DAILY ENERGY EXPENDITURE OF NORTHERN FUR SEALS: TECHNIQUES AND MEASUREMENTS

by

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Abstract

Seasonal changes in the daily energy expenditure (DEE) of captive northern fur seals (*Callorhinus ursinus*) and key components of their energy budget (cost of resting metabolism, thermoregulation, activity and growth) were examined to elucidate potential reasons for the species' population decline in the wild. The average DEE of 6 females was 527.8 ± 65.7 kJ kg^{-1} d^{-1} and fluctuated seasonally (~20% greater in the fall than in the winter). Resting metabolism also changed significantly with season, and was higher in the fall (potentially due to molting or as preparation for migratory activity). While resting metabolism was the largest component of the DEE (~80% on average), it did not follow the same seasonal trend as DEE, and therefore was not the source of the seasonal variation in DEE. Cost of activity was the second major component of DEE and may explain the observed seasonal variations. Energetic costs associated with thermoregulation appeared to be negligible. The northern fur seals were thermally neutral in all seasons for all water temperatures tested (2 °C – 18 °C), except during the summer when immersed in 2 °C water. Comparing this broad thermal neutral zone to the average sea surface temperatures encountered by fur seals in the wild during annual migrations indicates that fur seals can likely exploit a large geographic area without added thermal metabolic costs. While the direct energetic costs of growth appeared to be negligible compared to DEE, the higher growth rates in the summer and elevated resting metabolism in the fall suggests that inadequate nutrition could have greater negative effects during these seasons. Two alternative proxies for measuring energy expenditure were tested and calibrated against respirometry for potential application to wild individuals. The doubly labeled water (DLW) method over-estimated DEE by 13.1 ± 16.5% compared to respirometry. In comparison, accelerometry over-estimated DEE, using fine time scale intervals of 60 and 15 min, by an average of 5.4 ± 29.3% and 13.8 ± 39.5%, respectively. Importantly, seasonal effects (and time of day for accelerometry) must be accounted for when estimating energy expenditure from measures of DLW and acceleration in free-swimming northern fur seals.

Preface

I was the main designer of all the experiments described in this thesis, with suggestions from my supervisors Dr. David Rosen and Dr. Andrew Trites. All captive northern fur seal data was collected at the Vancouver Aquarium with approval from the Animal Care Committees of both the Vancouver Aquarium and the University of British Columbia (Permit #A10-0342). I participated in all aspects of the research and data collection in this thesis that included standard operating procedures. The collection of the data was a team effort involving the training and veterinarian staff at the Vancouver Aquarium, the research technicians of UBC's Marine Mammal Research Unit, my co-supervisor David Rosen and myself. Contour plots of the average sea surface temperatures (SST) throughout the Northeast Pacific Ocean for each month of the year, from 1982 to 2012, were obtained from Fisheries and Oceans Canada (http://www.pac.dfo-mpo.gc.ca/science/oceans/data-donnees/sst-tsm/index-eng.htm). Metabolic Solutions Inc. (Nashua, NH) conducted the isotope analysis of the serum and dose samples.

I preformed all the data analysis in this thesis, receiving statistical advice from D. Rosen, C. Gerlinsky, B. Wright, S. Fortune, E. Rechsteiner and V. Noble.

Chapters 2, 3, and 4 were prepared as manuscripts for peer-reviewed scientific journals. Each of these chapters benefited from the comments and editing of the co-authors, D. Rosen and A. Trites and committee member, B. Milsom.

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List of abbreviations

AIC	Akaike information criterion
BMR	Basal metabolic rate
DEE:	Daily energy expenditure
DEE_{DLW}:	Daily energy expenditure estimates obtained *via* the DLW method
DEE_{resp}:	Daily energy expenditure estimates obtained *via* respirometry
DLW:	Doubly labeled water
FEL	Fecal energy loss
FMR	Field metabolic rate
HIF	Heat increment of feeding
LCT	Lower critical temperature
LME	Linear mixed effects (model)
ODBA:	Overall dynamic body acceleration
PDBA:	Partial dynamic body acceleration
$PDBA_{xy}$:	Sum of the dynamic body acceleration obtained using the dorso-ventral (heave) and anterior-posterior (surge) axes
R^2	Coefficient of determination
RMR:	Resting metabolic rate
RQ:	Respiratory quotient
SST	Sea surface temperature
TNZ	Thermal neutral zone
UCT	Upper critical temperature
UEL	Urinary energy loss
$\dot{V}CO_2$:	Rate of carbon dioxide production
$\dot{V}O_2$:	Rate of oxygen consumption
$\dot{V}O_{2\ Air}$:	Average rate of oxygen consumption resting in ambient air conditions
$\dot{V}O_{2\,DEE}$:	Average rate of oxygen consumption over 5-day metabolic measurement trials
$\dot{V}CO_{2\,DEE}$:	Average rate of carbon dioxide production over 5-day metabolic measurement trials

$\dot{V}O_{2\,RMR}$: Average rate of oxygen consumption resting in ambient air conditions

$\dot{V}O_{2\,\text{Water}}$: Average rate of oxygen consumption resting immersed in water (all water temperatures tested)

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Chapter 1: Introduction

The northern fur seal (*Callorhinus ursinus*) is a species of *Pinnipedia*, believed to be the oldest extant member of the *Otariidae* family and the only extant species of its genus (Gentry 1998, Reeves *et al.* 2002, COSEWIC 2010). Inhabiting the North Pacific Ocean, the Sea of Okhotsk, and the Bering Sea, the northern fur seal is the most widespread and numerous otariid in the northern hemisphere (Gentry 1998). However, the northern fur seal has only six major breeding colonies, including two new island colonies established since 1786: San Miguel Island, California established in 1965 and Bogoslof Island, Alaska established in 1980 (DeLong 1982, Loughlin & Miller 1989, Gentry 1998).

Approximately 50% of the world's northern fur seals occur on Alaska's Pribilof Islands (St. Paul and St. George; Testa 2011). This population declined significantly from 1956 to 1980 and began declining again in 1998 (Towell *et al.* 2006, Towell *et al.* 2008). As of 2008, the average annual rate of population decline was approximately 6% on St. Paul Island and 3% on St. George Island (Towell *et al.* 2008). While the initial decline (1956 - 1980) has been linked to the commercial harvest of females, scientific pelagic collections and higher than normal juvenile mortality; explanations for the lack of population recovery since the cessation of those activities and for the current population decline remain elusive (Trites & Larkin 1989, Towell *et al.* 2006). Potential primary explanations include entanglement in discarded fishing gear and other debris, interactions with fisheries, prey limitation, and the indirect effects of ocean climate change; less likely explanations include contaminants, oil spills and predation (COSEWIC 2010).

Occurring concurrently with the decline of the northern fur seal, are population declines in other North Pacific Ocean marine mammal and seabird species, including the sea otter (*Enhydra lutris*), Steller sea lion (*Eumetopias jubatus*) and harbour seal (*Phoca vitulina*) (Springer *et al.* 2003, DeMaster *et al.* 2006). While several indicators suggest that the decline of the Steller sea lion in the 1980s and 90s was a result of nutritional stress, less direct explanatory evidence exists for the sea otter, harbour seal, or northern fur seal (Trites & Donnelly 2003, DeMaster *et al.* 2006, Rosen 2009). Reduced numbers of young females returning to the rookeries on St. Paul Island suggests that nutritional stress may also be contributing to the current decline of the Pribilof Islands' northern fur seal population (Spraker & Lander 2010).

The nutritional stress hypothesis suggests that inadequate nutrition resulting from changes in the quality and quantity of available prey impacts marine mammal populations (Trites

& Donnelly 2003, Rosen 2009). Nutritional stress, by definition, occurs when there is a mismatch between daily energetic requirements and nutritional intake (King & Murphy 1985). Nutritional stress can directly produce negative physiological and/or behavioural states, including reduced birth rates, reduced body size, increased neonate mortality, increased juvenile mortality, changes in blood chemistry and body composition, and behavioural modifications such as longer foraging bouts (Trites & Donnelly 2003). Longer foraging bouts can result in secondary consequences of nutritional stress, such as increased risks of killer whale predation, increased energy intake requirements, and decreased offspring provisioning (COSEWIC 2010).

One strategy for determining the risk of nutritional stress involves calculating the energetic or nutritional requirements of individuals and the likelihood of them fulfilling those obligations. In theory, it should be relatively straightforward to estimate the energetic requirements of members of a population. However, like many mammals, northern fur seals have seasonal life cycles and, thus, highly seasonal energy expenditures and nutritional requirements (which may be temporally offset from each other) that become more pronounced with age and development (Robeck *et al.* 2001, Rosen *et al.* 2012, Rosen & Trites 2014). Such bioenergetic cycles increase the difficulty in making an assessment of potential conditions for nutritional stress and highlight the necessity for estimates of season-specific energetic requirements and expenditures.

Unfortunately, it is virtually impossible to measure the daily energetic expenditures of wild northern fur seals from the late fall to early summer due to this species' pelagic migration (Kenyon & Wilke 1953, Bigg 1990, Gentry 1998, Ream *et al.* 2005, Melin *et al.* 2012). Each year, northern fur seals of the Pribilof and Bogoslof Islands migrate away from the Bering Sea throughout the North Pacific Ocean as far south as California. Adult males migrate only a short distance, wintering in the Bering Sea and the Alaskan and Russian waters of the North Pacific Ocean (Kenyon & Wilke 1953, Bigg 1990, Gentry 1998, Ream *et al.* 2005). Adult females and juveniles of both sexes make a more extensive migration to either the transitional region of the central North Pacific Ocean or across the North Pacific Ocean to the continental margins of North America (Kenyon & Wilke 1953, Bigg 1990, Gentry 1998, Ream *et al.* 2005). Similarly, northern fur seals of the western "population" (the Robben, Kuril and Commander Islands) also undertake a pelagic migration throughout the North Pacific Ocean and the continental margins of Russia (Gentry 1998). In contrast, females from San Miguel Island, California have a less

extensive pelagic migration (male distribution and migration remains unknown). They remain near the continental margins of North America, specifically California, throughout the year and simply shift their distribution northward and offshore during the winter (Melin *et al.* 2012). Therefore, methods of measuring the energy expenditure of northern fur seals in the field must be applicable during the migration including a capability of extended measurement periods.

1.1 Field measurements of energy expenditure

Two methods that are often used to measure field metabolic rates in a variety of marine mammal species, and which may have potential application to the northern fur seal, are the doubly labeled water (DLW) turnover and accelerometry methods (Nagy *et al.* 1999, Halsey *et al.* 2011). However, each of these methods has logistical constraints and predictive limitations that are often species-specific (Speakman 1997, Butler *et al.* 2004, Halsey *et al.* 2011). It is therefore necessary to validate the accuracy of each method's estimate of energy expenditure with more direct measures — such as those acquired *via* respirometry — prior to its application on members of a wild population (Butler *et al.* 2004, Halsey *et al.* 2011).

The doubly labeled water (DLW) method, based on isotope washout dynamics, estimates an individual's CO_2 production using the differential elimination of heavy hydrogen (^2H) and oxygen isotopes (^{18}O) introduced into the body water (Speakman 1997). The DLW method was first developed in the 1950s, and by the early 2000s nearly 120 studies using this technique were being published annually (Butler *et al.* 2004). Previous studies using the DLW method have measured the energy expenditure of a variety of mammalian (including pinnipeds), avian and reptilian species, in both wild and captive settings (Lifson *et al.* 1955, Nagy *et al.* 1999, Speakman *et al.* 2001, Sparling *et al.* 2008).

The DLW method is a relatively non-invasive technique that permits the estimation of energy expenditure in free-ranging, naturally behaving individuals, with the only disruptive requirements being the capture of the animal twice for the procurement of blood samples and the injection of the labeled isotopes (Speakman 1997, Butler *et al.* 2004). However, the cost of the labeled isotopes is high (particularly for large individuals) and the mathematics of converting the raw isotope turnover data into estimates of daily energy expenditure is complex and often not adequately considered (Speakman 1997, Butler *et al.* 2004). The specific mathematical method used for combining the raw data from an array of potential models must be determined prior to its use on wild individuals, as it can have a substantial impact on the final estimate of the energy

expenditure (Speakman 1997). Furthermore, only a limited number of validation studies for the DLW method have been attempted with marine mammals, and their results indicate additional species-specific calibrations are required (Costa 1987, Boyd *et al.* 1995, Sparling *et al.* 2008).

The use of measures of body accelerometry to estimate energy expenditure works on the principle that animals expend energy to contract their muscles during activity, leading to the acceleration of the limbs and body (Wilson *et al.* 2006, Halsey *et al.* 2009b). Therefore, accurate measures of the dynamic acceleration about the center of the animal's mass in all 3 body axes should theoretically be closely correlated with the individual's energy expenditure (Wilson *et al.* 2006, Halsey *et al.* 2009a). Summing the dynamic acceleration in each of the 3 body axes has been termed Overall Dynamic Body Acceleration (ODBA).

The concept of accelerometry was first introduced as a proxy for energy expenditure in the 1960s to better understand human biology and the mechanical work of walking (Cavagna *et al.* 1963, Green *et al.* 2009). However, the use of accelerometry to estimate energy expenditure in animals has been facilitated more recently by advancements in the miniaturization of data loggers (Wilson *et al.* 2006, Green *et al.* 2009, Enstipp *et al.* 2011). Since 2008, at least 10 studies involving ODBA (predominantly animal studies) have been published annually, with that number growing each year.

Interest in developing the accelerometry method as an effective means of estimating energy expenditure stems from the fact it is less expensive than the DLW method, provides data with finer temporal resolution, and can be applied over longer measurement periods (Fahlman *et al.* 2008b). Previous studies on a few species of marine mammals (Weddell seals *Leptonychotes weddellii*; Williams *et al.* 2004, and Steller sea lions; Fahlman *et al.* 2008b) have presented evidence for the usage of ODBA, with the caveat of – at minimum – the need for species-specific calibration equations. However, questions still remain about the overall ability of accelerometry to effectively predict energy expenditure (Halsey *et al.* 2011). Of particular concern is whether long-term estimates are compromised by the inability of acceleration to measure energetic changes that are independent of activity (*e.g.*, thermoregulation, growth, reproduction, and basal and resting metabolic rate; Halsey *et al.* 2011) — a question which must be adequately validated prior to using accelerometers to measure energy expenditure in wild individuals over extended periods of time.

1.2 Seasonal changes in energy requirements of the northern fur seal

The daily energetic expenditure of the northern fur seal throughout the year is predicted to be highly seasonal, as a cumulative response to seasonal variation in different individual components of the energy budget (Robeck *et al.* 2001, Rosen *et al.* 2012, Rosen & Trites 2014). Currently, however, there are no published measures of the daily energetic expenditure of the northern fur seal for extended periods, with the exception of one study that measured field metabolic rates (FMR) of fasting and foraging adult female northern fur seal during the breeding season (Costa & Gentry 1986). These FMRs were found to be 3.5 to 6.2 times higher than Kleiber's allometric prediction for the basal metabolic rate (BMR) of adult mammals, and are consistent with the FMRs obtained for Antarctic fur seals (*Arctocephalus gazella*), California sea lions (*Zalophus californianus*) and harbour seals (2.3 - 6.7 x BMR; Kleiber 1975, Costa & Gentry 1986, Costa & Trillmich 1988, Costa *et al.* 1989, Boyd & Duck 1991, Costa *et al.* 1991, Bowen *et al.* 1992, Arnould *et al.* 1996b).

In addition to seasonal changes in total daily energy expenditure and its component parts, fur seals should also make seasonal adjustments in the priorities they set for fulfilling the different components of their energy budgets (Rosen & Kumagai 2008). In other words, individuals that cannot completely satisfy all of their energy needs should show seasonal preferences for allocating energy to certain components of their energy budgets over others. As a result, shifts in energetic priorities would result in different consequences for an episode of nutritional stress, depending on the time of year that it occurs (Rosen & Kumagai 2008).

Four key components of the energy budget of the northern fur seal are believed to be the costs of resting metabolic rate, growth, thermoregulation and activity. Resting metabolic rate (a standard measure of baseline energy expenditure) is known to change seasonally, independent of other direct bioenergetic concerns (such as growth and thermoregulation) in both young northern fur seals (Rosen & Trites 2014) and other mature pinnipeds (Rosen & Renouf 1998, Sparling *et al.* 2006). The costs of growth are likely higher during the spring and summer months when the body size of immature fur seals increase (Trites & Bigg 1996). Similarly, higher metabolic rates to maintain core body temperatures are predicted when environmental temperatures fall outside of the northern fur seal's thermal neutral zone (TNZ) (Schmidt-Nielsen 1997, Williams & Worthy 2002). Past studies have, however, failed to reach a consensus on the thermal capabilities of young northern fur seals (estimates of the lower critical temperature in water have ranged

from ~4 to 18 °C; Miller 1978, Trites 1990, Donohue *et al.* 2000, Liwanag 2010, Rosen & Trites 2014). Metabolic rate is also predicted to be associated with the northern fur seals' activity levels (Darveau *et al.* 2002). High activity and metabolic rate are predicted in the late fall and early winter, corresponding to the time of year when rapid migration to the wintering grounds would naturally be occurring; in comparison, lower activity and associated metabolic rates are predicted during the late winter and summer when relatively localized foraging is occurring (Kenyon & Wilke 1953, Bigg 1990, Gentry 1998, Ream *et al.* 2005).

1.3 Research goals

The aim of my thesis was to fill data gaps in the current understanding of seasonal variation in the northern fur seals' daily energy expenditure (DEE) and changes in their seasonal energetic priorities, as well as to calibrate two techniques for the measurement of energy expenditure in wild individuals. I seasonally measured the daily energy expenditure of 6 captive juvenile female northern fur seals held at the Vancouver Aquarium. I took simultaneous measurements of energy expenditure *via* accelerometry, the DLW method and respirometry (oxygen consumption), with the latter considered to be the most accurate estimate of true energy output. Additionally, I measured the 4 key components of the animals' energy budget within each season: the costs of growth (*via* changes in body mass, length, and body composition), activity level, resting metabolic rate, and thermoregulation. This allowed me to evaluate:

1. Innate physiological changes in the energy budgets and energetic priorities of northern fur seals,
2. The potential effect of thermoregulatory costs across a range of environmental conditions on total energy requirements,
3. The effectiveness of two techniques – doubly labeled water turnover and accelerometry – to estimate metabolism, and whether this differs by season.

1.4 Captive animal studies

As a result of the substantial pelagic migration that northern fur seals undertake, there is limited information available on their biology from the late fall to early summer. Much of what is known was obtained from either the scientific harvests of the North Pacific Fur Seal Commission (1958 to 1974), or from studies conducted in Alaska during the breeding season. Studies with animals in laboratory conditions are another source of information and are beneficial due to their capacity for controlled experiments and the collection of detailed data

using techniques that cannot be applied in the wild. However, studies using captive animals are generally limited in their ability to simulate natural conditions, since they fail to mirror wild conditions. For example, bioenergetic studies conducted under such circumstances are unable to incorporate costs associated with prey capture, prey selection and deep dives, and therefore may ultimately be unable to directly determine if nutritional stress is occurring amongst wild populations (Rosen 2009).

Despite the inherent shortcomings of captive studies, intrinsic physiological changes have been shown to occur within a captive pinniped's energy budget, including seasonal shifts in the costs of resting metabolism, growth, thermoregulation, and, to a degree, activity (Rosen & Renouf 1998, Donohue *et al.* 2000, Sparling *et al.* 2006, Liwanag 2010, Rosen *et al.* 2012, Rosen & Trites 2014). Therefore, the pattern and costs of seasonal changes in both total energy use and in the major components that make up their energy budget can be experimentally quantified and provide insight into the same processes that are likely to occur in the wild population. Additionally, studies in the laboratory can identify the northern fur seals' energetic priorities, and inferences can be made about the seasonal nutritional status of wild fur seals.

1.5 Thesis structure

This thesis is organized into 5 chapters. The three central chapters (Chapters 2, 3 and 4) detail specific, inter-linked experiments within the overall study. They are written as self-contained manuscripts and have some necessary repetition of information.

Chapter 2 focuses on a single component of the northern fur seals' energy budget — the thermal costs of aquatic exposure — to calculate potential expenses associated with a pelagic existence and determine whether innate thermal capabilities might limit the seasonal distribution of the northern fur seal. The lower critical temperature (LCT) in water, the temperature below which homeotherms must expend additional energy to maintain their body temperature, for the northern fur seal based on previous literature is hypothesized to be as low as 4 °C (Donohue *et al.* 2000, Liwanag 2010, Rosen and Trites 2014), or as high as 15 °C - 20 °C (Miller 1978, Dawson and Fanning 1981, Costa and Kooyman 1982, Williams 1986, Rutishauser *et al*. 2004).

