; Field *et al.*, 2007; Lake *et al.*, 2003). Several drawbacks occur with these approaches: (1) remains in stomachs and faeces only represent prey consumed over a short period of time (i.e., days to weeks; Hammond & Rothery, 1996), (2) hard parts (e.g., fish otoliths, cephalopod beaks) are more recognizable and therefore, possibly over-represented than partially digested soft tissue (Hyslop, 1980), (3) differential passage rates of different prey species bias estimates of frequency of occurrence (Harvey & Antonelis, 1994), and (4) taxonomic identification can be difficult and time consuming.

Regardless of the weaknesses of traditional diet analyses, stomach content and faecal analysis can provide a reasonable assessment of diet composition, including: (1) direct information on prey size and/or meal size, (2) generate large sample sizes (i.e., faecal analysis), and (3) information on temporal and spatial changes in prey consumption. Furthermore, these data function in supporting data interpretation of new dietary techniques.

To alleviate problems associated with traditional diet analyses, biochemical approaches have been developed. Fatty acid signature analysis (FASA) has been of interest from both nutritional and tropho-dynamic perspectives, with the application of fatty acids (FA) as trophic markers to trace or confirm many different marine predator-prey relationships from secondary producers to upper trophic level predators (Ackman *et al.*, 1970; Auel *et al.*, 2002; Iverson *et al.*, 1997b; Lea *et al.*, 2002; Nelson *et al.*, 2001; Ruchonnet *et al.*, 2006). In essence, FASA assumes that base lipid constituents (i.e., fatty acids) are incorporated into the tissues of predators conservatively so that a predator's FA composition will reveal the dietary source of

lipids. If the prey-to-predator lipid transfer is traceable, identification of ingested species can enable a description of trophic interactions and food webs (Bradshaw *et al.*, 2003; Iverson *et al.*, 1997b).

Using FASA to determine diet composition is not straightforward, because (1) several FA are biosynthesised *de novo*, possibly altering the FA signature of the predator, (2) stratification of FA within the blubber has been observed in many species (Best *et al.*, 2003; Birkeland *et al.*, 2005; Grahl-Nielsen *et al.*, 2003; Olsen & Grahl-Nielsen, 2003), indicating components of blubber are synthesised independently of diet, (3) rates of mobilisation and breakdown of FA can vary according to life history stage and environmental context (Chapter 5; Iverson *et al.*, 1995b; Pierce & McWilliams, 2005; Samuel & Worthy, 2004), and (4) molecular structure can alter FA mobilisation patterns (Raclot, 2003; Raclot & Groscolas, 1993; Staniland & Pond, 2005). At higher trophic levels, markers may also become obscured because accumulated FA can originate from a variety of dietary sources and dietary FA signatures may be altered through *de novo* biosynthesis, metabolization and breakdown (Dalsgaard *et al.*, 2003). Quantifying trophic relationships using FA therefore requires species-specific information on FA dynamics such as stratification in sampled tissues (Best *et al.*, 2003), deposition rates and patterns (Iverson *et al.*, 2004; Budge *et al.*, 2004) and differential utilisation patterns (Chapter 5; Birkeland *et al.*, 2005).

Although some aspects of FASA have been applied successfully to phocid seals, their blubber composition is highly dynamic owing to their reliance on stored reserves for lactation. Further, highly stratified blubber (e.g., Best *et al.*, 2003) with differential mobilisation or deposition rates among species have important repercussions for diet estimation. The diet itself may also play an important role in modifying energy expenditure because specific lipids may offer different characteristics in terms of energy density and oxidation rates (Maillet & Weber, 2006). Weddell seals (*Leptonychotes weddellii*) in particular are subject to high inter-annual variability in resource abundance ensuing from environmentally mediated prey availability (Pinaud & Weimerskirch, 2002). The resulting variability in diet composition affects reproductive performance and population size (Hindell *et al.*, 2003; Le Boeuf & Crocker, 2005; Reid *et al.*, 2005).

Being easily accessible for capture and measurement during breeding makes this species an ideal candidate to examine over-winter diet, lactational changes in fatty

acid composition and feeding during lactation. We investigated the change in fatty acid composition of Weddell seal blubber during lactation specifically to assess characteristics of differential mobilisation and its implications for diet interpretation. We aimed to determine (1) the extent of fatty acid stratification in the blubber of female Weddell seals; (2) if particular fatty acids were selectively mobilised from the inner compared to the outer blubber layer during lactation and; (3) how mobilisation affected diet predictions.

6.2 Methods

6.2.1 Sample collection

This study was conducted at Hutton Cliffs, Antarctica (77° 51' S, 166° 45' E) during the austral summer (October to December) of 2003. Blubber samples were collected from lactating female Weddell seals, captured 1 to 6 (mean 3.8 ± 0.22) days post-parturition (n = 19) and again near the end of lactation (n = 10; 36 to 38 dpp; $\bar{x}$ ± SEM = 36.9 ± 0.26). Each animal was captured, immobilised and measured as described in Wheatley et al. (2006b).

Blubber biopsies were taken from the posterior flank of each animal by making a small (~ 1 cm) incision with a scalpel blade in an anterior – posterior direction. A 6-mm biopsy punch was inserted through the incision, and a core was taken from the whole blubber layer (i.e., through until the muscle layer was reached). In the laboratory, the blubber core was extended to its full length without stretching and cut into two approximately equal pieces, assessed visually. There were no visible differences (e.g., colour, opacity, texture) between the outer portion (closest to the skin) to the inner portion (closest to the muscle) of the cores. Each sample was stored in a pre-weighed glass vial (with a Teflon coated lid), containing a solution of 2:1 v/v chloroform and methanol, and 0.05 % (by weight) butylated hydroxytoluene (BHT; Sigma, St. Louis, USA). Vials were reweighed and all samples were stored at -20 ˚C until laboratory analysis. We found no difference between the weight of the outer and inner portion (generalised linear mixed-effects model, information-theoretic evidence ratio [see below] = 0.32); therefore, samples appeared to be separated evenly.

6.2.2 Laboratory and data analyses

Blubber lipids were extracted quantitatively using a modified overnight (Bligh and Dyer 1959) one-phase methanol/chloroform/water extraction. Following extraction, lipid was trans-methylated to produce fatty acid methyl esters (FAME) and analysed using gas chromatographic (GC) and GC-mass spectrometric analyses (see Chapter 5). The concentration of individual FA were converted to a per cent of total FA mass, and FA present in trace amounts (< 0.5 %) were excluded from analyses. These proportions were used for analyses between the inner and outer blubber layer. However, the overall lipid content of the blubber samples changed (decreased) over lactation, so FA expressed as a percentage of mass composition did not accurately show the changes in FA during the two sampling periods. Lipid class results indicated that blubber was composed virtually entirely of triacylglycerol (TAG; 99.9 %). TAG stored in the tissue consists of glycerol esterified with three FA molecules, and the FA moieties represent about 95 % of the mass of TAG (Groscolas, 1990). Therefore, the lipid stores (kg) of each animal, as determined from hydrogen isotope dilution techniques (see Wheatley *et al.*, 2006a), was multiplied by 95 % to obtain the mass (kg) of FA in each individual, at each capture. All proportional values were arcsine-square-root transformed before analysis.

From evidence that females do not feed during the first 3 weeks of lactation (Eisert *et al.*, 2005), we calculated a 'feeding index' for the second half of lactation based on differences in milk energy output values, which represented the number of times that milk energy (%) exceeded that of non-feeding individuals. This allowed us to rank the relative amount of feeding that occurred among individuals (see Chapter 4) and was used as a covariate in models constructed to explain variation in FA principal component scores (see below).

Principal Components Analysis (PCA) was used on proportional (% of total FA) and absolute (kg) values to investigate patterns of FA in the blubber layers and over time. Principal component scores were used in a series of generalised linear and mixed-effects models (GLM, GLMM) to examine differences in FA composition and mobilisation. Examination of the residuals for all models determined the statistical error distribution and link function. Model ranking was based on Akaike's Information Criteria corrected for small samples (AIC_c, Burnham & Anderson, 2002). For the GLMMs, female identity was set as a random effect to account for repeated

measurements of individuals. Specific model comparisons were based on the information-theoretic evidence ratio (*ER*) which is the AIC_c weight (*w*) of the full model divided by the *w* of another (in this case, the null) model (Burnham & Anderson, 2002). Higher *ER* values indicate higher likelihoods of the tested model relative to the null. We also calculated the per cent deviance explained (%DE) in the response as a measure of a model's goodness-of-fit.

The FA most responsible for the multivariate patterns were identified in SIMPER (similarity percentages) analysis (Clarke, 1993). The SIMPER procedure compares the average abundances and examines the contribution of each FA to the average Bray-Curtis dissimilarity between two defined groups of samples (i.e., blubber layer).

To estimate biases associated with the type of blubber sample taken (i.e., whole, inner or outer portion) and the time of sampling (i.e., post-parturition or end-lactation) we applied a linear discriminant function analysis (DFA) using cross-validation to identify distinct prey groups based on FA profiles. We used this approach to examine differences in diet predictions, but not to construct a complete assessment of diet itself because we lacked FA data for many known and possible Weddell seal prey species (see Appendix 1). Our principal aim was to assess the degree to which FA mobilisation affected DFA classification accuracy. However, we did believe it necessary to use prey species that closely reflected that of the diet, which is generally thought to consist mainly of nototheniid fishes, cephalopods and crustaceans (Burns, 1998; Lake *et al.*, 2003; Plötz, 1986). To do this we obtained FA profiles for 23 known and possible Weddell seal prey species, broadly classified as either nototheniid fish (8 species) or cephalopods (15 species), from various published and unpublished sources (see Bradshaw *et al.*, 2003). Many crustaceans were unavailable, and were not included in analyses. Separate functions were estimated to predict the group membership of the seal-blubber samples for each sampling time and region (i.e., whole or partial blubber samples). We only used FA profiles for seals that had separate blubber samples from both sampling times ($n = 10$).

6.3 Results

6.3.1 Fatty acid composition and vertical stratification

Twenty-four separate FA (comprising 94 – 98 % of the total FA) were found in greater-than-trace amounts (> 0.5 %) in the inner and outer blubber samples (Table 6.1). Monounsaturated fatty acids (MUFA) dominated both layers (outer = 69.1 %, inner = 62.6 %; Fig. 6.1) consisting mostly of short-chain MUFA (≤ 18 carbons; SC-MUFA; outer = 63.0 %, inner = 54.4 %) with only a small proportion of long-chain MUFA (> 18 carbons, LC-MUFA; outer = 6.1 %, inner = 8.2 %). There was no evidence for a difference between layers using the first component (PC1) (GLMM; *ER* = 0.44). Saturated fatty acids (SFA) were found in the next highest percentage in both layers, but in contrast to the MUFA, these FA were relatively more common in the inner (22.5 %) than the outer (16.2 %) layer, although there was still no strong evidence for a difference between the two (*ER* = 1.92). Polyunsaturated fatty acids (PUFA) occurred in the lowest relative amounts and at similar percentages in both layers (outer = 14.7 %, inner = 14.9 %). Sixty-two per cent of the dissimilarity between the two layers post-partum was accounted for by four FA: 18:1ω9c (17.0 %), 16:0 (16.1 %), 16:1ω7c (15.6 %), and 14:0 (13.6 %), with MUFA (18:1ω9c and 16:1ω7c) more abundant in the outer blubber layer and SFA (16:0 and 14:0) more abundant in the inner layer.

A plot of the first two principal components divided the post-partum inner and outer blubber samples into two distinct groups (Fig. 6.2). PC1 accounted for 49.0 % of the variation in FA composition among samples, and the second component (PC2) accounted for 28.0 %. The GLMM used to examine only the influence of *layer* on PC2 scores revealed that this factor explained 77.9 % of the variation in PC2, further supported by a high evidence ratio (1.7×10^{43}, $AIC_c = 167.3$) when compared to the null model.

Table 6.1 *Average fatty acid composition (%) of the inner and outer blubber layer of Weddell seals at post-parturition and end-lactation. SEM = standard error of the mean.*

Fatty acids	Post-partum				End-lactation				Change			
	Inner	N = 19	Outer	N =19	Inner	N = 11	Outer	N = 11	Inner	N =10	Outer	N = 10
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
14:1ω5c	1.1	0.08	2.3	0.15	1.1	0.11	2.3	0.21	-0.1	0.16	0.2	0.19
14:0	9.7	0.52	7.6	0.39	9.5	0.66	7.3	0.54	0.3	0.95	0.6	0.43
i15:0	0.4	0.01	0.4	0.01	0.5	0.02	0.4	0.02	-0.1	0.02	0.0	0.01
16:1ω9c	0.3	0.01	0.4	0.01	0.3	0.01	0.4	0.01	0.0	0.01	0.0	0.01
16:1ω7c	12.3	0.19	15.7	0.34	8.0	0.36	15.0	0.50	4.0	0.45	0.9	0.32
16:1ω5c	0.4	0.01	0.4	0.01	0.3	0.01	0.4	0.02	0.1	0.01	0.0	0.01
16:0	10.3	0.23	6.8	0.19	7.8	0.32	6.6	0.33	2.5	0.15	0.3	0.11
i17:0	0.2	0.02	0.2	0.01	0.2	0.01	0.2	0.02	0.0	0.01	0.0	0.01
18:4ω3	1.1	0.03	1.0	0.03	0.8	0.03	1.0	0.04	0.2	0.02	0.0	0.02
18:2ω6	1.8	0.02	2.0	0.02	2.2	0.05	2.0	0.02	-0.4	0.05	-0.1	0.02
18:1ω9c	30.8	0.51	34.2	0.31	32.4	0.57	35.0	0.47	-1.2	0.75	-1.3	0.37
18:1ω7c	7.4	0.15	7.6	0.18	7.0	0.14	7.7	0.15	0.3	0.16	-0.2	0.08
18:1ω5	0.6	0.02	0.6	0.01	0.6	0.01	0.6	0.01	0.0	0.01	0.0	0.01
18:0	1.3	0.05	0.8	0.03	1.5	0.05	0.8	0.03	-0.2	0.05	0.0	0.03
20:4ω6	0.4	0.02	0.4	0.02	0.4	0.01	0.4	0.02	0.0	0.01	0.0	0.01
20:5ω3 EPA	3.9	0.16	3.4	0.15	1.5	0.13	3.0	0.23	2.2	0.16	0.3	0.10
20:4ω3	0.3	0.04	0.4	0.04	0.3	0.04	0.4	0.05	0.0	0.01	0.0	0.02
20:2ω6	0.6	0.03	0.6	0.03	1.2	0.07	0.7	0.07	-0.5	0.02	0.0	0.07
20:1ω9c	5.4	0.15	4.4	0.16	8.9	0.34	4.8	0.27	-3.1	0.33	-0.3	0.18
20:1ω7c	0.6	0.01	0.5	0.02	1.0	0.04	0.5	0.02	-0.3	0.04	0.0	0.01
22:6ω3 DHA	4.9	0.25	4.9	0.19	6.0	0.21	5.0	0.20	-1.1	0.13	-0.2	0.10
22:5ω3 DPA	1.5	0.22	1.6	0.14	2.2	0.29	1.7	0.21	-0.6	0.05	-0.1	0.05
22:1ω11c*	0.9	0.04	0.5	0.04	1.4	0.10	0.5	0.04	-0.5	0.08	0.0	0.02
22:1ω9c	0.8	0.03	0.4	0.03	1.4	0.09	0.5	0.05	-0.6	0.08	-0.1	0.04
24:1	0.2	0.02	0.1	0.01	0.5	0.03	0.1	0.02	-0.3	0.03	0.0	0.02

* includes 22:1ω13c

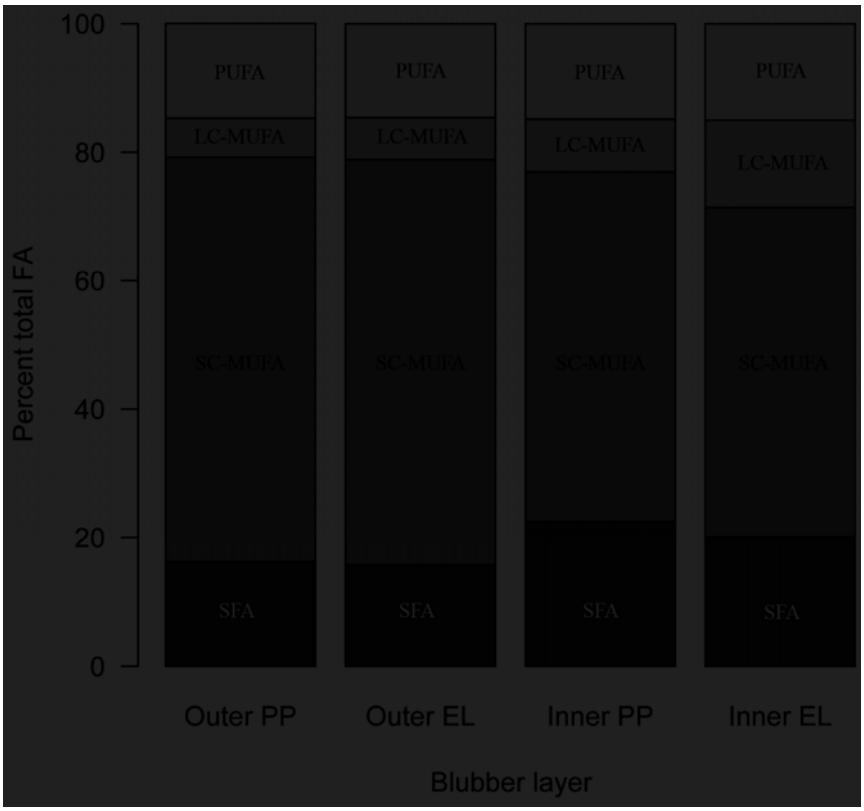

Figure 6.1 *Mean proportion of polyunsaturated (PUFA), long-chain monounsaturated (LC-MUFA), short-chain monounsaturated (SC-MUFA) and saturated (SFA) fatty acids in the inner and outer blubber layer at post-partum (PP) and end-lactation (EL).*

6.3.2 Layer variation in fatty acid composition

6.3.2.1 Inner layer

For the FA proportional changes within the inner blubber layer (comparing post-parturition to end-lactation cores), PC1 accounted for 50.2 % of the variation in FA composition, and 27.5 % in PC2. The FA driving the differences included 16:1ω7c, 16:1ω5c and 20:5ω3 with negative eigen values and 24:1, 22:1ω9c and 20:1ω9c with positive eigen values (Fig. 6.3). Testing only capture time, (*date*) explained 86.7 % of the variation in PC1 (GLMM; $ER = 5.0 \times 10^{42}$, $AIC_c = 75.3$). Polyunsaturated fatty acids remained relatively unchanged by the end of lactation, while the percentage of LC-MUFA increased, and SC-MUFA and SFA decreased over lactation (Fig. 6.1).