In Chapter 3, the more direct measures of daily energy expenditure *via* respirometry are compared to the alternative methods of accelerometry and doubly labeled water turnover, to evaluate the predictive capabilities and logistical constraints of these alternative methods for potential future use with wild individuals. Based on previously published literature, it is

hypothesized that the DLW method would provide a reasonably accurate measure (< 10% error) of the daily energy expenditure of northern fur seals (Boyd *et al.*, 1995; Sparling *et al.*, 2008; Speakman, 1997), and that accelerometry would provide an almost equally accurate measure within a given season (~ 10% error; Halsey *et al.*, 2009a).

Chapter 4 examines seasonal changes in both the daily energy expenditure and different components of the northern fur seals' energy budget (specifically the costs of resting metabolism, growth, thermoregulation, and activity). I identify the contribution of these components to the northern fur seal's energy budget, quantify how they vary throughout the year, and infer critical times throughout the year in the event of a potential nutritional stress event. The DEE of northern fur seals should change significantly throughout the year as a cumulative response to seasonal variations in individual components of their energy budgets. While seasonal shifts in the energetic costs associated with the different major components of an animal's energy budgets (*i.e.*, resting metabolic rate, thermoregulation, activity, and growth) are relatively straightforward to predict. The cumulative effect of simultaneous changes in these individual bioenergetic costs on total energy expenditure is much more difficult to foresee.

Finally, Chapter 5 provides a general conclusion and reiterates and synthesizes the key findings of the thesis. In addition, I explore the broader hypothesis that the decline of the northern fur seal on the Pribilof Islands is related to an inability to acquire and digest sufficient prey necessary to meet the energetic requirements.

Overall, my thesis aims to contribute to the current body of knowledge regarding the bioenergetics and ecology of northern fur seals by filling data gaps about seasonal variation in the daily energy expenditure and energetic priorities of the northern fur seal – information that is currently unobtainable from wild individuals. Additionally, the evaluation of different methodologies that measure energy expenditure in the captive settings might open new avenues of understanding for the northern fur seals in the wild.

Chapter 2: Broad thermal capacity facilitates the primarily pelagic existence of northern fur seals

2.1 Summary

Thermoregulatory capacity may constrain the distribution of marine mammals despite having anatomical and physiological adaptations to compensate for the thermal challenges of an aquatic lifestyle. We tested whether subadult female northern fur seals (Callorhinus ursinus) experience increased thermoregulatory costs in water temperatures potentially encountered during their annual migration in the Bering Sea and North Pacific Ocean. Metabolic rates were measured seasonally in 6 captive female northern fur seals (2.75 to 3.5 yr old) in ambient air and controlled water temperatures of 2 °C, 10 °C, and 18 °C. Rates of oxygen consumption in ambient air (1 °C – 18 °C) were not related to environmental temperature except below 2.5 °C (winter only). However, metabolism was significantly higher during the fall seasonal trials (Sept – Oct) compared to other times of year, perhaps due to the costs of molting. The fur seals appeared thermally neutral in all seasons for all water temperatures tested (2 °C – 18 °C) except during the summer when metabolic rates were higher in the 2 °C water. Comparing this broad thermal neutral zone to the average sea surface temperatures potentially encountered during annual migrations indicates wild fur seals can likely exploit a large geographic area without added thermal metabolic costs.

2.2 Introduction

Physiological and anatomical modifications are required to live in an aquatic environment and offset the thermal properties of water, which conducts heat away from the body 24 times faster than air of the same temperature (Dejours 1987). Specialized insulation is one common adaptation among marine mammals and other endotherms to reduce heat loss to the environment (Scholander *et al.* 1950, Williams & Worthy 2002). In many species of marine mammals, insulation from the aquatic environment is accomplished *via* a thick subcutaneous lipid (blubber) layer (Scholander *et al.* 1950, Strandberg *et al.* 2008, Liwanag *et al.* 2012b). However, some aquatic and semi-aquatic mammals, such as beavers, otters, and fur seals, have thin subcutaneous lipid layers, and rely instead upon a dense pelage and its associated structures for insulation during immersion (Irving *et al.* 1962, Williams *et al.* 1992, Liwanag *et al.* 2012a).

Unfortunately, even a dense pelage may not provide sufficient insulation against the cold-water conditions that furred mammals are likely to encounter. Lower critical temperatures (LCT) in water, the temperature below which homeotherms must expend additional energy to maintain their body temperature, are reported to be 20 °C for sea otters (*Enhydra lutris*; Costa & Kooyman 1982) and 14.4 °C for juvenile (pup and yearling) Antarctic fur seals (*Arctocephalus gazella*; Rutishauser *et al.* 2004). The fact that these lower critical temperatures are well above the usual water temperatures of their habitats implies that increased metabolic rates are required to maintain the core body temperatures of these and potentially other marine mammals that rely upon dense pelages for insulation (Costa & Kooyman 1982, Williams & Worthy 2002, Rutishauser *et al.* 2004).

Elevated resting metabolic rates, compared to similar sized terrestrial mammals, have been measured in many species of marine mammals, including several species of pinnipeds (Irving & Hart 1957, Costa & Kooyman 1982, Kasting *et al.* 1989, Hurley & Costa 2001, Williams *et al.* 2001, Boyd 2002). An increased metabolic rate is an effective short-term solution for maintaining core body temperatures and is a normal physiological response to environmental temperatures that are outside of the thermal neutral zone (TNZ) of an individual (Irving & Hart 1957, Gordon *et al.* 1972, Williams & Worthy 2002, Liwanag 2010). However, an increased metabolic rate represents a substantial added energetic expense if used as a long-term adaptation (Williams & Worthy 2002, Liwanag *et al.* 2009). As a result, the geographic range of most endotherms is likely constrained in part by thermal considerations (Angilletta 2009).

The northern fur seal (*Callorhinus ursinus*) is a species of pinniped that is assumed to depend primarily on its pelage for insulation, based on the thinness of its fat layer (Blix *et al.* 1979) and other morphological evidence (Liwanag *et al.* 2012a, Liwanag *et al.* 2012b). However, past studies have failed to reach a consensus on the thermal capabilities of young northern fur seals — although the lower critical water temperature is commonly estimated to be between 4 °C and 10 °C (Donohue *et al.* 2000, Liwanag 2010, Rosen & Trites 2014), one study placed the LCT at >18 °C (Miller 1978). This last estimate falls within the range of lower critical water temperatures of 15 °C - 20 °C reported for similar sized (non pup) aquatic and semi-aquatic mammals with dense pelages (Dawson & Fanning 1981, Costa & Kooyman 1982, Williams 1986, Rutishauser *et al.* 2004).

The thermal neutral zone of juvenile northern fur seals is of more than simply an academic interest. Environmental water temperatures below the LCT will cause northern fur seals to increase their metabolic rates to generate replacement body heat. These higher energy demands would have to be offset by increased food intake, and would potentially exclude the fur seals from exploiting habitats where satisfying the additional energetic costs of thermal regulation may be too high.

There are only three major breeding colonies of northern fur seals in North America (DeLong 1982, Loughlin & Miller 1989, Gentry 1998, COSEWIC 2010, Testa 2011). Each fall, individuals from two of these colonies — the Pribilof and Bogoslof Islands in Alaska — migrate from the Bering Sea to the North Pacific Ocean, travelling as far south as California (Kenyon & Wilke 1953, Bigg 1990, Gentry 1998, Ream *et al.* 2005). Adult females and juveniles of both sexes make a more extensive migration than adult males, to either the transitional region of the central North Pacific Ocean or across the North Pacific Ocean to the continental margins of North America (Kenyon & Wilke 1953, Bigg 1990, Gentry 1998, Ream *et al.* 2005). In contrast to the fur seals breeding in the Bering Sea, females from the third colony (San Miguel Island, California) have a less extensive pelagic migration (male distribution and migration remains unknown), remaining near the continental margins of North America throughout most of the year (primarily California), and shifting their distribution northward and offshore during the winter (Melin *et al.* 2012).

The lengthy migration of the northern fur seal (late fall to early summer) makes it difficult to evaluate the thermal capacity of this species in direct relation to environmental conditions (Kenyon & Wilke 1953, Bigg 1990, Gentry 1998, Ream *et al.* 2005). The extent to which their migration patterns are constrained by thermal capabilities is unknown, as is the degree to which environmental temperatures might affect thermoregulatory costs and subsequent prey requirements in certain foraging areas.

We measured the thermal costs of aquatic exposure in northern fur seals to quantify the potential energetic expenses associated with their pelagic existence and to investigate whether the thermal capabilities of this species might limit their seasonal distribution. Specifically, we measured the metabolic rates of captive northern fur seals (juvenile females) in water temperatures between 2 °C and 18 °C and compared them to metabolic rates when resting in dry ambient air conditions. We also investigated the thermoregulatory abilities of the individuals

over the course of the year to explore potential seasonal changes. These thermoregulatory abilities were then compared to the documented average sea surface temperatures along the generalized migration routes of female and juvenile northern fur seals. We examined whether their generalized migration route appeared constrained by these thermal costs, or whether these routes took them outside of their thermal neutral zone. This allowed us to identify potentially critical times of year when elevated metabolic rates due to thermoregulatory demands and increased food requirements might be expected.

2.3 Methods

2.3.1 Animals

Six female northern fur seals (ages 2.75 – 3.50 yr) participated in this study from March 2011 to January 2012 (Table 2.1). The animals came from a rookery on St. Paul Island, Alaska (one of the Pribilof Islands) in October 2008, following weaning at approximately 4 mo of age. The individuals were transported to the University of British Columbia's Marine Mammal Energetics and Nutrition Laboratory, located at the Vancouver Aquarium, British Columbia, Canada. They were raised in captivity and trained with positive reinforcement to be familiar with all necessary research protocols and equipment. The fur seals normally consumed a daily diet of herring and squid, supplemented with vitamins, and were housed in seawater pools with water temperatures that reflected the local ocean conditions (7 °C to 16 °C).

2.3.2 Protocol and timing

Four seasonal sets of metabolic measurement trials were conducted during the study period: Mar/Apr 2011 ("Spring"; individuals 2.75 yr old), Jun/Jul ("Summer"; Age 3 yr), Sept/Oct ("Fall"; Age 3.25 yr) and Dec 2011/Jan 2012 ("Winter"; Age 3.5 yr), with each set of trials taking approximately 4 - 6 wk to complete. Within each set of trials, the metabolic rate of each individual (see below) was tested both in ambient air conditions as well as at three different water temperatures: 2 °C, 10 °C, and 18 °C (± 0.5 °C).

On three separate occasions within each season (once for each water temperature treatment), each fur seal voluntarily entered a specially designed 340 L metabolic chamber (dimensions: 0.92 m. x 0.61 m x 0.61 m) under trainer control. The individuals were trained to remain calm, with minimal activity, within the chamber. Rates of oxygen consumption and

Table 2.1 Body mass (kg) and mass-specific rate of oxygen consumption resting in dry ambient air conditions ($\dot{V}O_{2\ air}$; mL O_2 kg^{-1} min^{-1}) during four seasonal trials (spring, summer, fall, and winter) for each of the six female northern fur seals from March 2011 to January 2012. Mean ± SD are presented for all six animals as well as for the truncated data set resulting from the removal of individual ME08 that did not behaviourally meet resting conditions.

Season	Spring		Summer		Fall		Winter	
Animal	Body Mass	$\dot{V}O_{2\ air}$	Body Mass	$\dot{V}O_{2\ air}$	Body Mass	$\dot{V}O_{2\ air}$	Body Mass	$\dot{V}O_{2\ air}$
AN08	19.8 ± 0.6	13.4 ± 2.0	21.2 ± 0.9	11.4 ± 0.1	22.2 ± 0.5	18.1 ± 4.5	21.2 ± 0.3	14.1 ± 8.3
AY08	14.2 ± 0.08	14.6 ± 1.0	17.0 ± 0.9	11.9 ± 1.3	18.4 ± 0.09	20.3 ± 2.6	17.7 ± 0.3	13.3 ± 4.5
KY08	15.3 ± 0.05	11.3 ± 0.4	17.6 ± 0.8	14.0 ± 2.0	19.5 ± 0.1	18.1 ± 4.6	15.6 ± 0.2	13.3 ± 2.0
ME08	15.6 ± 0.2	25.2 ± 2.8	17.1 ± 0.4	23.0 ± 5.7	19.8 ± 0.3	36.5 ± 2.5	19.3 ± 0.1	29.4 ± 10.1
TI08	18.9 ± 0.2	14.6 ± 1.0	22.6 ± 0.6	12.3 ± 2.4	26.6 ± 0.8	17.8 ± 2.2	25.1 ± 0.1	8.8 ± 3.1
TU08	15.2 ± 0.1	14.1 ± 1.7	18.6 ± 0.8	21.9 ± 3.7	21.4 ± 0.3	22.2 ± 2.5	20.3 ± 0.4	25.0 ± 5.3
Mean (all animals)	16.5 ± 2.3	15.2 ± 5.1	19.0 ± 2.3	15.7 ± 5.3	21.4 ± 2.9	22.1 ± 7.2	20.5 ± 2.5	17.3 ± 8.0
Mean (ME08 omitted)	16.7 ± 2.5	13.1 ± 1.4	19.4 ± 2.4	14.3 ± 4.3	21.7 ± 3.1	19.3 ± 1.9	20.8 ± 2.7	14.9 ± 6.0

carbon dioxide production of the fur seal were continuously measured in the metabolic chamber for 20 min in dry ambient air conditions. Immediately following the dry ambient air measures, the chamber was filled two-thirds full (sufficient to cover the animal's entire torso, but prevent swimming) with water at one of the three different treatment temperatures (2 °C, 10 °C, or 18 °C). It took approximately 5 min to fill the chamber and mechanical fail-safes prevented the water level from rising above the set point. Once immersed, rates of oxygen consumption and carbon dioxide production were again continuously measured for an additional 30 min (supported by the time required for heat flux density measurements through the skin of newborn northern fur seals to reach steady state, Blix *et al.* 1979, and consistent with Donohue *et al.* 2000) with water continuously being pumped through the chamber from a large temperature-controlled reservoir.

Trials were conducted in the morning and individuals were tested only once daily. Individuals were fasted overnight (>16 hr) to ensure a post-absorptive state had been reached. The order of water temperature treatments tested was consistent (10 °C, 2 °C, and 18 °C) within each trial block. Animal behaviour, air temperature and water temperature (when appropriate) were recorded every 5 min throughout each trial.

2.3.3 Metabolic rate

Open flow respirometry was used to measure rates of oxygen consumption and carbon dioxide production to determine metabolic rates. Measurements were made using one of two systems. First, ambient air was drawn through the metabolic chamber at 125 L min^{-1} *via* either the Sable Systems Model 500H Mass Flow Controller (Sable Systems, Las Vegas, NV) or the Sable Systems Field Metabolic Pump, both of which constantly corrected the flow rate to standard temperature and pressure. Next, subsamples of air from the excurrent airstream were dried through a canister of anhydrous $CaSO_4$ (Drierite; Hammond Drierite, Xenia, OH), before the O_2 and CO_2 concentration were analyzed by the Sable Systems FC-1B and CA-1B analyzers, respectively, or using the Sable Systems Field Metabolic System (P-Series). The resultant O_2 and CO_2 concentrations in the excurrent air were continuously monitored and recorded to a portable computer using Sable Systems' Expedata software.

The entire gas analysis system was calibrated with dry ambient air prior to and following each trial, such that changes in gas concentrations were determined against baseline (ambient) measures that accounted for system drift. The entire gas analysis system was also periodically

calibrated against gases of known concentrations. Rates of oxygen consumption were calculated for both the animal resting in dry ambient air conditions ($\dot{V}O_{2\,air}$) and while the animal was submerged in each one of the three different water temperatures ($\dot{V}O_{2\,water}$), using LabAnalystX software (M. Chappell, UC Riverside, Riverside, CA) and incorporating the appropriate equations from Withers (1977). The $\dot{V}O_{2\,air}$ and $\dot{V}O_{2\,water}$ were determined as the lowest continuous average oxygen consumption maintained for 10 min during the last 15 min of the dry phase and the last 25 min of the wet phase. A malfunctioning CA-1B analyzer (CO_2 sensor) was detected in a portion of the first two seasonal cycles of this study. For a few trials, with an average RQ value over the entire dry-wet metabolic session outside of a reasonable physiological range (0.65 - 1.05), $\dot{V}O_{2\,air}$ was calculated using a fixed RQ value of 0.8 rather than actual measures of expired CO_2.

2.3.4 Mass and body composition

The deuterium (D_2O) dilution technique was used once on each of the fur seals in each seasonal cycle to determine their total body water (Reilly & Fedak 1990). Body composition (total body lipid) was then estimated from the total body water using the "all animal – adult and pup" regression equation validated by Arnould *et al.* (1996a) for Antarctic fur seals. Briefly, blood samples were obtained from the animals while under veterinary supervised anesthesia (maximum 5% isoflurane). An initial blood sample was drawn into a serum separator tube prior to the administration of the D_2O, such that the background levels of ^2H could be assessed. The injection of 99.9% D_2O was then administered intramuscularly at a measured dosage of approximately 0.16 g kg^{-1} of animal. A second blood sample was drawn 2 hr post injection (permitting equilibration with the body water pool) to assess the increase in the concentration of ^2H. Animals were awake and kept in a holding run with a circular wading pool and running water during the 2 hr equilibration period; this did not confound the calculation of total body water as these individuals have never been observed to drink water.

Blood samples were centrifuged and the collected serum was stored at -70 °C until analysis. Metabolic Solutions Inc. (Nashua, NH) conducted the isotope analysis of the serum and dose samples, using a Europa Hydra continuous flow isotope ratio mass spectrometer and the methodology described by Scrimgeour *et al.* (1993). Body mass measurements of each fur seal (necessary for determining the proper dosage of D_2O and as a measurement of growth) were

collected daily prior to the first injection and first feed (at least 16 hr postprandial) by having the fur seal station on a platform scale (± 0.02 kg).

Blubber thickness measurements were collected once on each of the fur seals during each seasonal cycle using a Sonosite 180 Plus ultrasound system. The transducer probe was placed posteriorly to the pectoral flippers and ventral to the spinal column. Measures of blubber thickness were determined directly on the output image on the ultrasound system using the inbuilt measuring tools.

2.3.5 Data analysis

Seasonal changes in rates of oxygen consumption (metabolic rate) over the study period in either ambient air conditions or when immersed in water were determined using separate linear mixed effects (LME) models, with the random effect of the individual included to account for repeated measures. If significant differences were detected, we used a *post-hoc* Tukey contrasts simultaneous test for general linear hypotheses to determine between which seasons the significant differences occurred. Additionally, LME models, with individual again as the random effect, were used to investigate the effects of either the ambient air or water temperatures on the appropriate metabolic rates throughout the year and within individual seasons. As ambient air temperature was found to be significantly affecting the mass-specific $\dot{V}O_{2\,\mathrm{air}}$ in the winter, ambient air temperature was included as a covariate when testing the effects of water temperature on metabolic rate throughout the year and within individual seasons.

The potential costs of thermoregulation were determined as the paired difference between the dry ambient air and wet immersed mass-specific $\dot{V}O_2$ within an individual session. Innate rhythmicity is fundamental to a seasonal mammal's biology (Warner *et al.* 2010). Circadian oscillators regulate the day-night cycle of metabolic and behavioural processes, and photoperiod affects the annual rhythm of energy metabolism, molting, and reproduction (Warner *et al.* 2010). Therefore, this approach controlled for any variance in resting metabolism, which might obscure any real changes in metabolism due to costs of thermoregulation (*i.e.*, reduces Type II error). LME models, with the individual as the random effect, were used to investigate seasonal changes in the potential costs of thermoregulation over the course of the study. As season was found to be a significant predictor of the potential costs of thermoregulation, separate models within each season were also constructed to determine the effects of the water temperature on the potential costs of thermoregulation. If significant differences were detected, a *post-hoc* Tukey contrasts

simultaneous test for general linear hypotheses was used to determine between which temperatures the significant differences occurred. Single sample *t*-tests were used to determine if the potential costs of thermoregulation (*i.e.*, increase in metabolism) at any given temperature was significantly different than zero.