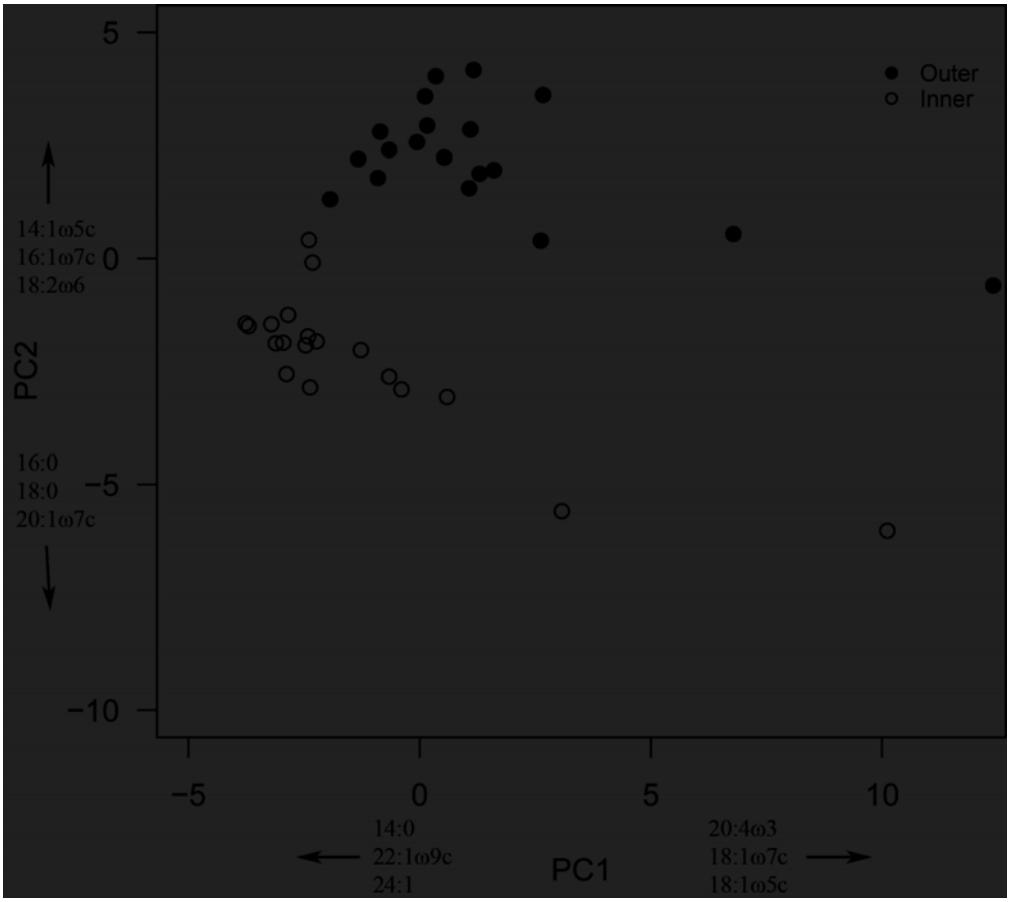

Figure 6.2 *Principal component plot for the inner and outer blubber layer of Weddell seals collected post-parturition. The first principal component (PC1) explained 49.0 % of the total variation and the second principal component (PC2) explained 28.0 % of the variation between the blubber layers. The three fatty acids with the most extreme positive and negative loadings (eigen values) for PC1 and PC2 are shown along the axes.*

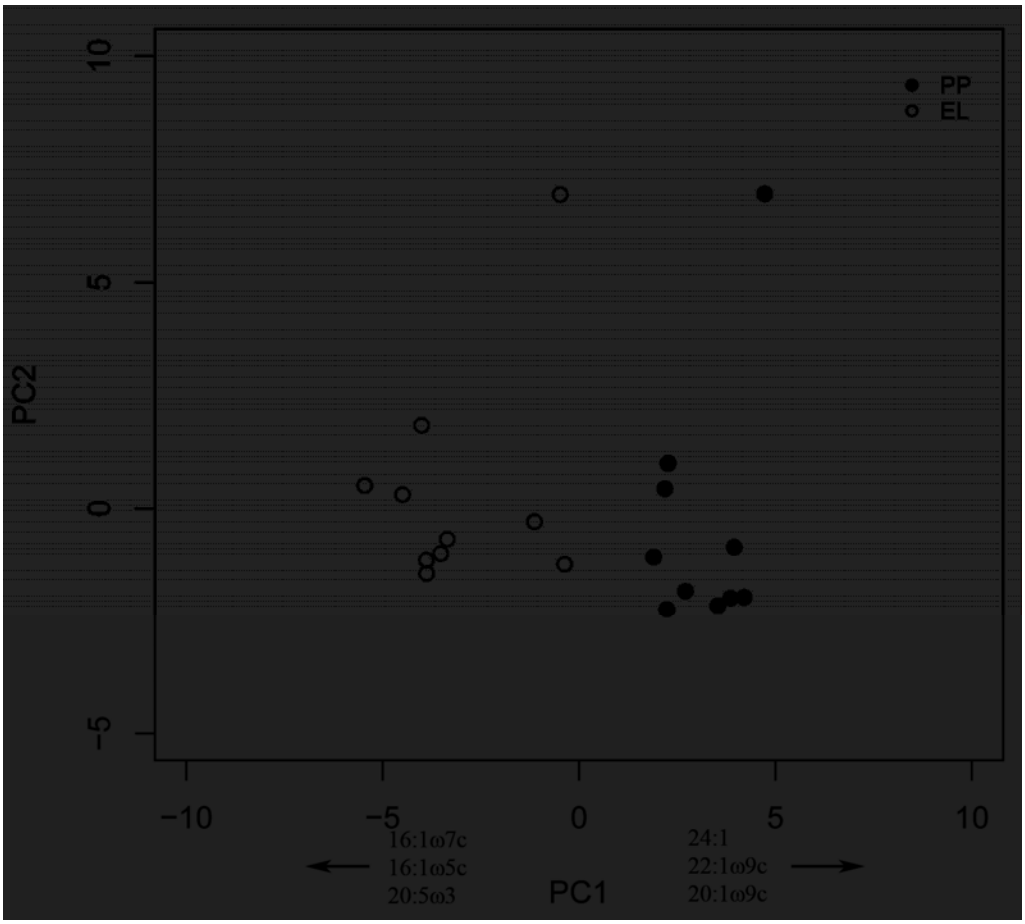

Figure 6.3 *Principal component plot for FA changes in the inner blubber layer of Weddell seals between post-partum (PP) and end-lactation (EL). The three fatty acids with the most extreme positive and negative loadings (eigen values) for the first principal component (PC1) are shown along the axis.*

In absolute terms, 69.7 % of the difference between the inner portion of the post-partum and end-lactation blubber layers was in SFA 14:0 and 16:0, and MUFA 18:1ω9c, 16:1ω7c. The most parsimonious model testing for the effect of total body lipid stores (kg) at post-parturition (TBL) and feeding index on the fractional mobilisation (i.e., the fraction of initial mass of the FA that was lost during lactation) of FA from the inner blubber layer between post-parturition and end of lactation included only the term *feeding* (AIC_c = 50.7, %DE = 33.5 %). Several FA were mobilised consistently more than others (Table 6.2), but in all females the essential fatty acid (EFA) 20:5ω3 had the highest fractional mobilisation (range: 69.6 – 91.6 %).

6.3.2.2 Outer layer

There was little evidence for a difference in the proportional FA of the outer blubber layer between post-parturition and end-lactation cores according to the PCA (Fig. 6.4). The GLMM used to only examine the influence of *date* on PC1 scores revealed that *date* described only a small component of the variation in samples (%DE = 0.48, AIC_c = 89.1) and little evidence for a temporal change (Fig. 6.1). The most parsimonious model testing for the effect of *TBL* and *feeding* on the fractional mobilisation of FA from the outer blubber layer included only the term *feeding* ($wAIC_c$ = 0.971), although the %DE by this model was lower (%DE = 14.6 %). FA 20:5ω3 still had the highest fractional mobilisation in some females, but the mobilisation of other FA in the outer layer was much more variable compared to the inner layer (Table 6.2).

6.3.3 Prey and seal blubber classification

Discriminant function analysis (jackknifed) using the two prey groups (nototheniids and cephalopods) correctly classified all (100 %) prey species. We identified six FA with the highest mobilisation properties (14:0, 16:0, 14:1ω5c, 16:1ω5c, 16:1ω7c, 20:5ω3) and six FA with low mobilisation properties (20:1ω7c, 22:1ω9c, 22:1ω11c, 20:2ω6, 22:5ω3, 20:1 ω9; Table 6.2) during lactation. The DFA was re-run first with the highly mobilised FA excluded (referred to as 'high-excluded' discriminant function) and then with the low mobilised FA excluded (referred to as 'low-excluded' discriminant function). For each analysis prey remained 100 % correctly classified.