Seasonal changes over the study period in body mass, body composition, blubber thickness, and their respective rates of change were also determined using LME models, with the random effect of the individual included to account for repeated measures. Again, if significant differences were detected, a *post-hoc* Tukey contrasts simultaneous test for general linear hypotheses was used to determine between which seasons the significant differences occurred. All statistical analyses were conducted using the R software package (R Development Core Team 2012).

2.3.6 Sea surface temperature encountered during the annual migration

Contour plots of the average sea surface temperatures (SST) were obtained for the Northeast Pacific Ocean by month (1982 to 2012) from Fisheries and Oceans Canada (http://www.pac.dfo-mpo.gc.ca/science/oceans/data-donnees/sst-tsm/index-eng.htm). Different iterations of the annual migration patterns of juvenile and adult female northern fur seals from North American rookeries were overlaid onto these contour plots. While the individual migratory studies covered various time periods from 1871 to 2006 (Kenyon & Wilke 1953, Bigg 1990, Gentry 1998, Ream *et al.* 2005, Melin *et al.* 2012), there is a high degree of consistency between descriptions. Specifically, they considered time frames of 1955 to 1977 (Bigg 1990), 1871 to 1977 (Gentry 1998), 1871 to 1911 and 1940 to 1948 (Kenyon & Wilke 1953), 2002 (Ream *et al.* 2005) and 2006 (Melin *et al.* 2012). Combined, the contour plots and descriptions of the migration pattern allowed us to estimate the minimum and maximum average SST that juvenile female northern fur seals encountered during each month of their annual migration through the Bering Sea and North Pacific Ocean (Fig. 2.1 — *e.g.*, month of November in 1982 with migration pattern as described by Ream *et al.* 2005).

2.4 Results

2.4.1 Metabolic rates in ambient air

Rates of oxygen consumption and other variables are presented as means ± 1 SD. The mean rate of oxygen consumption of the northern fur seals resting in dry ambient air conditions ($\dot{V}O_{2\,air}$) across all individuals and all seasons was 339.2 ± 148.6 mL O_2 min^{-1} which, on a mass-

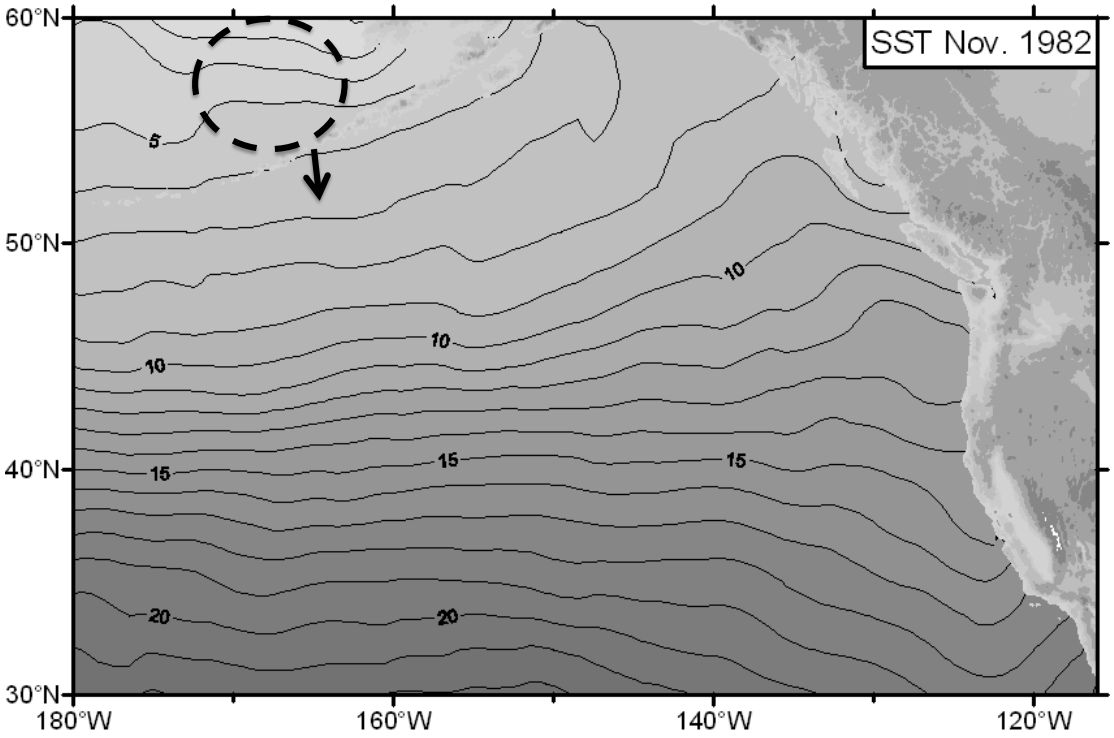

Figure 2.1 Example of the relationship between described locations of northern fur seals (within dotted zone and arrow) and monthly average sea surface temperature in November of 1982 (isocline bars in °C). Fur seal locations are based on the annual migration pattern movement data from Ream *et al.* (2005), for northern fur seals from the Pribilof Islands during a single month (November). The presumed location during each month as based on the variously described migration patterns (see text) is plotted onto a contour map of the northeast Pacific Ocean displaying the sea surface water temperatures (SST) for that month of the region, for each year from 1982 to 2012 (data from Fisheries and Oceans Canada, http://www.pac.dfo-mpo.gc.ca/science/oceans/data-donnees/sst-tsm/index-eng.htm). Collective results are summarized in Fig. 2.5.

specific basis, was 17.6 ± 7.3 mL O_2 kg^{-1} min^{-1} (Table 2.1). However, one individual (ME08) was unusually active during these trials, and yielded data that did not reflect resting conditions. Omitting the $\dot{V}O_{2\ air}$ data from this individual, the average mass-specific $\dot{V}O_{2\ air}$ of the remaining northern fur seals, across all seasons, was 15.4 ± 5.1 mL O_2 kg^{-1} min^{-1} (Table 2.1). This translated to an average in-air resting metabolic rate that was 3.2 times Kleiber's (1975) prediction for similar-sized adult terrestrial mammals. The average mass-specific $\dot{V}O_{2\ air}$ was significantly different among seasons (P = 0.001) and was significantly higher in the fall seasonal trials (19.3 ± 3.4 mL O_2 kg^{-1} min^{-1}; 4.2x Kleiber) compared to the other three seasonal trials (overall mean 14.1 ± 4.9 mL O_2 kg^{-1} min^{-1}; 2.9x Kleiber; P = 0.005), which did not differ significantly from one another (P = 0.5; Table 2.1).

Ambient air temperatures in the metabolic chamber during these trials did change significantly over the course of the study (P = 0.001). The highest average ambient air temperature of 13.0 °C ± 3.4 °C occurred during the summer trials, compared to the average ambient air temperatures of 8.6 °C ± 2.3 °C during the spring, 10.6 °C ± 2.7 °C during the fall and 6.6 °C ± 4.3 °C during the winter seasonal trials. Ambient air temperature, however, had no significant effect on the mass-specific $\dot{V}O_{2\ air}$ of the northern fur seals across all trials (P = 0.4), or within the spring, summer or fall seasons (ambient air temperatures range during spring, summer, and fall was 8 °C – 18 °C; P = 0.3). Ambient air temperature significantly affected the mass-specific $\dot{V}O_{2\ air}$ in the winter (P = 0.04), with the mass-specific $\dot{V}O_{2\ air}$ appearing to increase at temperatures below 2.5 °C (Fig. 2.2). Removing the mass-specific $\dot{V}O_{2\ air}$ data measured in ambient air temperatures below 2.5 °C, the average mass-specific $\dot{V}O_{2\ air}$ for the winter trials decreased to 11.4 ± 3.9 mL O_2 kg^{-1} min^{-1}, yet the overall seasonal trend in the mass-specific $\dot{V}O_{2\ air}$ did not change (*i.e.*, fall metabolism was higher than all other seasons).

2.4.2 Metabolism during wet thermal challenges

The mean rate of oxygen consumption of the northern fur seals across all individuals (omitting individual "ME08") and all tested water temperatures was 17.5 ± 5.1 mL O_2 kg^{-1} min^{-1} while resting immersed in water ($\dot{V}O_{2\ water}$; equivalent to 341.6 ± 108.6 mL O_2 min^{-1}). As with $\dot{V}O_{2\ air}$, the mass-specific rate of oxygen consumption while resting immersed in water differed significantly between seasons (P = 0.001). Within the individual seasons, no difference in the

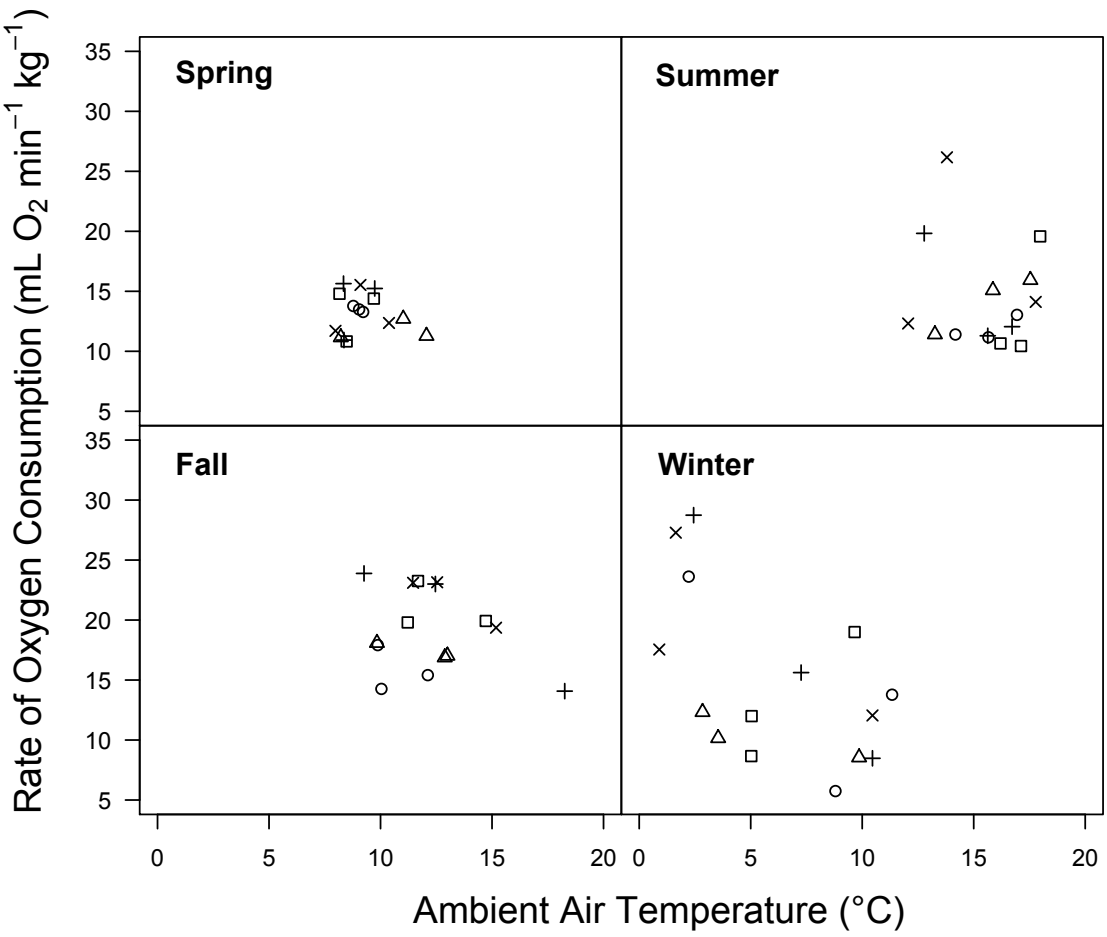

Figure 2.2 Seasonal mass-specific rates of oxygen consumption in ambient air (mL O_2 min^{-1} kg^{-1}) in comparison to the air temperatures at which the trials were conducted for five, 3 yr old female northern fur seals from March 2011 to January 2012. Individual symbols represent individual test subjects: AN08 (○), AY08 (△), KY08 (＋), TI08 (×) and TU08 (□). In the winter, the mass-specific $\dot{V}O_{2\ air}$ appears to increase at temperatures below 2.5 °C.

mass-specific $\dot{V}O_{2\ water}$ was attributable to water temperature in the spring, summer, and fall seasonal trials (P = 0.1). However, water temperature was a significant predictor of the mass-specific $\dot{V}O_{2\ water}$ during the winter (P = 0.02). The inclusion of ambient air temperature as a covariate into the linear mixed effects models found ambient air temperature to be a significant predictor of the mass-specific $\dot{V}O_{2\ water}$ in the winter (P = 0.01). As a result of including ambient air temperature, no difference in the mass-specific $\dot{V}O_{2\ water}$ was attributable to water temperature in the winter either (P = 0.9; Fig. 2.3).

We controlled for variance in resting metabolic rates associated with circadian oscillators and photoperiodic affects by calculating the paired difference between the dry ambient air and wet immersed mass-specific $\dot{V}O_2$ within an individual session. Overall, water temperature was not significant in the paired difference between the dry ambient air and wet immersed mass-specific $\dot{V}O_2$ within an individual session in any of the seasonal trials (Fig. 2.4). The cost of thermoregulation at each water temperature within each season was not significantly different from zero within each seasonal trial (P = 0.06), with the exception of the summer seasonal trials (P = 0.02). During the summer seasonal trials, the paired difference within an individual session between the $\dot{V}O_{2\ air}$ and $\dot{V}O_{2\ water}$ at 2 °C was significantly different from zero (P = 0.04). For these trials, the mass-specific $\dot{V}O_{2\ water}$ was 19.0 ± 6.5 mL O_2 kg^{-1} min^{-1} which was 36% higher on average than the associated $\dot{V}O_{2\ air}$.

2.4.3 Mass and body composition

Over the course of the study, the average body mass of the individual fur seals increased significantly by 3.9 kg (P = 0.001). The average body mass was lowest during the spring trials (16.7 ± 2.5 kg), significantly higher in the subsequent summer trials (19.3 ± 2.5 kg; P = 0.001), and higher again in the fall trials, when the average body mass was highest (21.2 ± 2.8 kg; P = 0.003). While the average body mass was slightly lower (but not significantly different) during the winter trials (20.6 ± 2.5 kg; P = 0.7), it was still significantly higher in the winter than in the spring (P = 0.001; Table 2.1).

Within the seasonal trials, the individual's growth rates (standardized as changes in the absolute body mass over the course of an entire 6 wk seasonal trial) were significantly positive

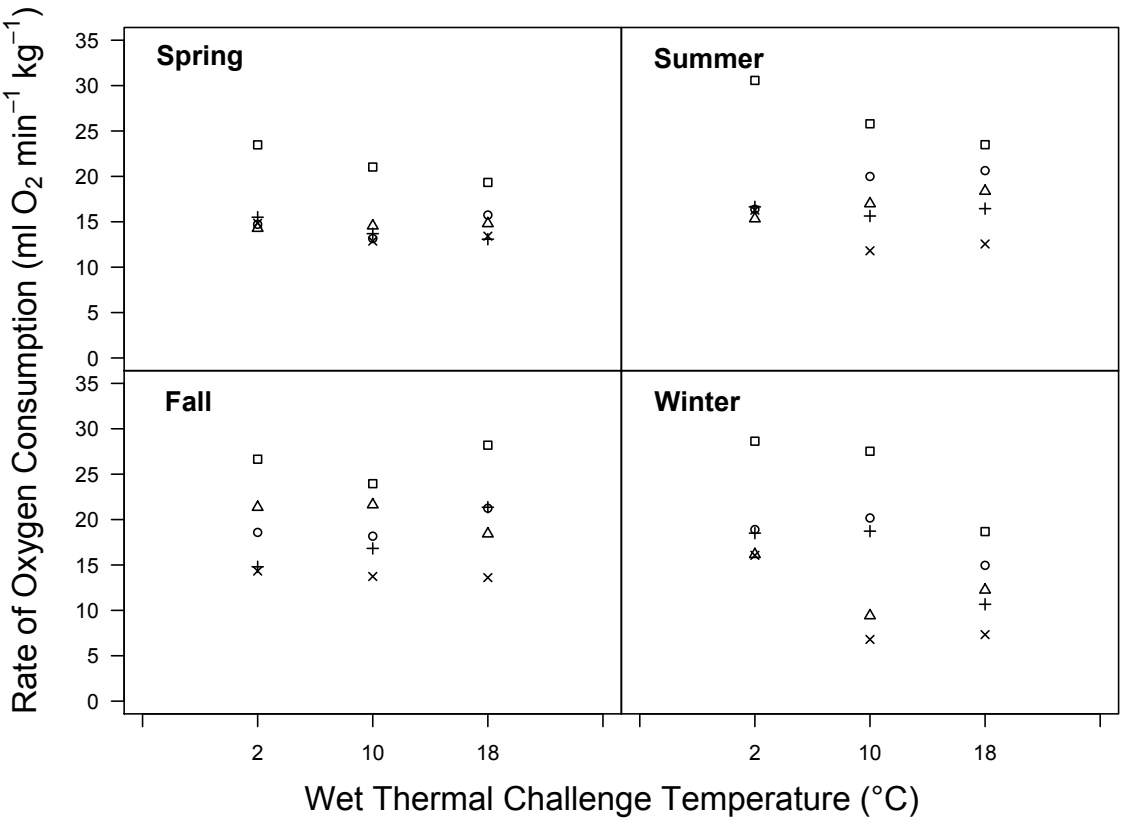

Figure 2.3 Seasonal in water mass-specific rates of oxygen consumption (mL O_2 min^{-1} kg^{-1}) in comparison to the three water temperatures (2 °C, 10 °C, and 18 °C) at which the trials were conducted for five, 3 yr old female northern fur seals from March 2011 to January 2012. Individual symbols represent individual test subjects: AN08 (○), AY08 (△), KY08 (+), TI08 (×) and TU08 (□). The inclusion of ambient air temperature as a covariate into the linear mixed effects models found ambient air temperature to be a significant predictor of the mass-specific $\dot{V}O_{2\ water}$ in the winter and therefore no difference in the mass-specific $\dot{V}O_{2\ water}$ was attributable to water temperature in any season.

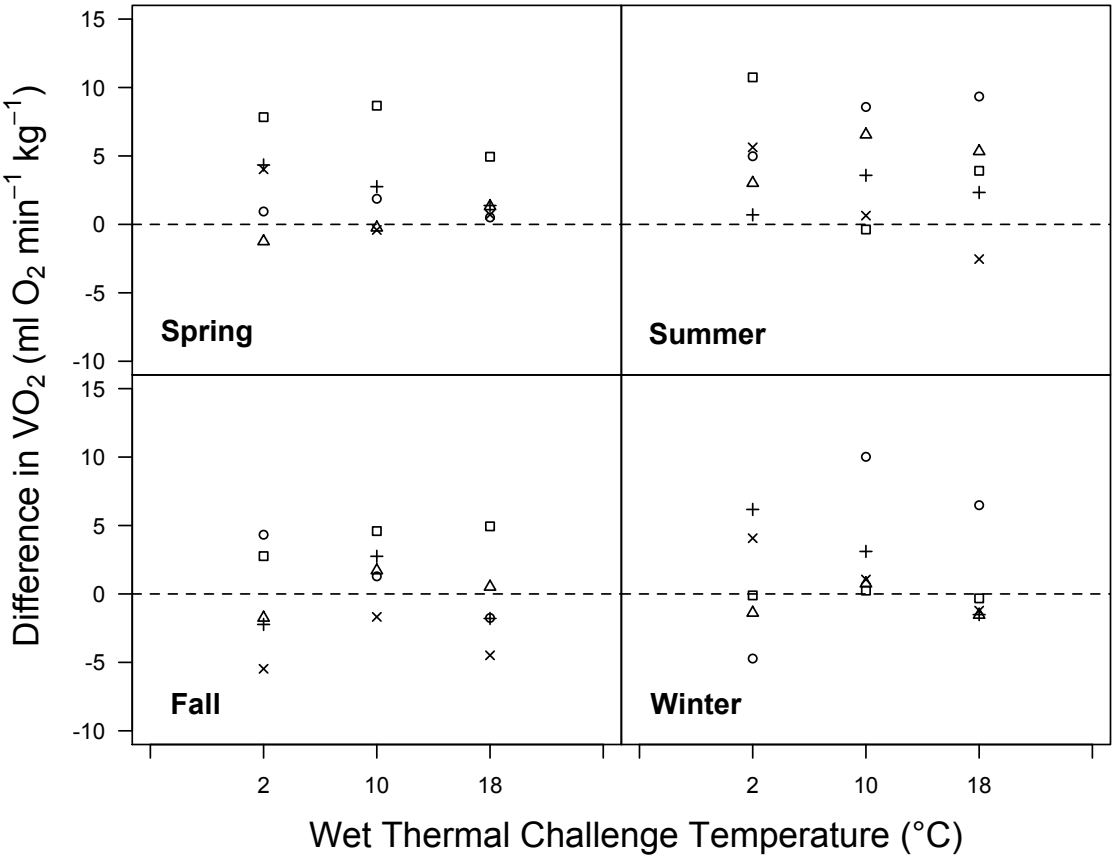

Figure 2.4 Seasonal potential costs of thermoregulation for five, 3 yr old female northern fur seals (from March 2011 to January 2012), controlling for variance in resting metabolic rates — determined as the paired difference between the dry ambient air and wet immersed mass-specific $\dot{V}O_2$ within an individual session (mL O_2 min^{-1} kg^{-1}). Data are presented for each of the three wet thermal challenge temperatures (2 °C, 10 °C, 18 °C). Individual symbols represent individual test subjects: AN08 (○), AY08 (△), KY08 (＋), TI08 (×) and TU08 (□). The cost of thermoregulation was not significantly different from zero within each seasonal trial for any water temperature, with the exception of the 2 °C thermal challenge in the summer seasonal trial.

during both the spring (P = 0.03) and summer trials (P = 0.04), while there were no significant changes in body mass during the fall (P = 0.1) and winter trials (p = 0.08).