Table 6.2 *Fractional mobilisation (%) of fatty acids (FA) from the inner and outer blubber layer during lactation. Bold designates the three FA with the highest fractional mobilisation for each individual.*

Fatty acids	Inner blubber layer									Outer blubber layer								
	Y536	W636	Y965	Pu194	Y4295	Pu114	P871	P130	Pu761	Y536	W636	Y965	Pu194	Y4295	Pu114	P871	P130	Pu761
14:1ω5c	9.01	53.70	61.77	52.65	51.05	**78.63**	**77.93**	**62.90**	59.74	28.01	45.03	43.58	68.95	26.03	40.00	**51.96**	**74.72**	**61.17**
14:0	41.74	64.31	63.88	66.05	58.37	**75.96**	72.60	**59.52**	56.28	39.39	48.27	36.89	72.43	35.04	43.67	**42.44**	**69.23**	55.94
i15:0	30.19	53.24	58.33	55.84	47.00	62.43	55.66	39.64	44.57	36.18	52.11	34.69	70.57	30.29	40.31	26.72	**57.85**	55.07
16:1ω9c	42.18	64.95	70.57	60.42	52.61	63.26	62.65	41.99	56.04	37.54	55.41	40.56	70.68	29.51	38.85	28.14	48.71	57.77
16:1ω7c	54.34	**78.38**	**84.47**	71.97	**72.65**	75.75	74.28	57.40	**75.19**	41.02	**60.67**	41.02	69.60	37.96	42.03	**32.87**	51.22	**62.28**
16:1ω5c	**56.50**	**78.80**	**83.43**	**72.51**	**72.48**	73.90	**79.81**	57.20	**73.48**	40.83	59.92	44.67	70.50	38.44	41.86	29.40	50.88	60.90
16:0	**57.02**	75.26	78.95	**71.99**	68.68	69.68	67.29	50.11	67.58	**45.93**	59.98	33.22	72.42	**41.16**	44.39	22.64	46.46	56.71
i17:0	50.10	62.25	73.62	44.05	56.81	49.59	59.46	33.37	56.19	**44.05**	59.12	44.50	66.83	32.30	**55.93**	12.23	37.88	59.24
18:4ω3	49.95	72.10	80.05	69.40	66.23	68.71	65.99	47.40	67.06	39.17	**61.22**	44.40	71.22	**38.88**	43.30	20.62	43.79	59.48
18:2ω6	28.24	57.01	65.73	55.81	47.92	50.36	45.54	22.01	45.31	38.09	57.46	35.34	70.32	29.38	40.39	17.09	39.44	54.42
18:1ω9c	35.30	63.91	72.47	60.66	55.47	57.62	54.48	32.22	56.60	40.12	58.07	33.70	70.35	29.97	40.76	18.57	39.80	54.99
18:1ω7c	42.89	67.05	74.94	63.70	58.85	60.74	57.32	35.88	59.52	40.76	58.55	34.62	70.08	31.46	41.51	18.80	40.25	55.66
18:1ω5	39.04	61.73	71.84	60.08	56.91	57.80	56.78	37.54	55.47	38.16	57.53	36.96	69.81	31.32	39.47	15.74	42.30	55.65
18:0	39.53	59.35	65.48	58.19	51.25	51.21	47.62	25.55	43.44	43.25	58.45	29.45	73.52	38.40	43.14	8.05	38.79	49.36
20:4ω6	45.13	71.82	74.83	63.40	61.43	63.09	59.16	43.56	59.61	33.70	56.57	42.24	70.78	35.75	40.90	9.23	43.85	57.53
20:5ω3 EPA	**71.90**	**88.24**	**91.58**	**81.43**	**83.77**	**83.39**	**83.42**	**69.59**	**85.86**	40.31	**66.78**	**46.80**	72.46	**49.83**	**46.52**	27.54	48.75	**65.74**
20:4ω3	38.16	68.82	77.97	63.15	59.81	58.65	63.87	50.13	63.30	29.78	51.98	**46.05**	**74.23**	30.63	40.54	5.29	47.35	58.68
20:2ω6	-4.11	35.62	43.98	34.09	23.92	34.01	25.25	-1.97	16.91	**94.77**	54.64	35.72	72.73	22.86	41.42	-2.00	32.80	46.14
20:1ω9c	30.77	35.55	52.13	46.30	29.86	38.00	29.06	3.03	26.21	40.97	48.26	27.09	72.00	25.92	43.17	2.44	35.36	46.15
20:1ω7c	19.55	42.55	50.06	45.82	27.20	37.00	26.02	0.83	25.25	37.97	55.10	30.93	71.52	25.93	42.42	4.27	37.03	46.55
22:6ω3 DHA	30.37	50.16	63.50	55.12	45.54	53.28	50.62	27.77	43.65	35.04	57.78	38.55	73.10	31.71	42.01	9.69	39.13	53.20
22:5ω3 DPA	24.22	36.98	53.93	43.13	33.39	42.89	40.15	13.38	27.72	33.63	55.40	41.25	**74.30**	24.77	40.16	6.60	35.90	51.83
22:1ω11c*	29.09	45.17	44.61	48.89	32.12	37.71	30.70	0.78	22.04	39.46	56.67	22.03	**74.48**	35.06	**45.83**	-3.96	36.57	41.92
22:1ω9c	18.85	41.44	41.14	41.70	23.71	26.66	13.39	-17.49	15.61	38.56	57.14	21.51	73.47	30.56	44.36	-2.55	4.66	39.02
24:1	-5.24	6.05	-4.57	32.71	10.19	13.30	17.57	-25.72	-10.00	33.03	52.78	**48.15**	51.72	34.00	26.08	7.81	34.59	53.23

* includes 22:1ω13c

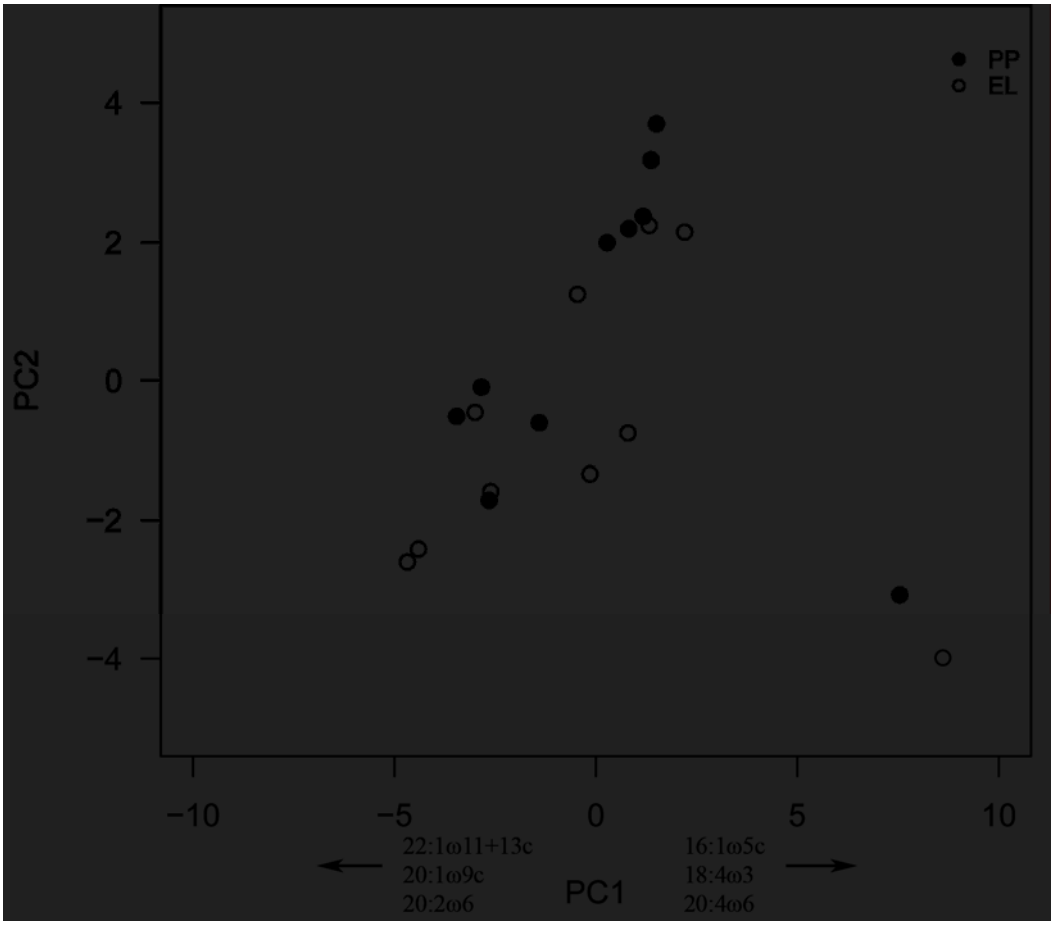

Figure 6.4 *Principal component plot for FA changes in the outer blubber layer of Weddell seals between post-partum (PP) and end-lactation (EL). The three fatty acids with the most extreme positive and negative loadings (eigen values) for the first principal component (PC1) are shown along the axis.*

Using the first discriminant function (DF) for the prey classification, we recalculated the DF for seal blubber by inserting the proportions of each of the FA from blubber samples (cf. Bradshaw *et al.*, 2003). This was done for whole samples and for layer samples (i.e., inner or outer) taken at post-parturition and end-lactation. This produced a classification of a seal blubber sample as 'nototheniids' or 'cephalopods' for each sampling time and sample section. This was repeated for the high- and low-excluded DFs. On average, the full and low-excluded DFs categorized blubber samples as ≥ 50 % cephalopod. The high-excluded DF classified samples as ≥ 50 % nototheniids. Using the number of samples classed into each prey group for each sample type, we calculated the per cent difference in diet classification predicted from the full DF and the high- and low-excluded DFs. The full and the low-excluded DF classified the seal blubber samples similarly (Table 6.3a). However, there were

considerable differences in classification with the high-excluded DF (Table 6.3b).

Table 6.3a *Summary of per cent differences in prey group classifications between the full discriminant function (DF; n = 22 fatty acids) and the DF with low mobilised fatty acids removed (n = 16 fatty acids). Subscript 'F' denotes results from the full classification and subscript '- L', denotes low-excluded DF classification. Boldface designates comparisons between like samples.*

Sample	whole PP_F	inner PP_F	outer PP_F	whole EL_F	inner EL_F	outer EL_F
whole PP_{-L}	**10%**					
inner PP_{-L}	50%	**10%**				
outer PP_{-L}	10%	50%	**10%**			
whole EL_{-L}	20%	40%	20%	**10%**		
inner EL_{-L}	10%	50%	10%	0%	**30%**	
outer EL_{-L}	20%	40%	10%	10%	40%	**20%**

Table 6.3b *Summary of per cent differences in prey group classifications between the full discriminant function (DF; n = 22 fatty acids) and the DF with high mobilised fatty acids removed (n = 16 fatty acids). Subscript 'F' denotes results from the full classification and subscript '- H', denotes high-excluded DF classification. Boldface designates comparisons between like samples.*

Sample	whole PP_F	inner PP_F	outer PP_F	whole EL_F	inner EL_F	outer EL_F
whole PP_{-H}	**80%**					
inner PP_{-H}	70%	**30%**				
outer PP_{-H}	70%	30%	**70%**			
whole EL_{-H}	90%	30%	90%	**80%**		
inner EL_{-H}	80%	40%	80%	90%	**50%**	
outer EL_{-H}	100%	40%	100%	90%	60%	**100%**

6.4 Discussion

We have shown that some fatty acids were fractionally mobilised more than others during lactation in Weddell seals (e.g., 20:5ω3), and that there are important fatty acid composition differences between the inner and outer blubber layers. Dietary predictions including the highly mobilised FA were questionable (i.e., a mostly cephalopod diet); however, removal of highly mobilised FA from discriminant function analysis reversed dietary predictions to a predominantly nototheniid diet, consistent with most Weddell seal dietary studies (Burns, 1998; Lake *et al.*, 2003; Plötz, 1986). This demonstrates that differences in FA mobilisation rates affect dietary predictions, and need to be accounted for in future work. The FA used in our analyses were those identified as having either elevated or reduced mobilisation rates during lactation. However, different FA may be of importance during other periods of fasting (i.e., moult). Therefore, future research should include examining selective use of FA during this time.

The proportion of FA accumulated during foraging trips depends on energy expenditure while previously ashore. This may reduce the power to detect temporal differences in FA composition unless mobilisation rates and selective use of FA are measured. We have shown that some FA are selectively mobilised to support both maintenance metabolism and milk production during lactation. These FA may be underestimated in the blubber if sampled throughout lactation, thereby affecting diet predictions. Failing to account for mobilisation during periods of high turn-over may seriously bias FASA diet estimates. We suggest that dietary predictions will be most reliable when full blubber core samples are taken at parturition.

Fatty acid mobilisation and changes in composition during lactation occurred mainly in the inner blubber layer. We also found absolute (although not proportional) changes in the outer layer – an observation not previously reported. The main FA depleted in the inner layer were the same that dominated post-parturition (18:1ω9, 16:1ω7, 16:0 and 14:0) and overall SC-MUFA and SFA were used the most. Similar to the inner layer, 18:1ω9, 16:1ω7, 16:0 and 14:0 were the FA that caused the most (absolute) dissimilarity between sampling times in the outer layer. Conversely, all groups of FA (PUFA, LC-MUFA, SC-MUFA and SFA) were used in similar proportions in the outer layer, but not the inner layer. This indicates that although

changes occurred within both halves of the blubber they did not change uniformly.

Several previous studies have described stratification in the blubber of marine mammals (Arnould *et al.*, 2005; Best *et al.*, 2003; Olsen & Grahl-Nielsen, 2003); however, this is the first study that describes the changes in stratification over time. The strong vertical stratification in Weddell seal blubber was similar to that found in other species (Andersen *et al.*, 2004; Arnould *et al.*, 2005; Olsen & Grahl-Nielsen, 2003). Higher concentrations of SFA (particularly 16:0 and 14:0) were present in the inner compared to the outer layer, while MUFA (particularly 18:1ω9c and 16:1ω7c) were more prevalent in the outer layer. This may be due to a more metabolically active inner layer. SFA offer more chemical energy per unit mass (Maillet & Weber, 2006), while the outer layer is more structural and so requires more stable FA with lower melting points (Fredheim *et al.*, 1995).

Blubber stratification in seals may arise because (1) fatty acids that enter the tissue first are more rapidly turned over and released compared to the cell's bulk lipid, i.e., last in – first out (Ekstedt & Olivecrona, 1970); (2) some FA are differentially mobilised according to chain length, unsaturation, positional isomerism and melting point (Raclot & Groscolas, 1993); and (3) there may be a gradient of use across the entire blubber layer (Andersen *et al.*, 2004) that might be related to the structural/physiological demands of both layers. Although all these may play a role in the differential mobilisation of FA we observed, selective mobilisation of FA should also arise in response to the energetic and specific nutritional demands of the mother and pup at different stages of development. For example, the essential fatty acid (EFA) 20:5ω3 had the highest fractional mobilisation from the blubber, specifically the inner layer, during lactation. It was also highest in the milk immediately post-parturition (Chapter 5), suggesting that mobilisation of this FA occurs early in lactation when females are fasting. It also suggests that some fatty acids may be selectively mobilised at different times depending on energetic or growth requirements of mothers and pups.

Evidence from milk energy output and FA mobilisation/transfer indicates and that some females fed during late lactation (Chapter 4; Chapter 5), although not all females apparently fed, and FA trends were similar for feeders and non-feeders. Even though the feeding index was an important contributor to fractional mobilisation of FA, our data suggest that some fractional mobilisation of fat reserves occurs within Weddell seals during lactation regardless of feeding.

To account for differential metabolism, deposition and biosynthesis of FA by predators, Iverson et al. (2004) developed calibration coefficients for individual FA (Quantitative Fatty Acid Signature Analysis - QFASA) to compare the FA of predator lipid stores to FA of various prey types. This technique allows one to estimate the proportional contribution of each prey type in a predator's diet (Iverson *et al.*, 2004). One of the fundamental requirements of the model is an understanding of, and accounting for, variable rates and patterns of lipid metabolism and deposition in the predator. The differential mobilisation of particular FA we found highlights that it may be more applicable to use more 'inert' (low mobilised) FA for predictions. Furthermore, differences in the degree of differential mobilisation may vary among species, and will need to be taken into consideration when estimating calibration coefficients for some FA. Accordingly, using FASA as a biomarker of dietary composition and change should be used cautiously because individual FA are mobilised differentially depending on the physiological state of the animal. This result emphasizes the need for further research to understand species-specific FA mobilisation and turnover, to provide more accurate and robust quantitative estimates of diet and its variation.

Chapter 7

General Discussion

7.1 Overview

Life history is commonly defined as a set of evolved strategies, including behavioural, physiological and anatomical adaptations, that influence survival and reproductive success directly (Ricklefs & Wikelski, 2002; Stearns, 1992). Both bird and mammal studies have been at the forefront of understanding life history diversification (Promislow & Harvey, 1990; Roff, 1992; Stearns, 1989) and physiological causes of trade-offs have been a central topic in most studies (Ricklefs & Wikelski, 2002; Roff, 1992; Stearns, 1992; Zera & Harshman, 2001). A core idea in life history physiology is that differential allocation of limited maternal resources has a central role in the cost of reproduction, maintenance, growth and storage (Harshman & Zera, 2006). In particular, the cost of reproduction is of fundamental importance in life history evolution. When studying the constraints of life history evolution at the physiological level individual differences provide good case studies for examining adaptive allocation of energy in fitness terms (i.e. lifetime reproductive success - Gittleman & Thompson, 1988).

Reproductive effort is defined as the proportion of total energy that an organism devotes to reproduction, and is usually viewed in a cost-benefit framework (Stearns, 1992; Williams, 1966). To be favourably selected, a phenotype for a given level of reproductive effort must enhance the fitness of the individual exhibiting this phenotype (Hirshfield & Tinkle, 1975). As such, determining the magnitude and variance of factors that influence reproductive effort are central to current ecological theory, relating physiological processes to life history strategies (Gadgil & Bossert, 1970; Tinkle, 1969; Williams, 1966).