The average absolute amount of lipid in the fur seals' bodies also significantly increased by 1.5 kg over the course of the study period (from 0.7 ± 0.3 to 2.3 ± 0.8 kg; P = 0.001), and the rate of increase in lipids was constant between the seasons (P = 0.2). The percent body mass that was lipid also increased constantly throughout the year (as would be predicted for growing animals), such that our study animals had the lowest absolute and relative amount of lipids during the spring trials (0.7 kg or 4.6 ± 2.1% of total body mass) and the highest absolute and relative amount of lipids during the winter trials (2.3 kg or 11.0 ± 3.7% of body mass).

The average blubber thickness of the northern fur seals across all individuals and all seasons was 1.8 ± 0.5 mm. The average blubber thickness was not significantly different between seasons (P = 0.3), ranging from 1.6 ± 0.4 mm in the fall to 2.1 ± 0.7 mm in the spring.

2.4.4 Average sea surface temperature (SST)

Estimates of the minimum and maximum average sea surface temperature encountered over the course of the fur seals' annual migration included high variability resulting from the spatial and temporal uncertainty of the exact location of female and juvenile individuals in the population (of the Pribilof Islands and Bogoslof Island) at different times of the year. This is represented in the different migratory routes and timings variously described by Kenyon and Wilke (1953), Bigg (1990), Gentry (1998), and Ream *et al.* (2005). Mild variation also resulted from the interannual spatial changes in SST throughout the Northeast Pacific Ocean for each month of the year from 1982 to 2012.

Average SST in the central and southern Bering Sea range from as low as 0 °C to as high as 14 °C between late May and late December when fur seals are present. However, adult females and juveniles encounter warmer water temperatures as they move southward through the Aleutian Islands into the North Pacific Ocean. The individuals then spread out and move over deep water en route to the wintering areas: either the transitional region of the central North Pacific Ocean or along the continental margins of North America from California to Sitka, Alaska. The northward return migration begins in early spring, as individuals move in a loose aggregation along the continental margins or offshore and eventually cross the Gulf of Alaska and the North Pacific Ocean to re-enter the Bering Sea. Most breeding individuals return to the Pribilof Islands in late May or June. Over the 8 – 10 mo migration, individuals can encounter

SST as low as 2 °C – 3 °C just south of the Aleutian Islands and in the Gulf of Alaska in January through May and as high as 19 °C – 21 °C off the coast of southern California or in the southern transition zone of the North Pacific Ocean in November through March (Fig. 2.5).

In comparison to fur seals that breed in the Bering Sea, females breeding in California remain near the continental margins of North America and shift their at-sea distribution northwards and offshore in the winter (Melin *et al.* 2012). As a result, the average SST encountered by female members of this population appear to stay within the range of 8 °C to 20 °C, with little variation throughout the year.

2.5 Discussion

The in-air metabolic rate of the northern fur seals in our study was equivalent to 3.2 times Kleiber's (1975) allometric prediction for adult mammals, which is consistent with other studies of northern fur seals that ranged in age from post-molt pups to 5 yr of age (Miller 1978, Donohue *et al.* 2000). As individuals develop, the mass-specific metabolic rate would generally be expected to decrease, due to reductions in the cost of growth as well as the allometric effects of increased absolute body mass (Kleiber 1975, Boyd 2002). Yet, within this general trend, seasonal variation would be expected due to changes in overall energy budgets (Robeck *et al.* 2001, Rosen *et al.* 2012).

Metabolism was highest in our study during the fall seasonal trials, but this was unlikely due to the costs of higher growth rates or a product of absolute body size. Absolute body mass was highest in the fall, but growth rates were higher during the spring and summer trials than during the fall or winter trials. In addition, the mass-specific $\dot{V}O_{2\,\mathrm{air}}$ from the winter to summer periods did not differ significantly between seasons, unlike the absolute body size of the individuals, which did change significantly.

We also found no evidence that the seasonal changes in the metabolic rate were related to thermoregulatory costs, as there was no relationship between the measured metabolic rates and ambient air temperatures within or between sets of trials, except during the winter seasonal trials. During the winter, the mass-specific $\dot{V}O_{2\,\mathrm{air}}$ appeared to increase with ambient air temperatures below 2.5 °C. Comparatively, a previous study of juvenile (2 – 5 yr old) northern fur seals failed to find metabolic changes across air temperatures of ~ -1 °C to 18 °C (Miller 1978). A different study, reported a lower critical air temperature for newborn northern fur seal pups between 0 °C

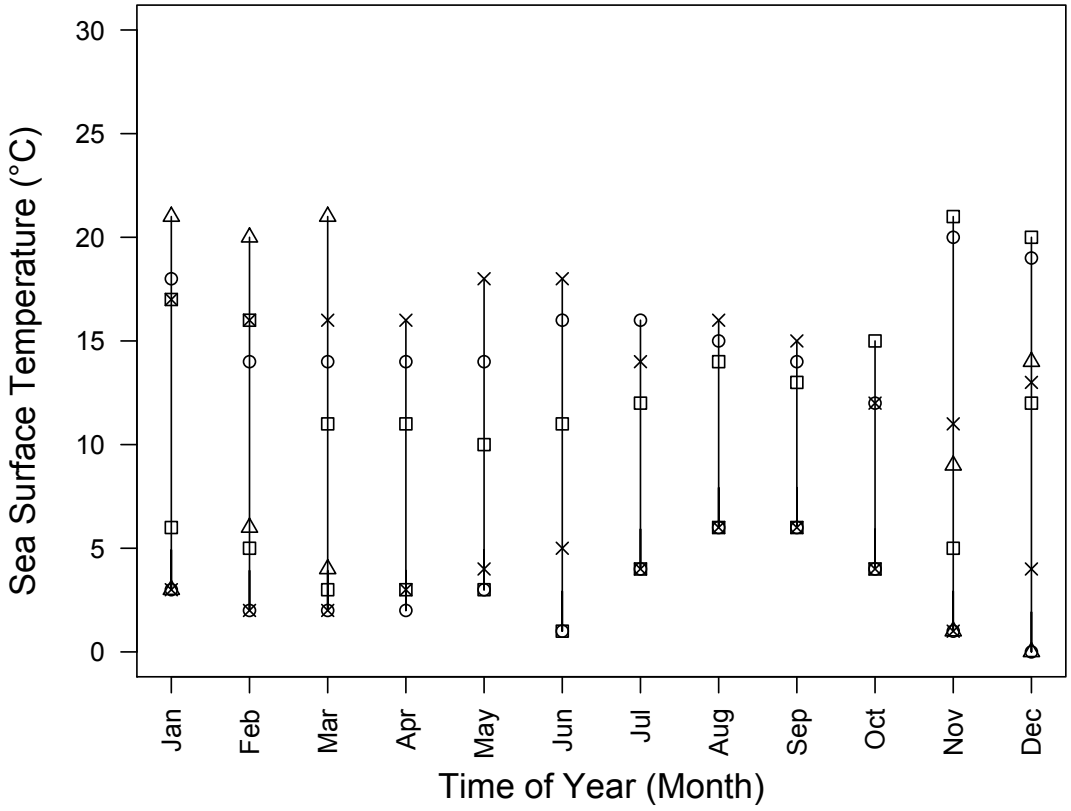

Figure 2.5 Range of average sea surface temperatures in degree Celsius (between 1982 and 2012) that adult female and juvenile northern fur seals from the Pribilof Islands and Bogoslof Island, Alaska are likely exposed to each month if they followed the migratory patterns described by Bigg (1990, ×), Gentry (1998, □), Kenyon and Wilke (1956, ○) and Ream *et al*. (2005, △). Individual symbols represent the maximum and minimum temperatures obtained from each of the migratory pattern iterations.

and 4 °C (Blix *et al.* 1979). On the Pribilof Islands (the location of the main breeding colony, Towell *et al.* 2008), documented average air temperatures below 2.5 °C only occurred between November and April (1956 to 1986) when female and juvenile northern fur seals are generally undertaking their annual pelagic migration (Trites & Antonelis 1994). Therefore, the suggested lower critical air temperature of our study supports the hypothesis that reduced air temperatures, in conjunction with increasing storm occurrences and winds speeds, which destroy a pelage's insulating air layer, are responsible for initiating the annual pelagic migration of these individuals (Trites & Antonelis 1994, Lea *et al.* 2009).

Molting, noted to be an energetically expensive process, is the most logical explanation for the elevated metabolic rate we observed in the fall (Boyd *et al.* 1993). Northern fur seals entering their third year (such as our study animals) have their molt centered in September (Scheffer 1962), which coincided with our fall trials. The 50% increase in metabolic rate exhibited by our fur seals in the fall relative to the other seasons was comparable to the 30% - 87% increase in metabolism associated with the molt in Steller and California sea lions (*Eumetopias jubatus* and *Zalophus californianus*; Kumagai 2004, Williams *et al.* 2007).

Molting is believed to decrease thermoregulatory capacity and add an additional energetic cost for many species of marine mammals (Boyd *et al.* 1993, Boily 1995). This could be substantial for northern fur seals, which take 4 or 5 mo to molt and rely primarily on their pelage for insulation (Scheffer 1962, Liwanag *et al.* 2012a, Liwanag *et al.* 2012b). However, we found that juvenile female northern fur seals in our study did not incur any additional energetic costs to being submerged in water (at any temperature) during the fall seasonal trails. This lack of thermoregulatory cost during the molt might be because northern fur seals do not lose old hairs until new replacement hairs have emerged (Scheffer 1962).

Similar to a lack of thermoregulatory cost during the fall seasonal trials, there was no increase in metabolic rates between the dry and wet conditions in any of the seasonal trials with the exception of the summer seasonal trials for the 2 °C water temperatures. While our study found a lack of thermal costs at three specific water temperatures (2 °C, 10 °C, and 18 °C), it is logical to assume that the same was true at the intermediary water temperatures. Thus, while we identified a zone of thermal neutrality—a portion of the overall TNZ—we failed to identify specific upper or lower critical water temperatures.

It should be noted that when we use the term "thermoregulatory costs" we are implicitly referring to supplementary costs incurred beyond the thermoneutral zone, where individuals must expend additional energy to maintain their core body temperatures. All homeotherms must devote energy to maintain core body temperatures even within their TNZ as part of their basic body expenditures (RMR), such as the routine maintenance of the protective insulative layer.

Our failure to detect an upper critical water temperature in any season was not surprising given that it is energetically inexpensive for marine mammals to dissipate excess heat in water (Williams & Worthy 2002). Otariids have specialized blood vessels (arteriovenous anastomoses) in their flippers that can readily dissipate excess heat through the skin surface while swimming (Bryden & Molyneux 1978, Williams & Worthy 2002). To date, only one study has found an upper critical water temperature (UCT) of 8 °C, and it was only during a single set of trials with younger northern fur seals (Rosen & Trites 2014). Comparatively, Liwanag (2010) could not identify an UCT in northern fur seal pups exposed to water temperatures as high as 25 °C.

In terms of the lower critical water temperature (LCT), we presume it is below or near to the 2 °C water temperatures we tested, which is lower than what we had predicted based on studies of other aquatic and semi-aquatic mammals that depend on a dense pelage for thermoregulation (Dawson & Fanning 1981, Costa & Kooyman 1982, Williams 1986, Rutishauser *et al.* 2004). Previous studies of younger northern fur seals immersed in water had reported LCTs ranging from 3.9 °C - 6.5 °C (Rosen & Trites 2014), to 8.3 °C ± 2.5 °C (Liwanag 2010), to ~ 10 °C (post-molt, pre-weaning pups; Donohue *et al.* 2000). It is noteworthy that all of these previous studies were undertaken with slightly different age groups. One other study that did use northern fur seals of similar ages to those we studied had a reported LCT in water of >18 °C (Miller 1978). However, this surprisingly high value may be confounded by the costs of diving, grooming, and swimming that occurred throughout the trials, as costs of movement can be substantial (Darveau *et al.* 2002).

Comparing the reported LCTs of other studies to our finding that it is near to or below 2 °C for juvenile females suggests that the apparent differences between the various studies may reflect a general decrease in LCT with age (Miller 1978, Donohue *et al.* 2000, Liwanag 2010, Rosen & Trites 2014). Energetically, developing a broad thermal neutral zone and explicitly a lower LCT may be necessary prior to bearing any additional energetic costs associated with

reproduction and lactation, which for the northern fur seal occurs with maturation between the ages of 3 and 7 yr old (Williams *et al.* 2007, COSEWIC 2010).

It is not immediately obvious how northern fur seals could have a LCT near to or below 2 °C when the sea otter, with a pelage containing many similar morphological traits but twice as dense, has a reported LCT of 20 °C (Costa & Kooyman 1982, Williams *et al.* 1992, Liwanag *et al.* 2012a, Liwanag *et al.* 2012b). The difference between species does not appear to be due to difference in surface area, as our fur seals (which increased in mass from 16.7 to 20.6 kg over the course of our study) would have had a calculated surface area of 0.52 – 0.60 m^2 (Irving *et al.* 1935, Blix *et al.* 1979, Innes *et al.* 1990, Trites 1990), which is similar to the estimated surface area of 0.50 – 0.81 m^2 for 16.6 to 20.0 kg sea otters (Costa & Kooyman 1982). Nor does it seem that our fur seals obtained increased insulation from their minimal blubber layer (average fat layer at birth is 2 – 4 mm, and 1.0 – 3.8 mm for captive 3 yr olds; Blix *et al.* 1979), in comparison to the sea otter's lack of a blubber layer (Davis *et al.* 1988).

Blubber is a relatively poor insulator, requiring high amounts and thick layers of blubber to establish effective insulation. Phocid seals, which depend primarily on blubber to minimize heat loss to the environment, have an average layer of ~ 40 mm — over 10 times thicker than in our fur seals (Scholander *et al.* 1950, Liwanag *et al.* 2012b). In our study, the absolute and relative amount of lipid mass of the fur seals increased over the course of the trials. However, having higher relative lipid layers would be more beneficial for thermoregulation in younger rather than older animals because smaller individuals have higher surface area to body mass ratios (Arnould *et al.* 1996a, Willmer *et al.* 2005). Thus, the change in lipid we observed was likely developmental or allometric in nature.

A potential explanation for the differences in LCT between sea otters and northern fur seals might be attributable to differences in several key features in their pelage, specifically the density of the guard hairs and the quantity of sebaceous glands present (Scheffer 1962, Williams *et al.* 1992). While sea otters possess higher absolute density of hairs, the density of guard hairs in the northern fur seal's pelage is ~ 20% – 90% greater (1425 – 2280 hairs cm^{-2}; between 2.5% – 4% of the total fur seal hairs) than for the sea otter (990 – 1200 cm^{-2}) (Scheffer 1962, Williams *et al.* 1992). Although guard hairs are believed to provide little insulation themselves, associated with each guard hair follicle are sebaceous glands that provide a hydrophobic barrier against pelage wetting through the excretion of sebum, a mixture of nonpolar lipids (Irving *et al.* 1962,

Williams *et al.* 1992). The sea otter has a single, large, bi-lobed sebaceous gland (Williams *et al.* 1992), while the northern fur seal in contrast has a pair of round sebaceous glands subdivided into two or three lobes for each guard hair follicle (Scheffer 1962). The northern fur seal could therefore have 2.4 to 3.8 times as many sebaceous glands compared to the sea otter. While the sebum composition is extremely species-specific (Zouboulis 2004) and an increased number of sebaceous glands does not necessarily equate to a greater volume of sebaceous secretions, this anatomical difference might explain why the northern fur seal has a broader TNZ than the sea otter.

The unpredicted resiliency of juvenile female northern fur seals to the effect of environmental water temperatures on metabolic rates would also suggest that the migration of juvenile female northern fur seals might not be constrained by water temperatures as commonly believed. Our investigation of the average sea surface temperatures encountered by northern fur seals in the Bering Sea and North Pacific Ocean during their annual migration (as described by Gentry 1998) suggests that they generally stay within a narrow range of temperatures from March to September, but experience higher, more variable water temperatures in the months of November to February. However, the migration patterns described by Kenyon and Wilke (1953), Bigg (1990) and Ream *et al*. (2005) suggests fur seals encounter greater variability of the average SST throughout their migration—and that they exploit their entire broad zone of thermal neutrality from November to June. The relatively narrow range of water temperatures they experience from July to October occurs predominantly in the Bering Sea and likely reflects a geographic aggregation for breeding where food is plentiful and survival of newborns is high, rather than a direct response to thermal conditions imposed by water temperatures (Trites 1990, Trites & Antonelis 1994).

The wide thermal neutral zone we observed implies that northern fur seals predominantly do not have to increase their metabolism to maintain a constant core body temperature while foraging or resting at sea, although changes in temperature with depth or the effect of microclimates cannot be discounted (Williams & Worthy 2002). Vertical oceanographic thermal properties, critical to the foraging success of the northern fur seal, can produce subsurface water temperatures as low as -2 °C (the freezing point of sea water) during dives (Kuhn 2011). Nevertheless, thermal substitution, the use of heat produced during activity or the processing of food, in combination with relatively short dive durations could minimize any additional energetic

disadvantage associated with diving to depth (Lovvorn 2007, Liwanag *et al.* 2009, Liwanag 2010, Kuhn 2011).

Ecologically, a broad thermal neutral zone implies that prey choice for juvenile females should not be limited by thermal considerations because they have the thermal capacity to alter their migration in response to changes in prey availability and distribution. Migration patterns of the northern fur seal were undoubtedly first established by moving between seasonal prey aggregations that provided predictable energetic resources (Sigler *et al.* 2009). Northern fur seals consume over 70 different species of fish, cephalopods, and crustaceans (Perez & Bigg 1986, Newsome *et al.* 2007, COSEWIC 2010) and presumably take advantage of seasonal pulses and spatial aggregations of prey (energy resources) during their annual migration (Perez & Bigg 1986, Lowry *et al.* 1991).

However, the broad distribution of northern fur seals across the North Pacific does not mean that northern fur seal behaviour is unaffected by thermal considerations. While abrupt changes in weather conditions (*e.g.*, increase in storm frequency, extent of ice cover) beginning in November are hypothesized to be an impetus to migration (Trites & Antonelis 1994, Lea *et al.* 2009), lower water temperatures likely also play a role. Females and juvenile northern fur seals are absent from the Bering Sea between January and May, when average sea surface water temperatures range between 0 °C and 4 °C. Individuals that remain in the Bering Sea during this time would likely experience an added energetic expense that depletes energy reserves, hinders growth and reproduction, and may ultimately lead to starvation (Rutishauser *et al.* 2004, Lea *et al.* 2009).

In comparison to fur seals that breed in Alaska, females from San Miguel Island remain near the continental margins of North America throughout the year, encountering a generally higher and constant range of temperatures (general temperature exposure range was 8 °C to 20 °C) due to the year-round upwelling of the California Current waters off southern California (Melin *et al.* 2012). Upwelling effects tend to be stronger in the summer, but change from day to day with wind speed and direction (Mann & Lazier 1991). Overall, this year-round upwelling constantly brings new nutrients to the water surface and appears to provide a productive ecosystem for a finite number of northern fur seals year round, such as the individuals from San Miguel Island or historical populations from mainland California and Oregon (Mann & Lazier 1991, Burton *et al.* 2001).

Despite their wide at-sea distribution in the North Pacific Ocean, northern fur seals (female and juvenile individuals) do not appear to migrate to waters that exceed 21 °C, suggesting there may be an upper critical water temperature (although one has yet to be identified). Northern fur seals (juvenile female individuals) nevertheless have an impressively wide zone of thermal neutrality (spanning at least near to or below 2 °C to 18 °C based on our measurements), and are physiologically well equipped to exploit the entire Bering Sea and North Pacific Ocean.

Chapter 3: Season and time of day affect the ability of accelerometry and the doubly labeled water methods to measure energy expenditure in northern fur seals

3.1 Summary

Estimates of energy expenditure for free-ranging animals are essential to answering a range of fundamental questions in animal biology, but are challenging to obtain and difficult to validate. We simultaneously employed three methods to measure the energy expenditure of 6 captive female northern fur seals (Callorhinus ursinus) during 5-day trials across 4 seasons: respirometry (oxygen consumption), doubly labeled water (DLW), and accelerometry. The DLW method estimated that the fur seals expended 13.1 ± 16.5% more energy than indicated by the more direct measures of oxygen consumption. Accelerometry failed to predict the average mass-specific rate of oxygen consumption ($\dot{V}O_{2\,DEE}$) within the individual seasons over entire 5-day trials. However, on a finer time scale (15 or 60 min) and adjusted for time of day, accelerometry estimated energy expenditure within an average difference of 5.4 ± 29.3% (60 min intervals) and 13.8 ± 39.5% (15 min intervals) of respirometry measured values. Our findings suggest that accelerometers have the potential to be more effective than the DLW method for measuring energy expenditure of free-ranging animals. However, rates of oxygen consumption varied with season, independent of overall activity. Seasonal effects (and time of day for accelerometry) must therefore be accounted for when estimating energy expenditure from measures of DLW and acceleration of free-swimming northern fur seals. Such corrections required for estimating energy expenditures in northern fur seals have implications for using accelerometers and DLW to estimate the energy expenditure of other species.

3.2 Introduction

Energy expended by animals can be reliably estimated by measuring the rate at which oxygen is consumed and, frequently, the rate at which carbon dioxide is produced in expired gases (Boyd 2002). Oxygen consumption can be converted relatively easily into energy expenditure, particularly when the respiratory quotient ($RQ = CO_2/O_2$) is known (Boyd 2002). As a result, respirometry (*i.e.*, indirect calorimetry) has become the "gold standard" for measuring the energy expended by a variety of marine and terrestrial mammalian species (direct calorimetry in marine mammals has fundamental concerns associated with the transfer of heat to

the environment acorss a blubber layer; Williams *et al.* 1993, Minetti *et al.* 1999, Boyd 2002, Fahlman *et al.* 2008a, Halsey *et al.* 2009a). Unfortunately, it is not possible to capture gas exchange in free-ranging animals such as diving marine mammals, except in rare cases when surfacing locations are isolated and predictable (Kooyman *et al.* 1973).

Energy expended by free-ranging marine mammals instead must be estimated using more indirect measures such as the doubly labeled water (DLW) turnover and accelerometry methods (Nagy *et al.* 1999, Halsey *et al.* 2011). However, these alternative methods come with their own logistical constraints and predictive limitations that are often species-specific (Speakman 1997, Butler *et al.* 2004, Halsey *et al.* 2011). It is therefore necessary to validate the accuracy of each alternative method of estimating energy expenditure with more direct measures, such as those acquired *via* respirometry (Wilson *et al.* 2006, Halsey *et al.* 2011). It is also important to know the limitations of each method before applying them to free-ranging animals (Butler *et al.* 2004, Halsey *et al.* 2011).

The doubly labeled water (DLW) method was developed in the 1950s, and has been used to measure the energy expenditure of a variety of mammalian, avian and reptilian species in both wild and captive settings (Lifson *et al.* 1955, Nagy *et al.* 1999, Speakman *et al.* 2001, Sparling *et al.* 2008). This isotope washout method estimates an individual's CO_2 production using the differential elimination of heavy oxygen (^{18}O) and hydrogen (^{2}H) isotopes introduced into the body water (Speakman 1997). The basic principle underlying the DLW method is that the ^{18}O isotope is eliminated from the body within the continuous flow of water (urine, saliva and evaporative cooling) and as respiratory CO_2, whereas the ^{2}H isotope is eliminated from the body only within water molecules (Speakman 1997). The difference in the elimination rate of these two isotopes (^{18}O to ^{2}H) correlates with the rate of CO_2 production, and can be converted to energy expenditure with an estimate of the RQ (Speakman 1997).

The DLW method has been used to measure the field metabolic rate of a number of marine mammal species (Nagy *et al.* 1999). However, Butler *et al.* (2004) suggested the DLW method could be ineffective in air breathing aquatic vertebrates because an increase in water production compared to CO_2 production minimizes the difference in the elimination curves of the two-labeled ions and increases the error. Unfortunately, the limited number of validation

studies attempted with marine mammals indicates the need for further species-specific calibrations (Costa 1987, Boyd *et al.* 1995, Sparling *et al.* 2008).

The concept of accelerometry – using measures of body acceleration to estimate energy expenditure – was introduced in the 1960s (Cavagna *et al.* 1963, Green *et al.* 2009). It is based on the principle that animals expend energy during activity to contract their muscles, leading to the acceleration of their limbs and bodies (Wilson *et al.* 2006, Green *et al.* 2009, Halsey *et al.* 2009a, Enstipp *et al.* 2011). This principle has led to the expectation that energy expenditure should closely correlate with the dynamic acceleration in all 3-body axes about the center of an animal's mass (Wilson *et al.* 2006, Halsey *et al.* 2009a). Increased use of accelerometry to estimate energy expenditure in animals has been facilitated by advancements in the miniaturization of data loggers (Wilson *et al.* 2006, Green *et al.* 2009, Enstipp *et al.* 2011).

One specific accelerometry method termed Overall Dynamic Body Acceleration (ODBA) sums the dynamic acceleration in each of the 3 body axes. Previous studies on a few species of marine mammals (Weddell seals; Williams *et al.* 2004, and Steller sea lions; Fahlman *et al.* 2008b) have presented evidence for the usage of ODBA, but questions remain about the overall ability of accelerometry to effectively predict energy expenditure (Halsey *et al.* 2011). Of particular concern is whether estimates are compromised by the inability of acceleration to measure energetic changes that are independent of activity (*e.g.*, thermoregulation, growth, reproduction, and basal and resting metabolic rate; Halsey *et al.* 2011). Interest in ODBA as a means to measure energy expenditure reflects the fact that it is less expensive than the DLW method, is less labour intensive to apply, provides data with finer temporal resolution, and can be applied over longer measurement periods (Fahlman *et al.* 2008b).

The following describes our simultaneous use of respirometry (oxygen consumption), accelerometry and the DLW methods to measure the energy expenditure of northern fur seals (*Callorhinus ursinus*) in a controlled setting. We considered the measured rates of oxygen consumption to be the most accurate and thus the "true" measure of energy expenditure, such that the accelerometry and DLW method could be evaluated and calibrated against it for potential future work on wild individuals. We also tested whether the ability of the DLW and accelerometry methods to estimate energy expenditure varied with time of year by taking measurements during the spring, summer, fall, and winter.

We predicted was that season would affect the predictive capabilities of the accelerometry method given that resting metabolism (zero activity) changes seasonally (Halsey *et al.* 2011). However, such physiological changes were not expected to affect the ability of the DLW method to predict rates of energy expenditure (Speakman 1997). Overall, based on previously published literature, we hypothesized that the DLW method would provide a reasonably accurate measure (< 10% error) of the daily energy expenditure of northern fur seals (Boyd *et al.* 1995, Speakman 1997, Sparling *et al.* 2008), and that accelerometry would provide an almost equally accurate measure within a given season (~ 10% error; Halsey *et al.* 2009a).

3.3 Methods

3.3.1 Animals

Six female northern fur seals were studied from March 2011 to January 2012 (Table 2.1). The animals were collected from a rookery on St. Paul Island, Alaska, in October 2008, following weaning at approximately 4 mo of age. The individuals were transported to the University of British Columbia's Marine Mammal Energetics and Nutrition Laboratory, located at the Vancouver Aquarium (British Columbia, Canada). They were raised in captivity and trained with positive reinforcement to be familiar with all necessary husbandry behaviours, research protocols and equipment. The fur seals were fed a daily diet of herring and squid (supplemented with vitamins) and were housed in seawater pools with water temperatures that reflected the local ocean conditions (7 °C – 16 °C). The animal trainers in combination with the veterinary staff determined the amount of herring and squid in the daily diet (with a goal of satiation within working conditions). The fur seals were fed twice over the course of the day: two-thirds of the daily diet in the morning and one-third in the afternoon. The Animal Care Committees for the Vancouver Aquarium and the University of British Columbia (Permit #A10-0342) approved all animal use and research protocols.

3.3.2 Timing and general protocol

We conducted four seasonal sets of trials: 1) Mar/Apr 2011 ("Spring"; Age 2.75 yr old), 2) Jun/Jul ("Summer"; Age 3 yr), 3) Sept/Oct ("Fall"; Age 3.25 yr) and 4) Dec 2011/Jan 2012 ("Winter"; Age 3.5 yr). Each set of trials took ~ 7 wk to complete. Within each seasonal set, the daily energy expenditure (DEE) of each individual was determined over 5 days simultaneously using respirometry and the doubly labeled water method (see section 3.2.3 to 3.2.5). In addition,

their activity was also measured using two types of accelerometers (see section 3.2.6). The order of individuals tested within a seasonal set was determined randomly.

Details of each DEE trial are described separately. In brief, each DEE trial began with drawing a blood sample before and after injecting the fur seals with the doubly labeled water while under anesthesia. Following the second blood sampling, a harness containing the accelerometers was placed on the fur seal, and the individual entered a metabolic chamber. The fur seal was free to undertake its normal daily activity either on land or in the water, while the rates of oxygen consumption, carbon dioxide production and activity were continuously measured. Each DEE trial lasted close to 5 full days, as the effective measurement period of the doubly labeled water method in the body water pool of pinnipeds was determined to be at least 1 isotope half-life or 4 - 6 days (Nagy 1980, Boyd *et al.* 1995). At the end of the trial, the individual exited the metabolic chamber, the harness and activity monitors were removed, and a final blood sample was obtained.

During the DEE trials, the test individual only interacted briefly with staff twice daily. Each morning, the individual received their morning feed and a quick physical health assessment (including measuring body mass) outside of the metabolic chamber (8.6 ± 4.0 min). Each afternoon, the individual received their second feed within the metabolic chamber *via* an access tube.

3.3.3 Metabolic chamber

The metabolic chamber consisted of a large, airtight dome, constructed of welded aluminum and Lexan^{TM}, placed over a holding pool and it's associated haul out space (Fig. 3.1). The approximate air space volume of the metabolic chamber was 3500 L. An internal air circulation system ensured proper air mixing within the chamber and an access tube over the haul out space permitted feeding directly into the chamber without compromising integrity. Air was drawn through the metabolic chamber at 125 L min^{-1} to a gas analysis system *via* the excurrent airflow pipe located above the pool, generating a 50% air turnover rate of ~ 19 min. A door in the chamber (located on the haul out space) permitted the animal controlled entrance/egress from the metabolic chamber. The entire metabolic chamber was tested for leaks and proper air circulation prior to use. A closed circuit digital video surveillance system

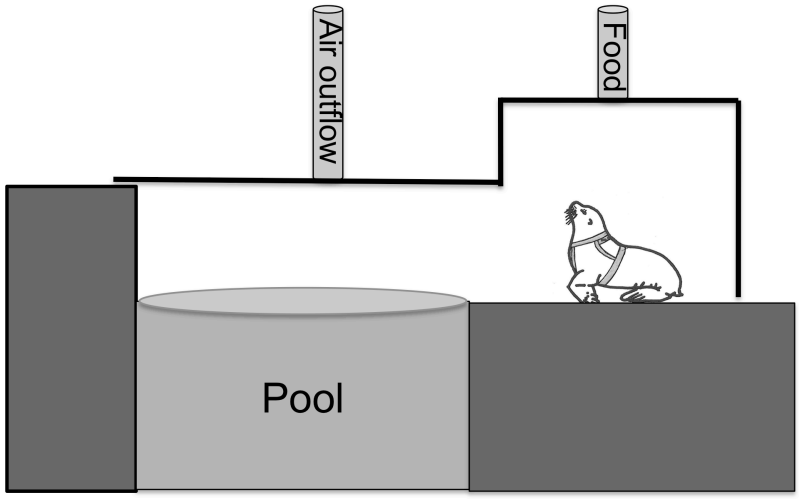

Figure 3.1 Metabolic chamber schematic for measuring daily energy expenditure (DEE) of northern fur seals *via* respirometry. Schematic of the metabolic dome (including airlock feeding tube and excurrent air tube) constructed over one of the holding pools for measurements of oxygen consumption rates during five-day metabolic measurement trials. The metabolic chamber consisted of a circular pool (8,500 L; depth 2.0 m, diameter 2.2 m), with an air space volume above the water of ~ 1900 L (depth 0.5 m, diameter 2.2 m) and dry haul out space volume of ~ 1600 L (length 2.4 m, width 1.1 m, height 0.6 m). Note: this schematic is not to scale.

(mounted above the chamber) recorded the entire experimental trial and provided a means to check animal behaviour in the event of an unusual metabolic event.

3.3.4 Respirometry

Rates of oxygen consumption and carbon dioxide production were measured using open flow respirometry to determine metabolic rates. Measurements were made using one of the two systems. First, ambient air was drawn through the metabolic chamber at 125 L min^{-1} *via* either the Sable Systems Model 500H Mass Flow Controller (Sable Systems, Las Vegas, NV) or the Sable Systems Field Metabolic Pump, both of which constantly corrected the flow rate to standard temperature and pressure. Subsamples of air from the excurrent airstream were dried through a canister of anhydrous $CaSO_4$ (Drierite; Hammond Drierite, Xenia, OH), before the O_2 and CO_2 concentrations were determined by either the Sable Systems FC-1B and CA-1B analyzers, respectively, or using the Sable Systems Field Metabolic System (P-Series). The resultant O_2 and CO_2 concentrations in the excurrent air were continuously monitored and recorded to a portable computer every 5 sec using the Sable Systems Expedata software.

The entire open flow respirometry system was calibrated with dry ambient air at the start and end of each trial as well as each morning, such that changes in gas concentrations were determined against baseline (ambient) measures to account for system drift. The entire system was also periodically calibrated against gases of known concentrations. Rates of oxygen consumption were calculated using LabAnalystX software (M. Chappell, UC Riverside, Riverside, CA) and incorporating the appropriate equations from Withers (1977). A malfunctioning CA-1B analyzer (CO_2 sensor) was detected in a portion of the first two seasonal cycles of this study. For trials with an average RQ value outside of a reasonable physiological range (0.65 - 1.05), the $\dot{V}O_2$ was calculated using a fixed RQ value of 0.8 rather than an RQ based on the erroneous measured rates of expired CO_2. Rates of oxygen consumption were converted to estimates of daily energy expenditure (DEE_{resp}) using the energy equivalents of $\dot{V}O_2$ for different RQ values as determined by Brody (1945).

3.3.5 Doubly labeled water (DLW) method

All blood samples were obtained under veterinary supervised anesthesia (maximum 5% Isoflurane) and were collected from the caudal gluteal vein. An initial blood sample was drawn into a serum separator tube prior to the administration of the DLW to assess background levels of the ^{2}H and ^{18}O isotopes. The DLW was then administered in two separate injections of ~ 98%

^{18}O water and 99.9% ^{2}H water. The DLW was injected intramuscularly at a dosage of 0.16 g kg^{-1} for each isotope. A second blood sample was drawn 2 hr post-injection (permitting equilibration with the body water pool; Costa 1987) to assess the effective dose (increase in the isotope concentration). Animals were awake and kept in a holding run with a circular wading pool and running water during the 2 hr equilibration period. A final blood sample was obtained immediately following the 5 days in the metabolic chamber.

Blood samples were centrifuged and the collected serum was stored at -70 °C until analysis. Metabolic Solutions Inc. (Nashua, NH) conducted the isotope analysis of the serum and dose samples, using a Europa Hydra continuous flow isotope ratio mass spectrometer and the methodology described by Scrimgeour *et al.* (1993). Calculations of the rate of carbon dioxide production ($\dot{V}CO_2$) and the accompanying daily energy expenditure (DEE_{DLW}) were conducted using the "Doubly-Labeled Water Calculation Program" (Lemen 1999; Natureware Ltd, Aberdeen, Scotland, UK) with an RQ of 0.80. For validation of the DLW method, conversion of $\dot{V}CO_2$ to energy expenditure was conducted as per studies in the wild, knowing only the initial and final weight of the individuals, the isotope enrichment in each of the blood samples, and using the best estimate RQ of 0.8. The DLW calculation program used 9 of the potential techniques (equations) available to estimate CO_2 production and differ in the way the parameters are combined (Lifson & McClintock 1966, Nagy 1983, Coward *et al.* 1985, Schoeller *et al.* 1986, Speakman 1993, Speakman *et al.* 1993, Racette *et al.* 1994, Schoeller *et al.* 1995, Speakman 1997- two pool and single pool estimate). In addition, these techniques can include the initial dilution space parameter calculated using either the plateau or intercept methods and the final dilution space parameter calculated using either the % mass or group scaled data procedures (Speakman 1997). Each of these techniques and parameter combinations produce a single estimate of DEE_{DLW} over each DEE trial for each fur seal.

3.3.6 Activity monitors (accelerometers)

Two different accelerometers were used to record the body acceleration (as a proxy for activity level) of the northern fur seals: 1) a Little Leonardo bi-axial acceleration data logger (M190L-D2GT; length = 53 mm, diameter = 15 mm, mass = 17 g; 12-bit resolution; recording range ± 3 g; Little Leonardo, Tokyo, Japan) and 2) an Actiwatch tri-axial acceleration data logger (length = 29 mm, width = 37 mm, height = 11 mm, mass = 16 g; recording range ± 2 g; Philips Healthcare, Bend, Oregon). Each logger was secured inside of a Velcro^{TM} sealed pocket

attached to a custom-made harness worn by the fur seal. The harness consisted of an adjustable fabric collar that fit closely to the individual without restricting movement, and lay anterior to the pectoral flippers. The loggers lay dorsal to the pectoral flippers, and were kept stationary by both the collar and an elastic bellyband attached to each pocket that encircled the animal posteriorly to the pectoral flippers (Fig. 3.2).

The Little Leonardo data logger was oriented to record acceleration in the dorsal-ventral (heave) and anterior-posterior (surge) axes at a frequency of 16 Hz. These data loggers were replaced every other day with identical loggers during the morning feed. Partial Dynamic Body Acceleration (PDBA) was calculated for each axis separately, following the technique described by Wilson *et al.* (2006). In each axis, the raw acceleration data was smoothed using a 2-s running mean with the resultant data representing the static acceleration. The difference between the static acceleration and the unsmoothed raw data provided the approximation of the dynamic acceleration. Summing the absolute dynamic acceleration values for each axis yielded the $PDBA_{xy}$ measure.

The Actiwatch data logger recorded acceleration over the entire length of the 5-day DEE trial. The Actiwatch provided data in the form of a single unit (count) of the number of times the test subject exceeded the threshold acceleration in any dimension (surge, heave or sway) during 15-s intervals, such that no additional data processing was required to obtain the dynamic acceleration measure.