One of the most accepted measures of reproductive effort is the fraction of body mass (or energy) expended in reproduction per year (Roff, 1992; Stearns, 1992). However, ecological differences between taxa, behavioural shifts by reproductive individuals, and seasonality in food supply can all make it difficult to determine what these costs are in different groups of organisms (Qualls & Shine, 1998). Given this complexity, attempts to model the costs of reproduction on the basis of any single index (other than lifetime reproductive success) will likely be applicable only to small groups of ecologically similar taxa.

This study has focused on the causes and consequences of intra-specific and inter-annual differences in the allocation of energy to reproduction in Weddell seals,

to determine how differences develop and influence an individual's reproductive success. Results are compared to a range of taxa, with particular emphasis on large mammals.

7.2 Reproductive effort

Fluctuations in productivity and food supply have been shown to affect foraging success (Frank & Slatkin, 1990; Olsson *et al.*, 2002) and body size (Boyce, 1978; Proffitt *et al.*, 2007; Sedinger *et al.*, 1995) in a number of species. A positive correlation between adult body size and reproductive effort has also been observed in both birds and mammals (Barbraud *et al.*, 1999; Clutton-Brock *et al.*, 1988; Pomeroy *et al.*, 1999; Weimerskirch *et al.*, 1997). Energy stores are an essential component in allocation processes (Drent & Daan, 1980) where they play a major role, especially for long-lived organisms, in allocation decisions between reproduction and survival (Weimerskirch *et al.*, 1997). Therefore, body mass and condition represent a link between foraging and allocation of resources.

Optimal foraging theory (McArthur & Pianka, 1966; Stephens & Krebs, 1986) predicts that animals should forage so as to maximize net energy gain. Species may adopt particular foraging strategies to compensate for unpredictability in the location of prey (Viswanathan *et al.*, 1999; Weimerskirch *et al.*, 2005), or may generally concentrate effort in more predictable foraging areas (Bradshaw *et al.*, 2004). Generalist predators tend to survive on many prey groups (Dell'Arte *et al.*, 2007), although they show marked preferences, to 'superior', energy-dense prey (Venzon *et al.*, 2002). Only when superior prey are not sufficiently abundant, will 'inferior' prey be included in the diet (Stephens & Krebs, 1986). However, this will also depend on the density of the inferior prey. Variation in sea-ice extent and the state of the Southern Oscillation has been shown to affect foraging success of adult female Weddell seals (Proffitt *et al.*, 2007), and in this study, individuals exhibited inter-annual differences in mass at parturition (where mass was higher in 2002 than in 2003), which were not related to dietary differences during the pre-breeding foraging trip (see Chapter 5). This suggests that differences in mass were not the product of foraging on less energy-dense prey in one year (2003) but that environmental variation influenced overall prey abundances, and that females were unable to

compensate for this disparity. As a consequence, allocation 'decisions' were affected by this decrease in foraging success.

In species without paternal care, characteristics such as maternal age and social rank may also influence maternal expenditure and offspring survival (Bowen *et al.*, 1994; Clutton-Brock *et al.*, 1985; Côté & Festa-Bianchet, 2001). Life history theory predicts that reproductive effort should increase with age as reproductive value decreases (Stearns, 1992); however, in some animals body mass has stronger effects on offspring development than does maternal age (Ellis *et al.*, 2000). Of the female Weddell seals captured for this study, there was no evidence for age effects with maternal post-partum body mass or reproductive effort. Furthermore, social rank does not appear to play a large role for females within breeding colonies of Weddell seals (Kooyman, 1981). Although females experienced inter-annual differences in foraging success, average pup birth mass was similar between the years, indicating that differential energy expenditure does not occur during gestation. Likewise, McMahon & Hindell (2003) found that birth mass did not differ between single and twin southern elephant seal pups, indicating that females can produce a total offspring output mass greater than that typically produced (i.e., for a single pup). This suggests that the size of pups at birth is not necessarily limited by the amount of resources available to the female during gestation, and therefore, not ultimately determined by maternal energy supply.

Although there was no relationship between body mass and age in this study, with a larger data set, Proffitt *et al.* (2007) found that body mass at parturition does increase with age (to an asymptote). Since larger females often produce larger offspring (Chapter 3), pup weaning mass also increases with female age. In addition, Hadley et al. (2007) found that the survival rate of Weddell seal offspring increases with maternal age. Although these age-related effects were most obvious for young animals, they identify a link between maternal post-partum mass, weaning mass and offspring survival rate. This is the first evidence for Weddell seals, and the third for a phocid seal (others being: grey seal - Hall *et al.*, 2001 and southern elephant seal - McMahon *et al.*, 2000b) that larger weaned pups have a better survival rate than smaller ones. Therefore, the resources transferred from mother to pup during lactation are important in determining survival (and ultimately population trajectory), and this will vary depending on a mother's previous foraging success and her body condition at parturition. If this is the case, females can most directly influence their lifetime

reproductive success and fitness by successfully rearing as large a pup as she is physically capable of producing (see below).

On average, small female Weddell seals did not compensate for the poor winter foraging by increasing energy expenditure during lactation to produce a larger pup at weaning. There appears to be a relatively fixed proportion of her mass to which a female will deplete her reserves before terminating lactation (*c.* 60 %; Chapter 3). This is comparable with that previously reported for elephant seals (58 – 66 %; Arnbom *et al.*, 1997; Carlini *et al.*, 1997; Crocker *et al.*, 2001), suggesting that there is an upper limit for energy export to offspring and that self-maintenance takes precedence over offspring condition (i.e., the classic 'K' reproductive strategy). This limitation in reproductive effort may represent an energy sparing mechanism which permits self-preservation and a chance at future reproduction (Rogowitz, 1996), so that the trade-off between long-term survival and offspring success is contingent on individual size.

Life history theory predicts that long-lived organisms with low fecundity should protect adult survival (i.e., future reproduction) at the expense of current reproduction (Stearns, 1992). Phenotypic trade-offs between life history traits are most easily seen under adverse conditions such as reduced food availability, where the amount of energy available to allocate to different functions is limited (Stearns, 1992). The frequency and duration of episodes of extremely low resource quantity or quality are likely to be important selective factors on traits such as energy storage and allocation. The large variation in reproductive output observed in a number of long-lived species (DFO, 2000; Erikstad *et al.*, 1997; Erikstad *et al.*, 1998; Reid, 1987; Siniff *et al.*, 1977; Stirling, 2005; Testa *et al.*, 1990) may indicate that delayed reproduction may ultimately yield higher lifetime reproductive success than breeding when conditions are poor (Covas *et al.*, 2004). Stochastic environments may therefore favour flexibility of reproductive effort (Erikstad *et al.*, 1997; Erikstad *et al.*, 1998).

Similar to other phocid seals (DFO, 2000; Stirling, 2005), Weddell seals have variable reproductive rates (Siniff *et al.*, 1977; Testa *et al.*, 1990), including sensitivity to environmental variation at the age of first reproduction (Hadley *et al.*, 2006). The magnitude of variability in polar environments may require that seals occasionally invest in reproduction at a cost to their own survival (i.e., flexibility in reproductive effort; Hadley *et al.*, 2007). This may be why, in contrast to life history predictions, smaller females (in poor condition) still produced and reared a pup, and

this was not confounded by age effects within this sample population (*i.e.*, no effects of age on mass at parturition, Chapter 3). Alternatively, reproductive success of smaller individuals may also be linked to the temporal effects of oceanographic variation on food supply. In grey seals, maternal condition influences foetal development, with females in poorer condition delaying implantation later than females in good condition (Boyd, 1984). In addition, an early increase in energy intake of some ungulate species will increase the probability of conception (White, 1983). If foraging conditions are favourable earlier in the implantation and gestation period, reproductive success is favoured and smaller females may reproduce that year. However, if conditions deteriorate late in gestation (affecting mass and condition) when it may be too late to abort, smaller females may be forced either to abandon their pup (i.e., sacrifice current reproduction) or complete lactation (with a possible sacrifice to future reproduction). Variability in reproductive rates within individual species suggests that females adjust their allocation to reproduction depending on prevailing conditions and fat reserves.

7.3 Lactation

There are a wide range of reproductive strategies employed among animal species. Lactation is one of the major defining characteristics of mammals and is a critical part of their reproductive strategies. Variation in foraging and previous energy acquisition is directly measurable as body reserves at the onset of lactation. However, energy expenditure on offspring during lactation varies between species depending on life history characteristics (McClure, 1987). In many ecosystems, inter-annual variation in environmental characteristics such as food supply can have considerable impacts on the evolution of reproductive traits such as offspring size and reproductive frequency (Ballinger, 1977; Monaghan *et al.*, 1989; Saba *et al.*, 2007). To cope with fluctuations in food resources, organisms have evolved a wide range of strategies for energy acquisition and allocation to reproduction (Drent & Daan, 1980). One fundamental dichotomy is between those species in which reproduction is fuelled by recently acquired energy ('income breeders') and those where storage constitutes the primary energy source ('capital breeding', Drent & Daan, 1980). Furthermore, the production of altricial and precocial offspring also represents extremes of a continuum

in characteristics of mammalian reproductive strategies (Derrickson, 1992). Where altricial young are born helpless, are totally dependent on maternal milk and require care for a comparatively long time (e.g., humans); precocial young are highly developed at birth, and they may consume solid food within a few days. Therefore, reproductive strategies involve trade-offs, and these trade-offs favour one mode over the other depending on the environmental context.