3.3.7 Resting metabolic rate (RMR)

Resting metabolic rate (RMR) was measured in a separate specially-designed 340 L metabolic chamber (dimensions: 0.92 m x 0.61 m x 0.61 m) on three separate occasions within each season that were near to, but exclusive from, the times each individual underwent the DEE measurements. The individuals entered this metabolic chamber voluntarily under trainer control and were previously trained to remain calm, with minimal activity, within the chamber. Rates of oxygen consumption and carbon dioxide production were continuously measured for 20 min in ambient air conditions *via* respirometry (see section 3.2.4). Trials were conducted in the morning and individuals were tested only once each day. Individuals were fasted overnight (>16

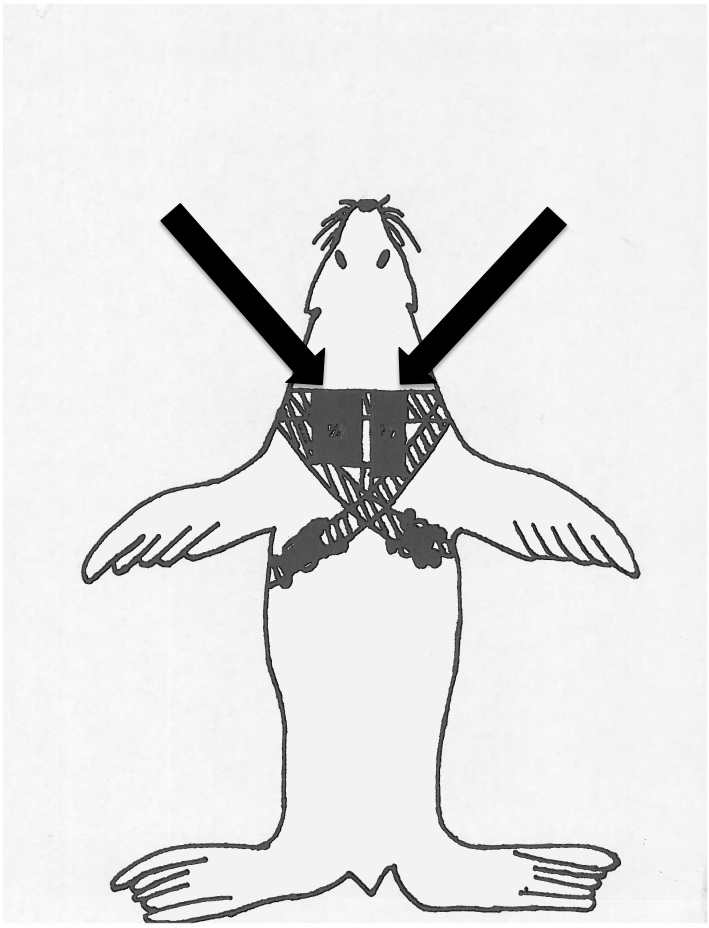

Figure 3.2 Schematic of the harness for the activity monitor data loggers. Schematic of the harness, contain the activity monitor data loggers, worn by the northern fur seals during the five-day metabolic measurement trials. The two arrows point towards the Velcro^{TM} sealed pockets, which contained the Little Leonardo bi-axial acceleration data logger and Philips' tri-axial acceleration data logger.

hr) to ensure a post-absorptive state had been reached. The RMR was determined as the lowest continuous average oxygen consumption maintained for 10 min during the last 15 min of these trials. In reality, the animals generally remained calm throughout the entire trial. Animal behaviour and air temperature were also recorded every 5 min throughout each trial.

3.3.8 Data analysis

Seasonal changes between trials in daily energy expenditure estimated *via* respirometry or DLW turnover (DEE_{resp} or DEE_{DLW}, respectively), Actiwatch score, $PDBA_{xy}$ score and RMR were determined separately using linear mixed effects models (LME; NLME library in R from Pinheiro & Bates 2000), with the individual included as the random effect to account for repeated measures. If significant overall differences were detected, a *post-hoc* Tukey Contrast simultaneous test for general linear hypotheses was used to determine between which seasons the significant differences occurred.

To determine if $\dot{V}O_2$, $\dot{V}CO_2$, RQ, Actiwatch Score or $PDBA_{xy}$ score were changing significantly throughout the course of the day, LME models, with the individual included as the random effect, were constructed separately for each variable against time of day within each season. The LME models were constructed at both the finer scale time intervals of 15 and 60 min.

LME models, with the individual included as the random effect, were also separately constructed to determine the ability of each of the potential techniques (equations) available to estimate CO_2 production and DEE from DLW turnover to predict the DEE from respirometry (O_2 consumption) over the entire DEE trial. Season was subsequently included into the model as independent variables to determine if the time of year was a significant factor in each of the techniques' predictive capabilities. As season was found to be a significant factor in each of the techniques' predictive capabilities, paired *t*-tests were used to determine within which seasons significant differences were occurring.

LME models, with the individual included as the random effect, were also constructed to determine the ability of each independent activity variable (Actiwatch score or $PDBA_{xy}$ score) to predict the mass-specific $\dot{V}O_2$ over the entire DEE trial. Season and average RMR were subsequently included in the model as independent variables to determine their impact and ability to improve the model's predictive capabilities. As season was found to be a significant

predictor of the mass-specific $\dot{V}O_2$, separate LME models within each season were constructed to determine the ability of either activity score to predict the mass-specific $\dot{V}O_2$. Average RMR was again also subsequently included in each seasonal model as an independent variable to determine its impact and ability to improve the model's predictive capabilities.

Regression equations were also constructed to determine the ability of each independent activity measure (Actiwatch score or $PDBA_{xy}$ score) to predict the mass-specific $\dot{V}O_2$ in individual fur seals at a finer scale (15 and 60 min intervals). The selected time intervals of 15 and 60 min balanced the desire for truly fine scale estimates with limitations imposed by the rate of air change within the metabolic chamber. Rates of oxygen consumption were appropriately lag-corrected to synchronize the data by accounting for the time it took respired gas to flow from the metabolic chamber to the gas analyzers as well as the lag time between the measured activity and the oxygen uptake required to replace the energy expended in the measured movement. The shift was empirically determined as the time that yielded the highest average coefficient of determination (R^2) across all individuals and all seasons.

Temporal auto-correlation between successive blocks of time was accounted for by using one 15 or 60 min block of data every 4.75 hr. The time between successive blocks of time was determined using the auto correlation estimation function (stats library in R from Venables & Ripley 2002) with consideration for covering all times of the day throughout a 5-day data collection period.

We again used a LME model (with the random effect of the individual included to account for repeated measures) to determine the ability of each activity measure to predict the mass-specific $\dot{V}O_2$ on the finer scale. Season, average RMR and time of day were also included to determine their impact and ability to improve the model's goodness of fit. As season was again found to be a significant predictor of the mass-specific $\dot{V}O_2$, we constructed LME models within each season, and subsequently included average RMR and time of day. We also included a sinusoidal wave correction in each model after finding time of day to be a significant predictor of the mass-specific $\dot{V}O_2$ within the seasonal trials. These new LME models were constructed using the response variable of the difference in the mass-specific $\dot{V}O_2$ from the average sinusoidal wave.

We determined the amplitude of the wave and wave shift parameters using the lowest Akaike information criterion (AIC) value for each subset accounting of temporal auto-correlation, across all individuals and all seasons. Model evaluation tests were conducted by constructing the different models using 5 of the 6 test individuals' data and treating the 6th individual ("ME08") as a complete unknown (predicting $\dot{V}O_2$ knowing only the initial weight and activity score throughout the trial).

3.4 Results

3.4.1 Energy expenditure *via* respirometry

Rates of oxygen consumption and other values are presented as mean ± 1 SD (Tables 2.1, 3.1). The average rate of oxygen consumption ($\dot{V}O_{2DEE}$) of the northern fur seals across all individuals and all seasons throughout the DEE trials was 351.6 ± 58.8 mL O_2 min^{-1}, which on a mass-specific basis was 18.1 ± 2.4 mL O_2 min^{-1} kg^{-1}. $\dot{V}O_{2DEE}$ was significantly different between seasons (P = 0.002). The average $\dot{V}O_{2DEE}$ was lowest during the spring (305.2 ± 24.5 mL O_2 min^{-1}) and highest during the fall seasonal trials (432.4 ± 36.1 mL O_2 min^{-1}; P = 0.001; Fig. 3.3). Converted to estimates of daily energy expenditure (DEE_{resp}), the overall average DEE_{resp} was 10,238.5 ± 1,647.0 kJ d^{-1} (Table 3.1). The average mass-specific $\dot{V}O_{2DEE}$ was also at its highest in the fall seasonal trials (20.5 ± 1.7 mL O_2 min^{-1} kg^{-1}); however, unlike the average $\dot{V}O_{2DEE}$, the average mass-specific $\dot{V}O_{2DEE}$ was lowest in the winter (Fig. 3.4).

The average rate of carbon dioxide consumption ($\dot{V}CO_{2DEE}$) of the northern fur seals across all individuals and all seasons (omitting trials during which the RQ had to be estimated) throughout the DEE trials was 289.4 ± 133.7 mL CO_2 min^{-1}, which was 14.9 ± 6.7 mL CO_2 min^{-1} kg^{-1} on a mass-specific basis. $\dot{V}CO_{2DEE}$ was also significantly different between seasons (P = 0.001; Fig. 3.3). The resultant average RQ values within each season are 0.80 in the spring, 0.77 in the fall and 0.97 in the winter.

The average resting rate of oxygen consumption ($\dot{V}O_{2RMR}$) of the northern fur seals, across all individuals and all seasons was 332.1 ± 146.9 mL O_2 min^{-1} (17.3 ± 7.3 mL O_2 min^{-1}

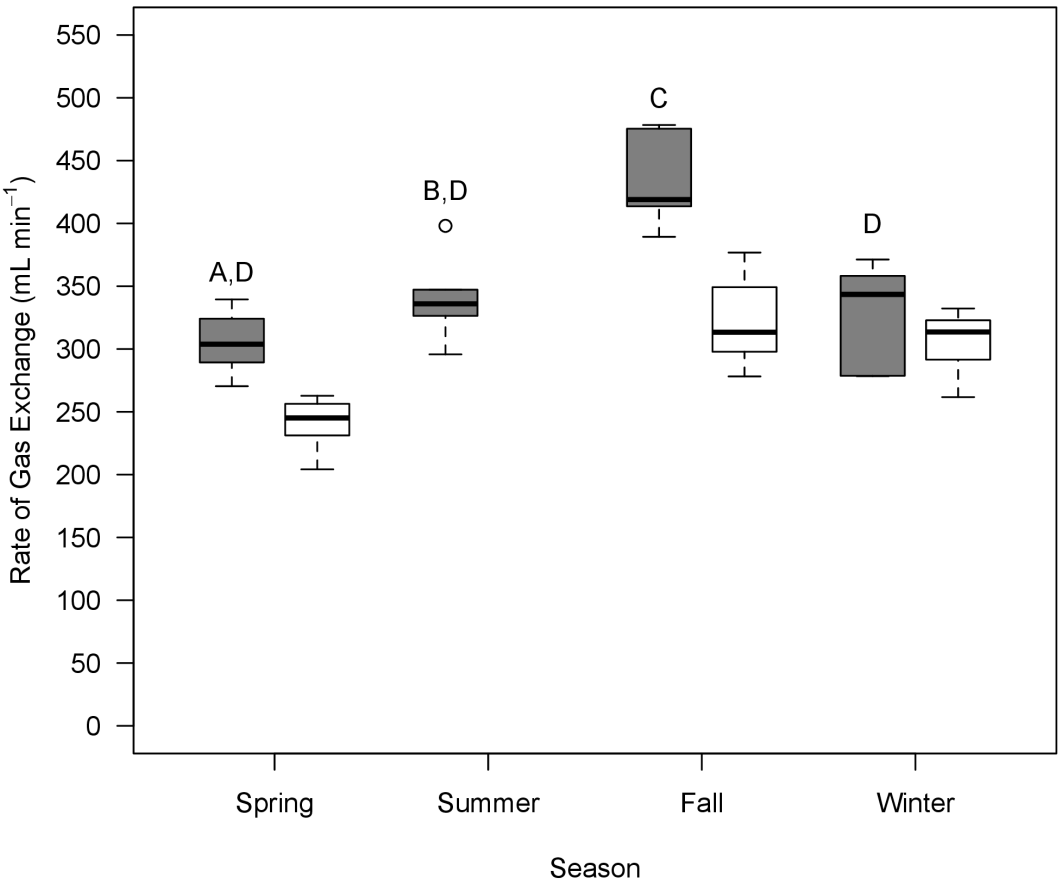

Figure 3.3 Average $\dot{V}O_2$ and $\dot{V}CO_2$ throughout the daily energy expenditure trials for 3 yr old female northern fur seals. The average $\dot{V}O_{2DEE}$ (gray boxes) and $\dot{V}CO_{2DEE}$ (white boxes) throughout 5-day daily energy expenditure trials are presented on a seasonal basis for six individuals. The average $\dot{V}O_{2DEE}$ was lowest during the spring seasonal trials, significantly higher in the subsequent summer trials (P = 0.01) and significantly higher again in the fall trials when the average $\dot{V}O_{2DEE}$ was at its zenith (P = 0.001). The average $\dot{V}O_{2DEE}$ in the winter was significantly lower than the fall (P = 0.001), but did not differ significantly from the spring and summer seasonal trials (P = 0.2).

Table 3.1 Daily energy expenditure (DEE) estimates for 3 yr old female northern fur seals derived from respirometry and the doubly labeled water (DLW) method over the course of the year. Average ± 1 S.D. estimates of daily energy expenditure (DEE) in six individuals measured in 4 seasonal sets of trials from March 2011 to January 2012. Values are provided for estimates derived from both the rates of oxygen consumption (respirometry) and 8 different techniques (equations) available to estimate CO_2 production based on the way the Doubly Labeled Water (DLW) method parameters are combined. The latter calculations used the plateau method and group scaled method for calculating the initial and final dilution space parameters, respectively. The average percent difference (overestimation) of each DLW estimate of the DEE, compared to respirometry is underneath each absolute measure. The *** columns represent the 3 most accurate DLW techniques on average.

Season	Respirometry	Lifson and McClintock (1966)	Coward *et al.* (1985)***	Schoeller *et al.* (1986)	Speakman (1993)***	Speakman *et al.* (1993)***	Racette *et al.* (1994) and Schoeller *et al.* (1995)	Speakman (1997) Two Pool	Speakman (1997) Single Pool	Average
Spring	8835.7 (± 720.3)	11415.3 (± 1339.5)	10225.2 (± 1524.7)	10965.8 (± 1210.1)	10623.5 (± 1171.8)	10256.0 (± 1134.2)	10262.5 (± 1134.8)	11126.8 (± 1227.9)	11829.0 (± 1394.2)	10838.0 (± 598.4)
	-----	29.4% (± 12.5)	15.7% (± 13.8)	24.3% (± 11.5)	20.4% (± 11.1)	16.3% (± 10.8)	16.3% (± 10.8)	26.1% (± 11.7)	34.0% (± 13.1)	22.8% (± 6.8)
Summer	9836.7 (± 979.1)	12733.4 (± 2178.9)	12503.7 (± 2180.9)	11969.4 (± 2041.7)	11532.2 (± 1914.7)	12530.0 (± 2206.2)	12623.4 (± 2233.8)	13617.4 (± 2453.0)	13293.3 (± 2354.1)	12600.3 (± 664.0)
	-----	29.1% (± 13.1)	26.8% (± 14.5)	21.3% (± 12.0)	16.9% (± 11.4)	26.9% (± 12.9)	27.9% (± 13.1)	37.9% (± 14.3)	34.7% (± 14.0)	27.7% (± 6.7)
Fall	12432.4 (± 1103.6)	13909.3 (± 1298.8)	12465.2 (± 1651.7)	13280.2 (± 1280.9)	12794.1 (± 1259.5)	12635.9 (± 1256.9)	12790.7 (± 1259.4)	13777.3 (± 1335.1)	14519.6 (± 1333.6)	13271.5 (± 730.3)
	-----	12.1% (± 6.9)	0.8% (± 13.8)	6.9% (± 6.4)	3.1% (± 7.0)	1.8% (± 7.2)	3.0% (± 7.0)	11.0% (± 6.9)	16.9% (± 6.0)	6.9% (± 5.8)
Winter	9849.3 (± 1126.7)	12331.9 (± 1274.7)	10639.7 (± 853.9)	11863.3 (± 1283.1)	11410.7 (± 1199.1)	10875 (± 1114.3)	10948.0 (± 1124.8)	11892.0 (± 1243.3)	12900.2 (± 1399.8)	11607.6 (± 782.8)
	-----	25.8% (± 12.6)	9.1% (± 15.0)	21.0% (± 11.8)	16.4% (± 11.4)	11.0% (± 11.1)	11.7% (± 11.1)	21.3% (± 11.9)	31.6% (± 13.2)	18.5% (± 7.9)
Average	10238.5 (± 1647.0)	12597.5 (± 1725.9)	11458.4 (± 1847.1)	12019.7 (± 1628.0)	11590.1 (± 1544.0)	11574.2 (± 1750.5)	11656.1 (± 1789.4)	12603.4 (± 1920.4)	13135.5 (± 1845.5)	-----
	-----	24.1% (± 13.0)	13.1% (± 16.5)	18.4% (± 12.1)	14.2% (± 11.8)	14.0% (± 13.6)	14.7% (± 13.6)	24.1% (± 14.6)	29.3% (± 13.4)	-----

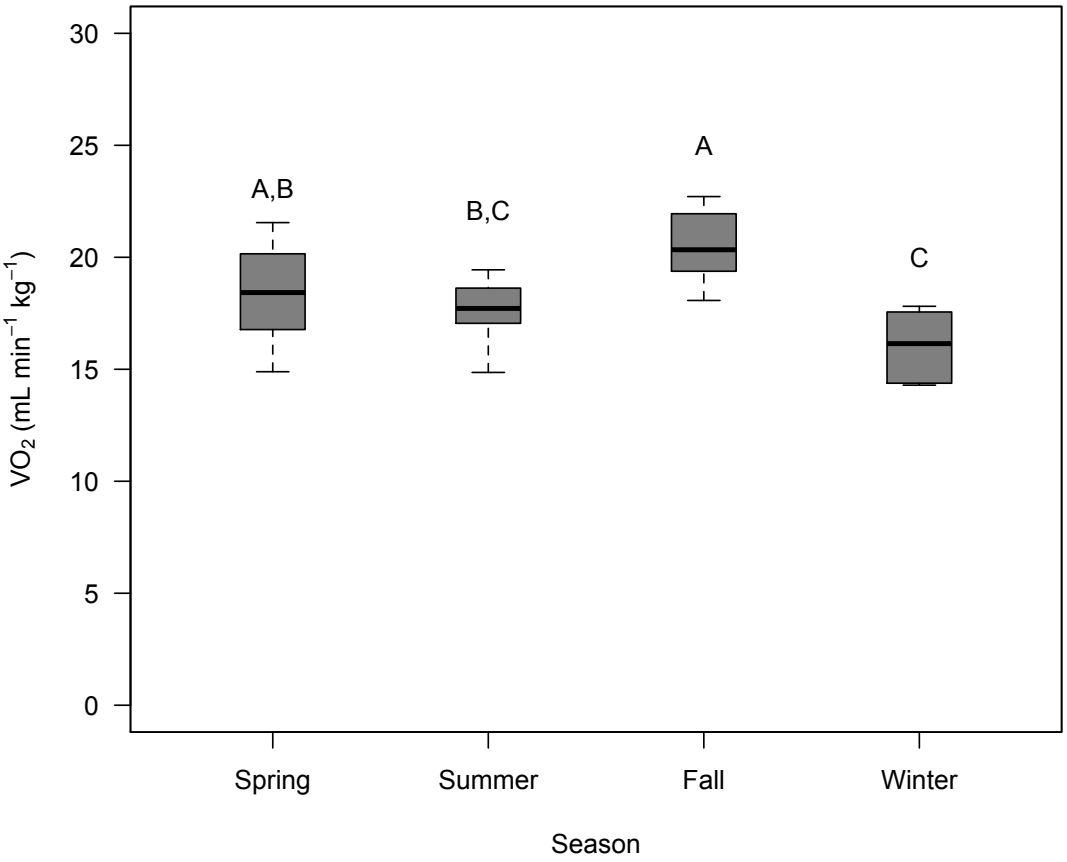

Figure 3.4 Average mass-specific $\dot{V}O_2$ throughout the daily energy expenditure trials for 3 yr old female northern fur seals. The average mass-specific $\dot{V}O_{2DEE}$ throughout 5-day daily energy expenditure trials are presented on a seasonal basis for six individuals. The average mass-specific $\dot{V}O_{2DEE}$ was at its zenith in the fall seasonal trials, significantly higher than either the summer or winter (P = 0.002), which are not significantly different (P = 0.3). However, the fall was not significantly different than the spring (P = 0.06). The spring seasonal trial itself was significantly higher (P = 0.02) than the winter but not significantly different than the summer (P = 0.7).

kg^{-1} on a mass-specific basis). However, one individual "ME08" was unusually active during these trials, and therefore that data does not reflect resting conditions. Omitting the $\dot{V}O_{2RMR}$ data from this individual, the average mass-specific $\dot{V}O_{2RMR}$ of the remaining northern fur seals, across all seasons, was 15.4 ± 5.1 mL O_2 min^{-1} kg^{-1}. The average $\dot{V}O_{2RMR}$ was significantly different between seasons (P = 0.001), and was significantly higher in the fall seasonal trials (19.3 ± 3.4 mL O_2 min^{-1} kg^{-1}) compared to the other three seasonal trials (overall mean 14.1 ± 4.9 mL O_2 min^{-1} kg^{-1}; P = 0.005), which were not significantly different from one another (P = 0.5).

Within each individual season the average $\dot{V}O_2$, and $\dot{V}CO_2$ (15 and 60 min intervals) changed significantly with the time of day (P = 0.001). In general, the average $\dot{V}O_2$ and $\dot{V}CO_2$ appeared to be increasing between the hours of 6 AM and 6 PM and decreasing between the hours of 6 PM and 6 AM (Fig. 3.5). As a result, the average RQ did not change significantly with the time of day (P = 0.1), except in the fall when using both the 15 and 60 min intervals and in the spring when using only the 15 min intervals (P = 0.01). The average hourly RQ only varied throughout the course of the day by 0.1 in the spring and fall and 0.2 in the winter.

3.4.2 Energy expenditure *via* doubly labeled water (DLW) method

Daily energy expenditure estimates obtained *via* the DLW method (DEE_{DLW}), incorporating the plateau and group scaled methods in combination with the different potential techniques (8 equations) are presented in Table 3.1. The average DEE_{DLW} estimates from the various techniques varied between 13.1 ± 16.5% and 29.3 ± 13.4% higher than the average estimates of DEE_{resp} (P = 0.05; Table 3.1). However, season was also a significant factor in the ability of the techniques to predict the DEE_{resp} (P = 0.001). Within the individual seasons, the average difference between the DEE_{DLW} estimates from each model and the DEE_{resp} estimates were lowest in the fall (6.9%) and highest in the summer (27.7%), with overestimates of 22.8% in the spring and 18.5% in the winter (Table 3.1).

3.4.3 Activity

The average Actiwatch activity score (15 s sample intervals) of the northern fur seals, across all individuals and all seasons, over the entire DEE trials was 76.3 ± 10.1 $interval^{-1}$, and differed significantly between seasons (P = 0.04). However, the only significant difference occurred between the fall (84.3 ± 6.3 $interval^{-1}$) and winter seasonal trials (69.6 ± 9.9 $interval^{-1}$; P

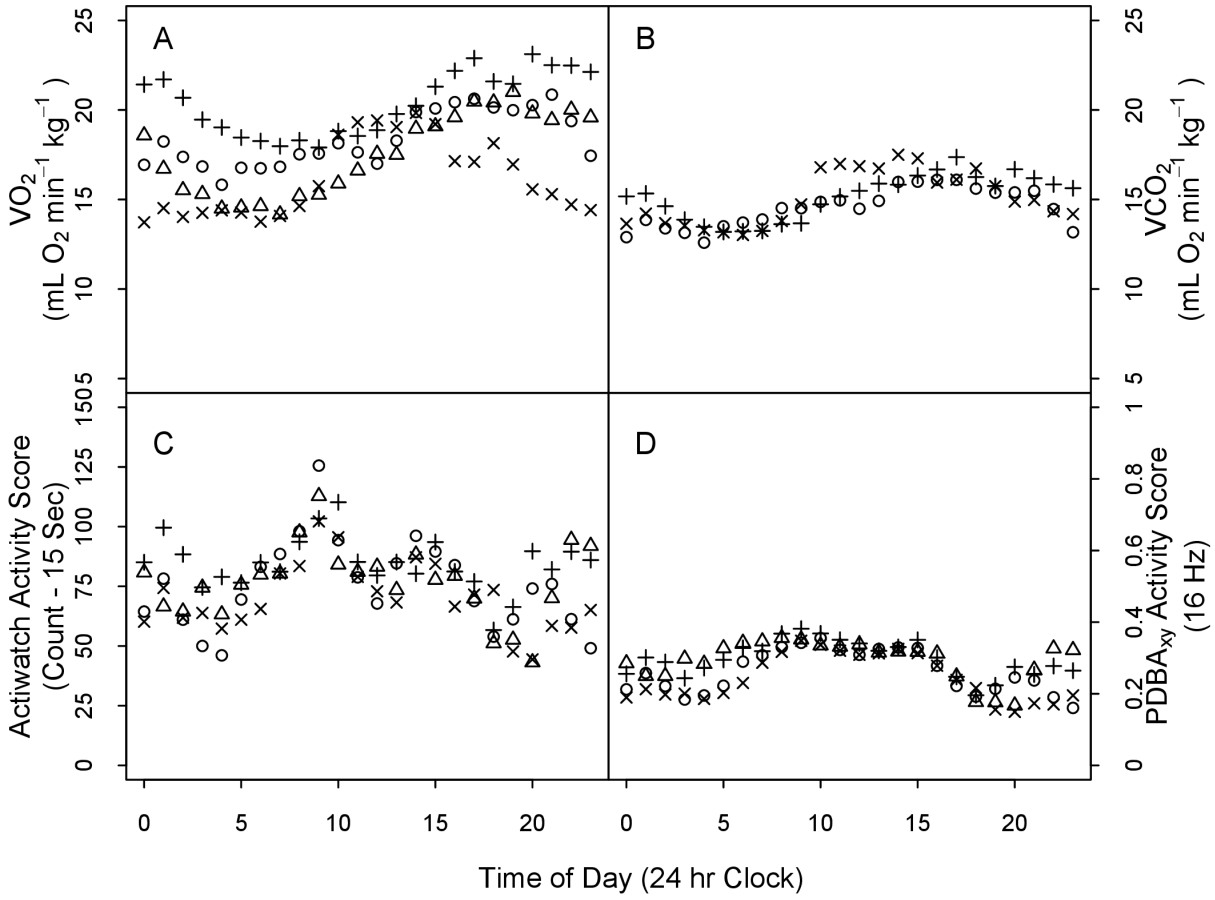

Figure 3.5 Hourly changes in $\dot{V}O_2$, $\dot{V}CO_2$, and activity score (Actiwatch and $PDBA_{xy}$) throughout the daily energy expenditure trials for 3 yr old female northern fur seals. Average hourly A) $\dot{V}O_2$, B) $\dot{V}CO_2$, C) Actiwatch activity score and D) $PDBA_{xy}$ activity score estimates throughout the day of six individuals from March 2011 to January 2012 during 5-day daily energy expenditure trials. Individual symbols represent the four seasonal sets of trials: Mar/Apr 2011 ("Spring"; ○), Jun/Jul ("Summer"; △), Sept/Oct ("Fall"; ＋) and 4) Dec 2011/Jan 2012 ("Winter"; ×).

= 0.001), neither of which were significantly different from the activity scores in the spring (74.5 ± 7.7 interval^{-1}) or summer seasonal trials (76.3 ± 11.6 interval^{-1}; P = 0.1; Table 3.2).

The average $PDBA_{xy}$ activity score derived from the Little Leonardo accelerometers across all individuals and all seasons during the DEE trials was 0.27 ± 0.06 g. The average $PDBA_{xy}$ score was not significantly different between seasons (P = 0.3), ranging from 0.30 ± 0.03 g in the fall to 0.24 ± 0.05 g in the winter (Table 3.2).

On the finer scale of 15 min sampling, the average Actiwatch activity score within each season changed significantly with the time of day during the fall and winter trials (P = 0.001) but did not during the spring and summer trials (P = 0.07). On the same scale, the average $PDBA_{xy}$ activity score also changed significantly with the time of day during the fall and winter seasonal trials as well as the summer (P = 0.002), but did not change significantly with the time of day during the spring (P = 0.4). The average activity scores (both Actiwatch and $PDBA_{xy}$) also changed significantly with the time of day on the scale of 60 min, within all seasons (P = 0.06) except for the average $PDBA_{xy}$ activity score during the summer seasonal trials (P = 0.02).

3.4.3.1 $\dot{V}O_2$ *via* accelerometry (calibration over the entire DEE trials)

Average Actiwatch and $PDBA_{xy}$ activity score separately, over the entire DEE trials, across all individuals and all seasons, are significant predictors of the average mass-specific rate of oxygen consumption ($\dot{V}O_{2DEE}$; P = 0.001). However, using either activity score, season was also a significant predictor of the mass-specific $\dot{V}O_{2DEE}$ (which was also significantly changing throughout the year) when added to the different models (P = 0.04). Within each individual season, neither Actiwatch nor $PDBA_{xy}$ activity score were significant predictors of the average mass-specific $\dot{V}O_{2DEE}$ (P = 0.07).

3.4.3.2 $\dot{V}O_2$ *via* accelerometry (calibration at 15 and 60 min intervals; fine scale)

Both activity measures, Actiwatch and $PDBA_{xy}$, were significant predictors of the mass-specific $\dot{V}O_2$ on the finer time scales of 15 and 60 min, when all individuals (individual "ME08" omitted – used in validation) and all seasons were pooled (P = 0.001). Additionally, season, time of day and resting metabolic rate were also significant predictors of the mass-specific $\dot{V}O_2$ when added to each model (P = 0.001).

Table 3.2 Average Actiwatch and $PDBA_{xy}$ activity score estimates from six, 3 yr old female northern fur seals during 5-day daily energy expenditure trials conducted on a seasonal basis.

Season	Average Actiwatch Activity Score	Average $PDBA_{xy}$ Activity Score
Spring	74.5 ± 7.7	0.26 ± 0.10
Summer	76.3 ± 11.6	0.29 ± 0.05
Fall	84.3 ± 6.3	0.30 ± 0.03
Winter	69.6 ± 9.9	0.24 ± 0.05

Within each of the individual seasonal trials, both the Actiwatch and $PDBA_{xy}$ activity scores were also significant predictors of mass-specific $\dot{V}O_2$ on the 15 and 60 min time scales (P = 0.002). However, RMR was not a significant predictor of the mass-specific $\dot{V}O_2$ in any season of the activity models (P = 0.05), except in the winter when using the Actiwatch activity scores with the 60 min time intervals (P = 0.04). Time of day, however, remained a significant predictor of the mass-specific $\dot{V}O_2$ when added to the models (P = 0.001). Accounting for temporal auto-correlation (by using one 15 or 60 min block of data every 4.75 hr), these significant results (P = 0.05) remained consistent for the majority of the subsets (>74%). The exceptions were 1) Actiwatch and $PDBA_{xy}$ activity were not significant predictors of the mass-specific $\dot{V}O_2$ in the summer seasonal trials for the $PDBA_{xy}$ activity score using the 15 and 60 min intervals and for the Actiwatch activity scores using only the 60 min intervals (< 37% of subsets significant) and 2) Time of day was only a significant predictor of the mass-specific $\dot{V}O_2$ using the Actiwatch activity monitors at the 15 min intervals in 68% of the subsets.

Validation of the models without the inclusion of a time of day correction found the models to generally under-predict the rates of oxygen consumption during the daytime hours and over-predict the $\dot{V}O_2$ during the nighttime hours (Fig. 3.6). Therefore, the sinusoidal wave correction was determined to be the most appropriate correction for time of day. The amplitude of the wave was 2.2 to 2.3 mL O_2 min^{-1} kg^{-1}, with a wave shift from zero of 0.95 to 0.99, for the two different time intervals (15 and 60 min) and the two different activity monitors. The predictive equations for the $\dot{V}O_2$ from the Actiwatch activity scores (Eq. 1a = 15 min intervals and Eq. 1b = 60 min intervals) are:

$$VO2 = ((C) + (2.2 \times Sin(2\pi T/24) + 0.98\pi) + (AAR)) \times (M) \qquad \text{(Eq. 1a)}$$

$$VO2 = ((C) + (2.3 \times Sin(2\pi T/24) + 0.99\pi) + (AAR)) \times (M) \qquad \text{(Eq. 1b)}$$

The predictive equation derived to estimate the rates of oxygen consumption from the $PDBA_{xy}$ activity scores (Eq. 2a = 15 min intervals and Eq. 2b = 60 min intervals) are:

$$VO2 = ((C) + (2.3 \times Sin(2\pi T/24) + 0.95\pi) + (PAR)) \times (M) \qquad \text{(Eq. 2a)}$$

$$VO2 = ((C) + (2.3 \times Sin(2\pi T/24) + 0.97\pi) + (PAR)) \times (M) \qquad \text{(Eq. 2b)}$$

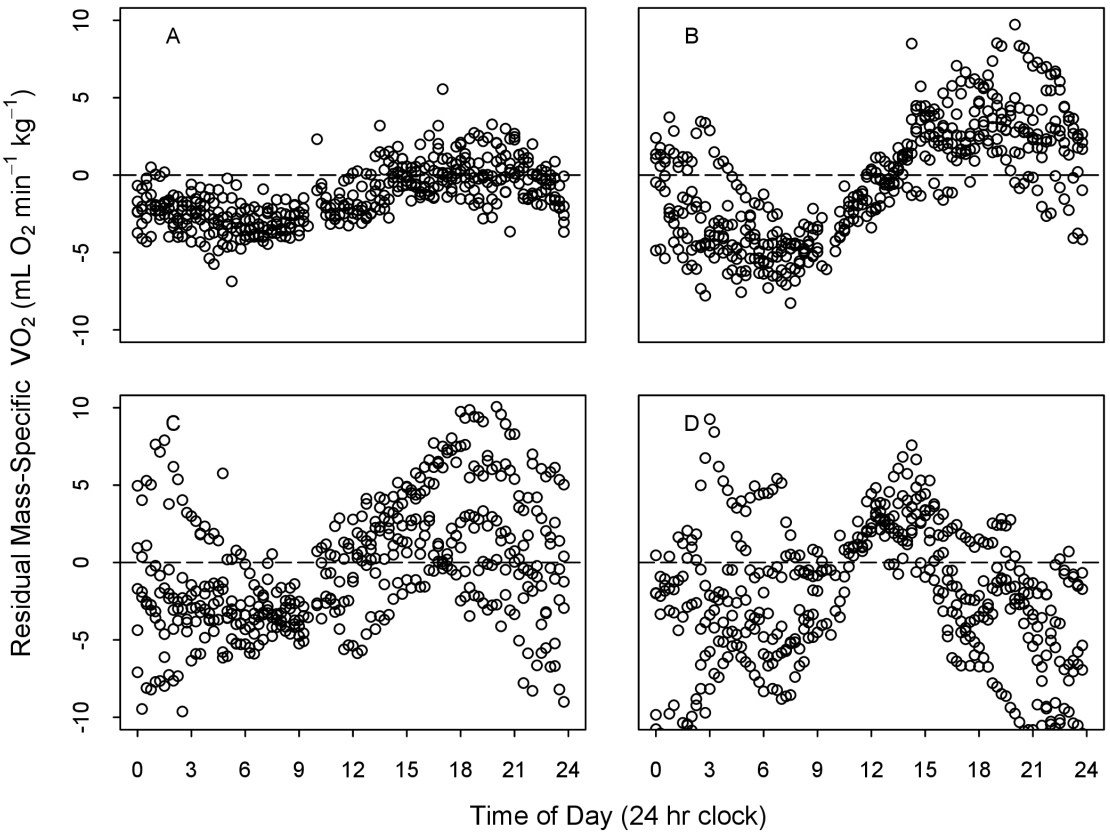

Figure 3.6 Model validation, 15-min time interval – Philips' Actiwatch, no time of day correction. The difference (residual) in the estimated mass-specific rates of oxygen consumption (mL O_2 min^{-1}) predicted from the Philips' tri-axial Actiwatch acceleration data logger from the measured mass-specific rates of oxygen consumption (mL O_2 min^{-1}) for test northern fur seal "ME08" throughout the course of the day, using the 15-min time interval. Comparisons were constructed separately to account for the different times of year: Spring (A), Summer (B), Fall (C) and Winter (D). The residuals appear to display an overlying sinusoidal wave pattern.

The season-specific components for Eqs. 1 and 2 are shown in Table 3.3. Within both equations, *C* is a seasonal constant, *T* represents the time of day on a 24 hr clock, and *M* is the animal's mass in kg. AAR is the Actiwatch activity regression and PAR is the $PDBA_{xy}$ activity regression. Using these predictive equations, the lowest average percent difference between the measured and predicted $\dot{V}O_2$ was 5.4 ± 29.3%, based on the Actiwatch activity score with 60 min time intervals (Eq. 1b; Table 3.4, Figs 3.7 and 3.8).

3.5 Discussion

Accurate estimates of energy expenditure in free-ranging animals, acquired using indirect techniques such as the DLW and accelerometry methods are essential to answering a range of fundamental questions in animal biology (Wilson *et al.* 2006, Green *et al.* 2009). Such data can provide insight into fields of interest that range from the behavioural ecology and life history strategies of individuals (harem reproduction strategies and energetics; Schulke 2001, Galimberti *et al.* 2007) to population energy budgets (nutritional status of wild animals; Winship *et al.* 2002, Rosen 2009). However, appropriate application of data derived from these merhods is dependent on understanding their innate accuracy and limitations. In our study, both of the indirect methods (DLW and accelerometry) demonstrated the potential to reliably estimate daily energy expenditure (as measured *via* respirometry). However, the accuracy of the measures was contingent on a number of factors, including the DLW model selected, the temporal resolution of the accelerometer, the time of year (season), and the time of day.

3.5.1 Doubly labeled water (DLW) method

The DLW method is a standard means for estimating the field metabolic rates of a variety of species, including pinnipeds (Speakman 1997, Nagy *et al.* 1999). However, these estimates of energy expenditure are rarely discussed in terms of their accuracy or their associated assumptions. For example, the model by which the raw isotope turnover data is combined to estimate CO_2 production and daily energy expenditure for the DLW method can have a substantial impact on the final value obtained (Speakman 1997). In addition to at least 9 different techniques (equations) available to estimate CO_2 production based on the way the parameters are combined, there are two different methods (plateau vs. intercept) for estimating the dilution space parameter, and two different methods (% mass vs. group scaled data) for approximating the final dilution space parameter (Speakman 1997). This results in 36 different potential estimates of the daily energy expenditure (DEE) for every DLW treatment (Speakman 1997).

Table 3.3 The season-specific components of the predictive equations (Equations 1, 2) for predicting the mass-specific rate of oxygen consumption ($\dot{V}O_2$) from two different activity measures (Actiwatch and $PDBA_{xy}$) in captive northern fur seals during two different time intervals of 15 and 60 min. With Acti representing the average Actiwatch activity score and $PDBA_{xy}$ representing the Little Leonardo $PDBA_{xy}$ activity score.

Time Interval	Season	C	Actiwatch Activity Regression	$PDBA_{xy}$ Activity Regression
15 Min	Spring	18.7345077	-2.64452 + 0.03643(Acti)	-2.16602 + 11.51964($PDBA_{xy}$)
	Summer	19.3589593	-1.65297 + 0.02122(Acti)	-0.71738 + 0.96113($PDBA_{xy}$)
	Fall	20.5692262	-4.09563 + 0.04861(Acti)	-2.99860 + 10.03522 ($PDBA_{xy}$)
	Winter	16.4081458	-2.19225 + 0.03122(Acti)	-1.73503 + 7.09068($PDBA_{xy}$)
60 Min	Spring	18.7345077	14.65145 + 0.04546 (Acti)	16.26087 + 12.35061($PDBA_{xy}$)
	Summer	19.3589593	16.51864 + 0.01461(Acti)	18.83477 - 4.00921($PDBA_{xy}$)
	Fall	20.5692262	15.95397 + 0.05424 (Acti)	18.33005 + 7.40302($PDBA_{xy}$)
	Winter	16.4081458	13.90306 + 0.03593(Acti)	14.63835 + 7.49722($PDBA_{xy}$)

Table 3.4 The average (± 1 S.D.) percent difference between the energy expenditure predicted from two different activity measures (Actiwatch and $PDBA_{xy}$) and measured $\dot{V}O_2$ in captive northern fur seals during two different time intervals of 15 and 60 min over the course of four seasonal trials. Positive numbers indicate that estimates based on accelerometers were greater than those measured via respirometry. The model validations of the two different activity measures, with time of day corrections, during the 15-min time intervals are shown in Fig. 3.7 and Fig. 3.8.