Many current models examining lactation strategies support either body size and/or phylogenetic history as influencing factors (Blackburn, 1993; Boyd, 1998; Oftedal, 2000; Trillmich & Weissing, 2006). In terms of body size, large animals have greater energy storage capacity relative to the demands of lactation, than do small animals; however from an evolutionary perspective, lactation strategies may be similar between species not because of the energetic demands of the offspring, but because of their particular phylogenetic history (Blackburn, 1993). In general, comparative biologists can often extrapolate general characteristics of organisms from those of related species; however, lactation strategies can be modified to suit different environments, diets, reproductive cycles, and breeding seasons within a species (Blackburn, 1993).

Phocid seals generally combine features of both altricial and precocial patterns, including inter-specific differences in the extent of precocial development. For some species, pups are more developed and are able to enter the water soon after birth (e.g., bearded seals - Hammill *et al.*, 1994; harbour seals - Burns *et al.*, 2005 and Weddell seals - Testa *et al.*, 1989). In other species, pups require an extensive post-weaning fast where they learn to swim and dive (e.g., northern and southern elephant seals - Le Boeuf & Laws, 1994). This increase in precocial development of some species may be favoured when offspring are more vulnerable to predation or environmental variability (e.g., ice floes, flooding tides). It also appears to represent a trade-off in the allocation of resources to different developmental pressures. For instance, high energy expenditure devoted to behavioural development to enhance hunting ability or predator avoidance during the early period of nutritional independence will limit the storage of body lipids that could be important for maximising post-weaning survival (Arnould *et al.*, 2003; Birgersson & Ekvall, 1997).

Some studies have demonstrated a physiological ceiling to mammary gland output, thereby limiting lactational performance of the mother regardless of her phenotype, age or experience (Hammond & Diamond, 1992; Hammond *et al.*, 1996).

In grey seals, a combination of mammary gland capacity (e.g. size, metabolic activity), nutrient availability and metabolic compensation appear to maximize the capacity for milk energy output (Mellish *et al.*, 2000). Increased energy output is also a function of increased mammary capacity in hooded seals (Mellish et al. 1999b). This confirms that larger females can produce more milk (Chapter 4), but also suggests that smaller mothers are physiologically limited to the degree that they can increase daily milk output ($kg \cdot day^{-1}$). To allocate more resources to offspring, a small female would have to improve milk composition or increase the duration of the lactation period itself.

Mammals differ according to litter size and frequency, the degree of development of the offspring at birth, postnatal thermoregulatory and nutritional needs, and maternal resource availability. These features are reflected in such aspects of lactation as milk quantity, time until weaning, and milk composition (Blackburn, 1993). Furthermore, the energy demands of offspring increase throughout pregnancy and lactation so that late lactation is the energetically most demanding period in the mammalian breeding cycle (Bronson, 1985; Millar, 1977). For Weddell seals, milk composition and milk output changed during lactation to support both energetic and physiological demands of females and pups. This observation may help to explain why Weddell seals have a longer lactation period than similar-sized phocids (e.g., elephant seals). In general, milk composition of larger mothers is similar to that of smaller mothers (Chapter 4), suggesting that variation in energy transfer during lactation is not a result of milk compositional changes. It appears that although larger females cannot increase energy transfer by modifying milk composition, they do have the physiological capabilities for a longer lactation and increased energy output so that pups will be weaned larger, with higher survival rates, thereby increasing reproductive success.

Despite their categorisation as capital breeders, there is evidence that some female Weddell seals do engage in feeding during late lactation (mixed capital-income; Chapter 4; Chapter 5; Eisert *et al.*, 2005; Hindell *et al.*, 2002). In comparison with smaller phocid seals (Bowen *et al.*, 2001), this lactation-feeding phenomenon was originally thought to provide a nutritional refuge for smaller females incapable of securing sufficient reserves during pre-partum foraging. However, facultative income breeding has been shown in other capital breeding animals (e.g., the aspic viper - *Vipera aspis*, Lourdais *et al.*, 2002), and appears to be a flexible strategy to adjust

reproductive investment to local resource levels by taking advantage of periods when prey are occasionally abundant. It appears that Weddell seals may also exhibit this flexible strategy, although it is restricted to larger females possessing the physiological capacity to dive for longer and exploit different resources during lactation. This supports the assumption that although body mass and phylogenetic history explain most of the variation in lactation patterns (20 – 69 %), the remaining variation has likely resulted from physiological adaptations to local environmental conditions (Ferguson, 2006). If individuals are to adjust their life history strategies according to temporal variation in the environment, they must recognize signals reflecting the state of the environment, as well as information on state variables such as body condition (Ergon, in press). Understanding what cues animals use in their reproductive decisions and how they respond to these cues are particularly important when seeking to predict the effects of environmental change (Ergon, in press).

When offspring have acquired sufficient energy stores from their mother they can achieve some developmental autonomy, and ontogenetic patterns of energy use should be tailored to the offspring's genotype. Patterns of energy use during development should therefore reflect adaptations maximizing offspring fitness (Berg *et al.*, 2001). At weaning, total body lipid stores of female pups were higher than those of male pups, although males received proportionately more lipid during lactation. This suggests that differential resource use may exist for pups during lactation, and that males may be predisposed to use more lipid. Sex differences in body condition and metabolism have been observed in many species (Beck, 2003; Biuw, 2003; Field *et al.*, 2005; Winship *et al.*, 2001). For pups and juveniles, these sex differences have been related to size dimorphism (i.e., males being larger than females) and females reaching sexual maturity earlier than males (Biuw, 2003; Field *et al.*, 2005). However, in Weddell seals, the age at maturity is similar for males and females (Testa & Siniff, 1987) and they exhibit reverse sexual dimorphism (males are typically smaller than females). Therefore, these traits may not be the driving forces behind sex differences in body condition and metabolism. Another possibility to both size-dimorphic species and Weddell seals is that because larger females have higher reproductive success (because they produce larger pups with higher survival rates), it may be advantageous for females to evolve lipid-sparing mechanisms early in development to improve size at maturity.

Differences in milk composition and yield found between mammals may be

influenced by many factors, including genetics, diet and stage of lactation (Blackburn, 1993). Some aspects of Weddell seal milk composition are different to that of other species. For instance, lipid content at post-parturition (39.9 %) was higher than that reported for southern elephant seals (16.1 %; Hindell *et al.*, 1994) but similar to that of harbour seals (40.8 %; Lang *et al.*, 2005) and grey seals (34.5 %; Mellish *et al.*, 1999). However, temporal changes in lipid differed between species and protein changes (low at post-parturition then subsequently tripling) were the most dissimilar to any other species. There was evidence for differences among individuals; however, milk was on average lipid-rich at parturition to support the pup's thermoregulatory needs at birth and possibly to facilitate pups accompanying their mothers in the water at an early age (starting 10-12 days post-parturition). Increases in lipid later in lactation most likely facilitated an increase in blubber thickness to support higher energy requirements after weaning. Milk at parturition also contained higher levels of saturated fatty acids, providing maximum catabolisable energy for the pup. As lactation progressed milk protein increased for lean tissue growth, but lipid contained higher proportions of monounsaturated fatty acids, offering optimal characteristics for energy storage. Few other studies have examined milk fatty acids over lactation in phocid seals, but instead have sampled on only one occasion (Grahl-Nielsen *et al.*, 2000). Of those studies that have sampled more frequently, Iverson et al. (1995b) found changes in some milk fatty acids between the beginning and end of lactation in hooded seals, although mid-lactation (i.e., day 2) composition did not differ to end-lactation (i.e., day 4). Conversely, Debier et al. (1999), found that fatty acid profiles in harp and hooded seals did not appear to undergo large changes over the course of lactation. Both papers do not include data on all fatty acid changes making it difficult to make broad comparison to Weddell seals. However, the changes in milk lipid, protein and fatty acid composition found here support the notion of a shift in the function of milk and the physiological priorities of the pups during lactation. This may be related to the relatively long lactation and early introduction to swimming and diving for the pups.

7.4 Energy source – fatty acids

Animals obtain energy and nutrients from food, so their diet can be considered

a key element affecting all life history traits (Taylor *et al.*, 2005). The most obvious way that environmental variation may influence body condition and fecundity is through variability in food availability. In general terms, diet effects can be classified as either quantitative (i.e., food availability) or qualitative (i.e., food consumption). The quantitative effects are evident because animals obtain energy from food so there is a positive correlation between food availability and body condition. Qualitative effects may also be evident in body condition if prey species differ in nutritional value (see Trites & Donnelly, 2003), but may also be apparent as variation in the fatty acid composition of lipid stores that constitute the bulk of stored energy reserves in phocid seals.

Fatty acids represent a large group of molecules that comprise the majority of lipids found in all organisms (Budge *et al.*, 2006), and are mobilised to provide metabolic fuel in situations of negative energy balance (Raclot, 2003). Their origin, diversity and biochemical properties has encouraged research ranging from assessment of animal nutrition and metabolism (Budge *et al.*, 2006; Clements *et al.*, 1994; Hagena *et al.*, 2007) to investigating trophic interactions and ecosystem structure from microorganisms (Colaço *et al.*, 2007) to upper trophic level predators (Iverson *et al.*, 1997b). In both terrestrial and marine food webs, the basic fatty acid pattern is laid down by primary producers (Jefferies, 1970) that provide the major metabolic energy in ecosystem food webs. Fatty acid patterns are transferred to higher trophic levels via grazing and predation (Dalsgaard *et al.*, 2003), and it is through this process that fatty acids have been identified as useful trophic markers with which to examine diet.

Based on fatty acid signatures of the blubber at parturition, there were no inter-annual differences in diet for female Weddell seals in this study. Maternal post-partum mass did differ between years, so these differences were most likely related to variability in food availability. There were however, inter-annual differences in the mobilisation of fatty acids during lactation influencing the fatty acid composition of the pup blubber at weaning. Although variation in milk fatty acid transfer could not be examined (milk was only collected in 2003), temporal blubber changes did determine that more saturated fatty acids (14:0 and 16:0) and monounsaturated fatty acids (16:1ω7c, 18:1ω9c and 18:1ω7c) were mobilised from the female blubber in 2002 (the 'good' year, compared to 2003 the 'bad' year), which corresponded to more 14:0, 16:0 and 16:1ω7c in the pup blubber of that year. Differences in fatty acid

mobilisation were related to the dissimilarity in overall condition (total body lipid, kg) of females at parturition. This suggests that the effects of environmental variability (and female condition) can be seen in fine-scale physiological responses such as fatty acid mobilisation.

Temporal changes within the inner and outer blubber layer have not been previously reported for any animal, and this study found that mobilisation of fatty acids and compositional changes were evident in both. However, changes did not occur uniformly. Feeding during lactation (but not total body lipid stores) was an important contributor to fractional mobilisation (i.e., the fraction of initial mass of the fatty acid that was lost during lactation) in both the inner and outer layer (Chapter 6). This indicates that the lack of foraging by smaller females during lactation causes more fatty acids to be mobilised from the outer layer, which is generally less metabolically active and more structural in nature (Fredheim *et al.*, 1995).

Blubber is a dynamic, complex and multifunctional tissue. For marine mammals it serves many roles such as defining the hydrodynamic shape of the body, adjusting buoyancy, insulation and as a source of stored energy (Ryg *et al.*, 1988). The ability of larger females to reduce the amount of fatty acids mobilised from the outer 'structural' layer may be advantageous in decreasing stress to some of the blubbers non-energetic functions, until lipid stores can be replenished during the post-breeding foraging trip.

Similar to hooded seals (Iverson *et al.*, 1995b), the essential polyunsaturated fatty acid 20:5ω3 (strictly a dietary fatty acid) had the highest fractional mobilisation from the whole blubber layer (more specifically the inner layer) during lactation. The proportion of 20:5ω3 was also the highest in the milk immediately post-parturition, indicating that mobilisation of this FA most likely occurred early in lactation when the females were fasting, and that there is a temporal component to fatty acid mobilisation (i.e., some fatty acids may be selectively mobilised at different times depending on energetic or growth requirements of the mothers and pups). In addition, pup blubber fatty acids at weaning did not match those of the mother at post-partum or the milk during lactation, indicating that differential use of fatty acids for growth and storage by the pup occurred.

Understanding the influence of maternal diet and lipid reserves on milk transfer is important for interpreting reproductive patterns, foraging ecology and life history strategies of these species (Iverson, 1993). Fatty acid signature analysis

(FASA) is one of several methods used to determine trophic relationships within ecosystems, and a number of studies have already demonstrated this technique useful in detecting dietary changes qualitatively (Bradshaw *et al.*, 2003; Iverson *et al.*, 1997b; Walton & Pomeroy, 2003). Recent work has focussed on quantitative development of the FASA technique (QFASA, Iverson *et al.*, 2004) to study predator diets, but relies upon calibration coefficients of individual fatty acids to account for biosynthesis, deposition and mobilisation rates within animals. This study identified certain fatty acids as having either high or low mobilisation properties, and these differences had a major impact on qualitative dietary predictions. Dietary predictions including highly mobilised FA are questionable, and their removal from analyses gives more reliable results, consistent with other dietary studies (Burns, 1998; Lake *et al.*, 2003; Plötz, 1986). This demonstrates that differences in FA mobilisation rates affect dietary predictions, and need to be accounted for in future work. However, these findings may be specific to lactation and to Weddell seals. Further research will need to examine other species and other periods when there is a high degree of fatty acid mobilisation (e.g., moult). Nonetheless, this identifies an inherent shortcoming with the present QFASA model, which may require possible recalibration of coefficients or elimination of fatty acids with high mobilisation properties, as they may not be accurately represented within the blubber at the time of sampling. In summary, for dietary investigations, whole blubber samples should be taken immediately at parturition, because samples taken during lactation will not give a good representation of the fatty acid composition from the pre-breeding foraging trip.

Iverson et al. (1995b) found that ingested fatty acids were deposited directly and without modification into the blubber of hooded seal pups. However, this was not the case for Weddell seals, and most likely reflects the longer lactation period (5-6 weeks) compared to the brief one of hooded seals (4 days). Therefore, fatty acid composition of the pup blubber will also not give a good representation of female fatty acid stores. Furthermore, in the case of breeding mammals, milk has been suggested to be a source of fatty acids with which to study diet (Iverson, 1993). In species where a mother fasts throughout lactation, such as with most phocid seals or during the perinatal period of otariid seals, milk fatty acids are thought to reflect the diet during the pre-breeding foraging trip (Iverson, 1993; Iverson *et al.*, 1995b). This has prompted a number of studies investigating the applicability of using milk to estimate maternal diet, often producing conflicting results (Iverson *et al.*, 1997a;

Staniland & Pond, 2004). Although feeding did occur with some study females, fatty acid changes were still evident in the milk of those that did not feed. Therefore, these changes in milk fatty acids throughout lactation supports previous research (Grahl-Nielsen *et al.*, 2000; Staniland & Pond, 2004; Staniland & Pond, 2005) demonstrating that the use of milk to estimate diet is also problematic. Our results highlight that QFASA is only applicable to estimate diet from maternal blubber, and if it is to be applied broadly, species-specific differences will need to be taken into consideration to provide more accurate and robust quantitative estimates of diet.

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Appendix I

Table A1.1 List of fatty acids within each fatty acid group. Boldface indicates essential fatty acids.

Fatty acid group	Fatty acid
SFA	14:0
	i15:0
	16:0
	i17:0
	18:0
SC-MUFA	14:1ω5c
	16:1ω9c
	16:1ω7c
	16:1ω5c
	18:1ω9c
	18:1ω7c
	18:1ω5
LC-MUFA	20:1ω9c
	20:1ω7c
	22:1ω11c*
	22:1ω9c
	24:1
PUFA	**18:4ω3**
	18:2ω6
	20:4ω6
	20:5ω3 EPA
	20:4ω3
	20:2ω6
	22:6ω3 DHA
	22:5ω3 DPA

* includes 22:1ω13c

Appendix II

Table A2.1 *List of species of possible prey items of Weddell seals for which there were no fatty acid profiles for.*

species	
fishes	family
Aethotaxis mitopteryx	Nototheniidae
Artedidraco loennbergi	Artedidraconidae
Artedidraco orianae	Artedidraconidae
Artedidraco skottsbergi	Artedidraconidae
Bathydraco macrolepis	Bathydraconidae
Bathydraco marri	Bathydraconidae
Bathyraja eatoni	Rajidae
Bathyraja maccaini	Rajidae
Chaenodraco wilsoni	Channichthyidae
Chionodraco hamatus	Channichthyidae
Chionodraco myersi	Channichthyidae
Cryodraco antarcticus	Channichthyidae
Cygnodraco mawsoni	Bathydraconidae
Dolloidraco longedorsalis	Artedidraconidae
Gerlachea australis	Bathydraconidae
Gymnodraco acuticeps	Bathydraconidae
Gymnoscopelus opisthopterus	Myctophidae
Histiodraco velifer	Artedidraconidae
Lepidonotothen squamifrons	Nototheniidae
Lycodichthys dearborni	Zoarcidae
Mancopsetta maculata	Achiropsettidae
Neopagetopsis ionah	Channichthyidae
Notolepis coatsi	Paralepididae
Ophthalmolycus amberensis	Zoarcidae
Pachycara brachycephalum	Zoarcidae
Pagetopsis macropterus	Channichthyidae
Pagetopsis maculatus	Channichthyidae
Pagothenia brachysoma	Nototheniidae
Pogonophryne marmorata	Artedidraconidae
Pogonophryne scotti	Artedidraconidae
Prionodraco evansii	Bathydraconidae
Racovitzia glacialis	Bathydraconidae
Rhigophilia dearborni	Piscicolidae
Trematomus eulepidotus	Nototheniidae
Trematomus lepidorhinus	Nototheniidae
Trematomus loennbergii	Nototheniidae
Trematomus nicolai	Nototheniidae
Trematomus scotti	Nototheniidae
Trematomus tokarevi	Nototheniidae
Trematomus vicarius	Nototheniidae
cephalopods	
Pareledone spp.	Octopodidae
Psychroteuthis glacialis	Psychroteuthidae