Time Interval	Season	Actiwatch Activity Score	$PDBA_{xy}$ Activity Score
15 Min	Spring	12.2 ± 19.7%	17.1 ± 52.2%
	Summer	3.9 ± 23.9%	2.1 ± 23.9%
	Fall	5.6 ± 25.3%	6.4 ± 26.3%
	Winter	33.5 ± 64.4%	36.2 ± 68.2%
	Average	13.8 ± 39.5%	15.4 ± 49.3%
60 Min	Spring	1.1 ± 5.8%	4.9 ± 45.0%
	Summer	1.8 ± 16.1%	2.0 ± 15.7%
	Fall	4.3 ± 20.5	4.7 ± 21.1%
	Winter	14.2 ± 51.2%	17.0 ± 55.1%
	Average	5.4 ± 29.3%	7.3 ± 39.9%

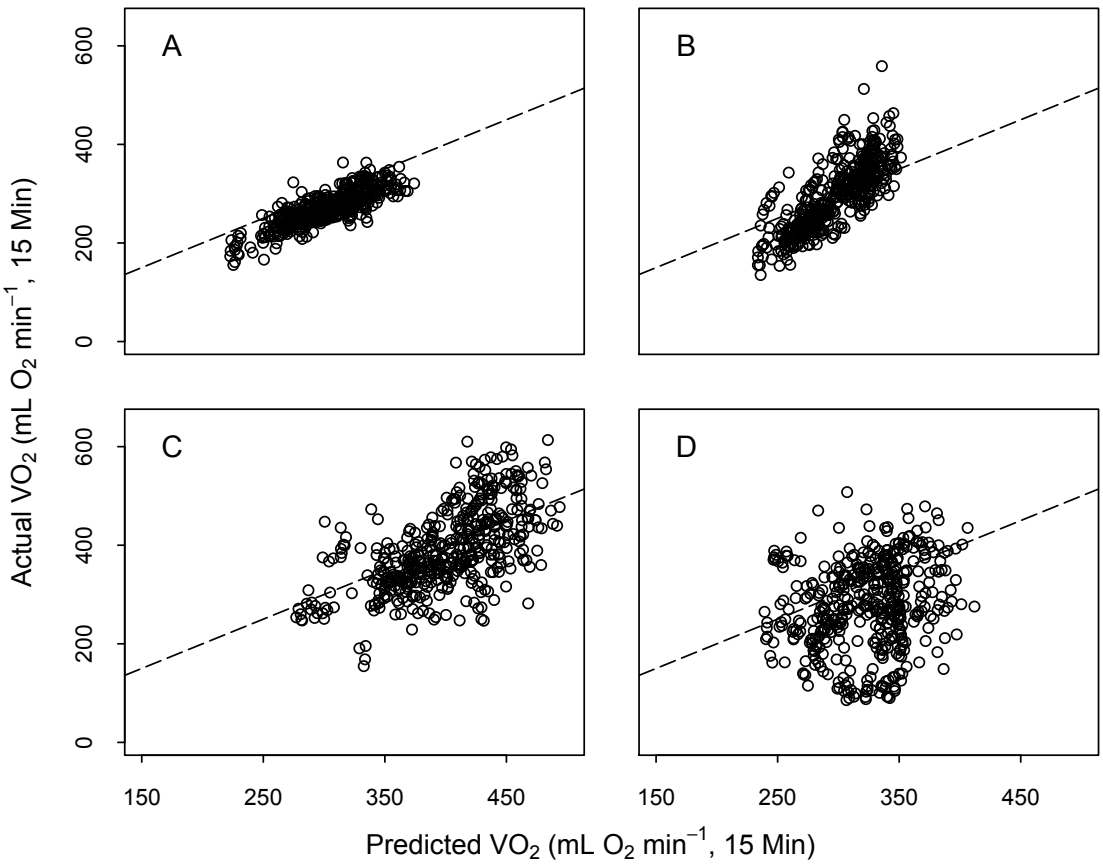

Figure 3.7 Model validation, 15-min time interval – Philips' Actiwatch, with time of day correction. The measured $\dot{V}O_2$ (mL O_2 min^{-1}) for test northern fur seal "ME08" compared against the estimated $\dot{V}O_2$ (mL O_2 min^{-1}) predicted from the Philips' tri-axial Actiwatch acceleration data logger using the 15-min time interval (see Eq. 1a and Table 3.3). The predictive equation included a time of day, sinusoidal wave correction. Comparisons were constructed separately to account for the different times of year: Spring (A), Summer (B), Fall (C) and Winter (D). The dashed lines represent an exact (1:1) predicted $\dot{V}O_2$ in comparison to the actual measured $\dot{V}O_2$.

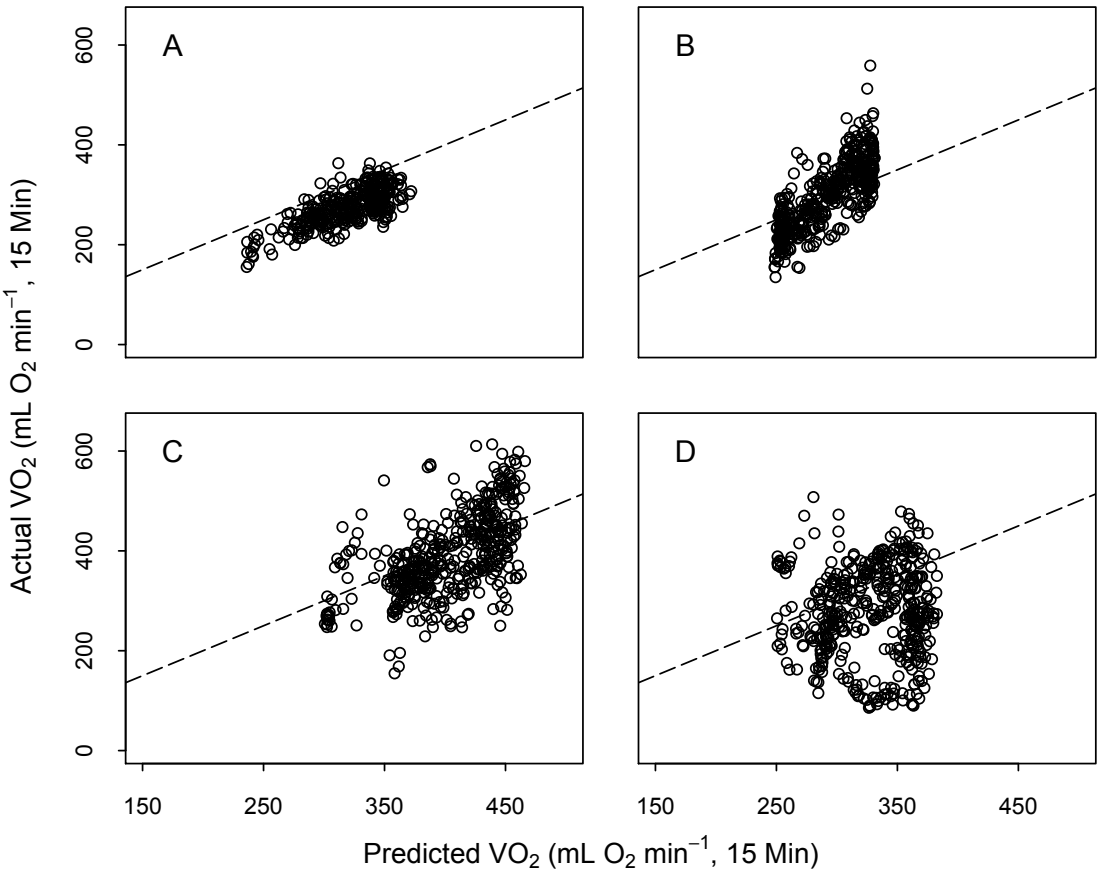

Figure 3.8 Model validation, 15-min time interval – Little Leonardo bi-axial acceleration data logger ($PDBA_{xy}$), with time of day correction. The measured rates of oxygen consumption (mL O_2 min^{-1}) for test northern fur seal "ME08" compared against the estimated rates of oxygen consumption (mL O_2 min^{-1}) predicted from Eq. 2a and Table 3.3 using the $PDBA_{xy}$ activity scores and the 15-min time intervals, obtained from Little Leonardo bi-axial acceleration data logger. These predictive equations include a time of day, sinusoidal wave correction. Predictions are constructed separately for each season: Spring (A), Summer (B), Fall (C) and Winter (D). The dashed lines represent an exact (1:1) predicted $\dot{V}O_2$ in comparison to the actual measured $\dot{V}O_2$.

The results of our study confirm Speakman's (1997) contention that the average difference between the smallest and largest of the DEE_{DLW} estimates was greater than 20%. Most of this variation is due to differences in the way model factors are combined, and only a minor amount of the difference resulted from the methods (plateau vs. intercept and % mass vs. group scaled) by which the dilution space and body water pool size parameters were calculated. As a result, accepting published field metabolic rates from the DLW method at face value without considering which technique (equation) was used to combine the parameters is precarious, as part of any observed "difference" may simply be the result of the calculation method used (Speakman 1997). For example, in our study we compared the different estimates of CO_2 production and DEE_{DLW} obtained only using the plateau and group scaled methods. We chose the plateau method because our fur seals were held in a run with only a small pool during the equilibration period, meaning that their activity levels during the equilibration period did not correspond well with their normal activity levels during the DEE trials, which is a requirement of the intercept method (Speakman 1997). In addition, we chose the group scaled estimate because an increased sample size increases the predictive power of the final dilution spaces (Speakman 1997).

In our study, the three estimates of the DEE_{DLW} that corresponded most closely to the measured rates of oxygen consumption (DEE_{resp}) were those based upon the models of Coward *et al.* (1985), Speakman (1993) and Speakman *et al.* (1993) — all of which are constructed on a two-pool approach (Speakman 1997). This was not surprising, given that two-pool approaches have been previously shown to be more appropriate for larger animals (> 5 – 10 kg) and humans (Speakman 1997). On average, these three best DLW models overestimated the DEE_{resp} by 13.1 – 14.2% (Table 3.1).

Previous validation studies on terrestrial mammals have reported an average discrepancy between DEE_{DLW} and DEE_{resp} estimates of only 2.2 ± 6.3% (Speakman 1997). Sparling *et al.* (2008) similarly reported that the DLW method overestimated the DEE of grey seals (*Halichoerus grypus*) by an average of 0.5%, although individual estimates ranged from underestimating by 39% to overestimating by 44%. Another validation study by Boyd *et al.* (1995) for California sea lions (*Zalophus californianus*) found the DLW method overestimated the DEE by on average 36% – 46%, although the short trial time only permitted a partial depletion of the hydrogen (14%) and oxygen (9%) isotope (compared to an isotope depletion of

> 35% in this study and that by Sparling *et al.* 2008). Therefore, the accuracy of the DLW method when applied to our northern fur seals is slightly less than generally previously found in terrestrial mammals, but within the range of discrepancy for previous marine mammal validation studies.

While our best overall estimates of DEE_{DLW} were 13% - 14% higher than the DEE_{resp} estimates, it is important to note that, no matter which model was chosen to calculate the DEE_{DLW}, the accuracy of our estimates were seasonally dependent. We found no significant difference between the DEE_{DLW} and DEE_{resp} estimates in the fall trials when using any of the aforementioned 3 best models. However, seasonal differences were apparent between DEE_{DLW} and DEE_{resp} estimates during all other seasons for all 3 models, except for estimates in the winter trials using the Coward *et al.* (1985) model. These seasonal differences in accuracy might reflect a seasonal violation of one of the 6 major assumptions of the DLW method, as described thoroughly by Speakman (1997). However, we could find no reason to believe that any of the assumptions had been violated, and certainly not on a seasonal basis.

A seasonal change in the respiratory quotient (RQ) is one potential explanation for the seasonal differences we observed in the relationship between the DEE_{DLW} and DEE_{resp}. Estimates of DEE_{DLW} assumed a constant RQ of 0.80 for the conversion of $\dot{V}CO_2$ to energy expenditure. Using the measured RQ of 0.97 instead of the best guess estimate of 0.80 for our winter trials improves the average difference of the DEE_{DLW} from 18.5% ± 7.9 to 1.5% ± 6.7 of the DEE_{resp}. During the spring, however, the measured RQ was 0.80 and therefore the observed significant difference between the DEE_{DLW} and DEE_{resp} estimates remain unchanged at 22.8% ± 6.8. Improving estimates of DEE_{DLW} using seasonally appropriate RQ values requires that they be estimated in each season from captive studies because it is not possible to capture gas exchange in free-ranging animals.

Changes in the ambient air and water temperatures are another potential explanation for the seasonal differences we observed in the relationship between the DEE_{DLW} and DEE_{resp}. Differences in air temperature can affect physical fractionation and the concentration of heavy isotopes (^{2}H or ^{18}O) entering the gaseous phase (leaving the body) compared to the concentration remaining in the body water (Speakman 1997). This includes both the processes of equilibrium fractionation (the gaseous water evaporating and condensing in close contact with the liquid

phase) and kinetic fractionation (the differential rate of isotope evaporation; Speakman, 1997). Previous studies have noted the importance of these fractionation corrections, and their ability to affect the accuracy of estimates of CO_2 production by 10% – 15%, which is consistent with the level of seasonal discrepancies in our study (Lifson *et al.* 1955, LeFebvre 1964, Tiebout & Nagy 1991).

Our fur seals experienced their highest average ambient air and water temperatures during the summer (air = 12.6 °C; water = 16.3 °C) and fall (12.8 °C; 15.2 °C) trials, and were exposed to lower temperatures during the spring (9.1 °C; 7.1 °C) and winter (9.2 °C; 3.7 °C) trials. The largest and smallest average difference between the DEE_{DLW} and DEE_{resp} estimates therefore occurred during summer and fall with temperatures that are quite comparable. It thus seems unlikely that fractionation differences due to ambient temperatures can explain the observed inaccuracies in energy estimation.

Resolving the source of the seasonal inaccuracies and achieving a better understanding of the biochemistry associated with the DLW method will improve the accuracy of this technique. Until then, studies of the daily energy expenditure of northern fur seals during the spring, summer, and winter months require more cautious interpretation, as there is a high likelihood of considerably over-estimating their true energy expenditure.

In reality, seasonal application of the DLW method to measure DEE for northern fur seals is impractical because northern fur seals undertake a substantial pelagic migration, and the effective time frame and quick turnover of the DLW would result in the requirement of at least one at-sea capture for measurements of the DEE from October to June (Kenyon & Wilke 1953, Nagy 1983, Bigg 1990, Gentry 1998, Ream *et al.* 2005, Sparling *et al.* 2008). Additionally, measurement of the DEE during the annual migration, particularly the initial migration (from the Aleutian Islands throughout the North Pacific Ocean as far south as California) using the DLW method could violate the assumption that the background levels of the isotopes are constant (Speakman 1997), as well as introduce fractionation variation due to large changes in average sea surface temperatures. Either factor would affect the accuracy of the DEE estimate (Speakman 1997).

3.5.2 Accelerometry

The failure of both measures of activity to predict $\dot{V}O_2$ over entire 5-day DEE trials within each season was not surprising. The concept of ODBA is that acceleration can be used to measure movement, providing a proxy for the $\dot{V}O_2$ required by muscular contractions to aerobically power the movement (Wilson *et al.* 2006). Strong relationships are predicted during episodes of physical activity, as the costs of movement can exceed any other energetic function by a factor of 10 or more (Darveau *et al.* 2002, Wilson *et al.* 2006). Hence, predictive relationships between activity and energy expenditure have been established for a variety of species during different types of activity (Wilson *et al.* 2006, Fahlman *et al.* 2008a, Halsey *et al.* 2009b). However, for an accurate estimate of energy expenditure over an extended period of time, the predictive equation must include animals at rest (acceleration values of 0).

Individuals can spend extended periods of time inactive, during which their energy expenditure can be highly variable due to expensive physiological processes such as growth, specific dynamic action, reproduction or molting (Kumagai 2004, Wilson *et al.* 2006, Green *et al.* 2009). Nevertheless, Green *et al.* (2009) established that activity measures during periods of supposed inactivity, such as digestion or thermoregulation, were still able to predict oxygen consumption when small amounts of movement occur, but the rate of change of oxygen consumption to activity (during digestion or thermoregulation) was far greater and larger inaccuracies resulted if precise activity measurements were not achieved. This suggests that the concept of using a single estimate of activity level over an extended period to predict the average $\dot{V}O_2$ is flawed, as the relationship between activity and the mass-specific $\dot{V}O_2$ may be a two-phase relationship, such that greater periods of inactivity will result in greater inaccuracies (Wilson *et al.* 2006, Green *et al.* 2009). Alternately, averaging the activity and $\dot{V}O_2$ over extended periods (5 days) will tend to decrease variability in the data and thus predictive power.

Although unable to yield a single simple estimate of total energy expenditure, these miniaturized accelerometers store high-resolution data that can be used to estimate activity-specific and fine scale estimates of energy expenditure (Wilson *et al.* 2006). In our study, we found a significant correlative relationship between activity and the mass-specific $\dot{V}O_2$ when averaged over both 15 and 60 min intervals.

Overall, the predictive relationships we found between activity and energy expenditure were significantly improved when season, time of day and RMR were incorporated into our models. Modeling energy expenditure within a season as a function of acceleration was similarly improved by accounting for time of day. In retrospect, the importance of correcting for season and time of day should not be surprising. The majority of calibration studies conducted to date have only compared activity to oxygen consumption using terrestrial species, exercising for a relatively short period of time on a treadmill, during a specific time of year (Wilson *et al.* 2006, Green *et al.* 2009, Halsey *et al.* 2009a, Halsey *et al.* 2009b). Additionally, none of these validation studies appear to have accounted for circadian oscillators that regulate the day-night cycle of metabolic and behavioural processes, or the effects of photoperiod on the annual rhythm of energy metabolism, molting and reproduction (Warner *et al.* 2010).

In essence, incorporating season (with an independent constant) and time of day into our predictive models corrected for changes in zero-activity metabolism that was independent of changes in body movement. Although we had expected these changes could be accounted for by including RMR into our models, we found instead that the seasonal predictive equations superseded RMR as a significant predictor of the mass-specific $\dot{V}O_2$. The results of our study, therefore, point to the need to further investigate the potential influence of seasonality and time of day on the relationship between activity and oxygen consumption, in addition to the effects of all (zero activity) metabolic processes such as thermoregulation or digestion.

Our study is not the first to suggest that the relationship between activity and energy expenditure varies with season. Enstipp *et al.* (2011), for example, found that the increase in the $\dot{V}O_2$ of adult green turtles (*Chelonia mydas*) during swimming when compared to submerged resting was 50% higher on average in the winter than in the summer. However, our study may be the first to identify time of day as being a significant predictor of metabolism — and to include a sinusoidal wave correction into the predictive equations.

The predictive relationships we found between activity and energy expenditure were also improved by lengthening the sampling interval from 15 to 60 min. This decreased the differences between the measured and predicted $\dot{V}O_2$ from 13.8% to 5.3% based on the Actiwatch activity score and from 15.4% to 7.1% based on the $PDBA_{xy}$ activity score. The improved accuracy that results from lengthening the sampling interval is likely due to the averaging or smoothing of fine

scale peaks in activity levels and metabolic rate, as well as eliminating any effects related to air turnover in the metabolic chamber.

Our experimental setup likely limited the range of activity levels that our fur seals could have attained, given that the maximum and minimum rates of oxygen consumption differed by an average factor of 3.6. The higher estimated costs of movement observed in other species suggests that the fur seals in our study never reached their peak activity levels (Darveau *et al.* 2002, Wilson *et al.* 2006). Other calibration studies have used human interaction to motivate increased activity levels (Halsey *et al.* 2009a, Enstipp *et al.* 2011), but we purposely designed our study to incorporate as little human interaction and influence during the trials as possible. Achieving a broader range of activities without inducing physiological stress may therefore be needed to ensure the calibrations can be meaningfully applied to predict the energy expenditure of wild individuals equipped with activity monitors.

Another important finding from our study was that the accuracy of predictions stemming from the $PDBA_{xy}$ and Actiwatch activity measures were not identical. The logical explanation is the number of axis in which acceleration was measured. Most notably, the Little Leonardo accelerometers monitored acceleration in only the dorso-ventral (heave) and anterior-posterior (surge) axes. In comparison, the Philips Actiwatch also monitored the 3rd axis' acceleration, the lateral axis (sway), and provided data more akin to measures of Overall Dynamic Body Acceleration (ODBA).

Halsey *et al.* (2009a) found ODBA to be only moderately better than any single axis for predicting rates of oxygen consumption. Their finding suggests that there should have been little consequence to the predictive abilities of the two devices (one with the 3rd axis and the other without). However, daily activities of our fur seals went far beyond simple travelling and diving behaviours, and included complex activities like grooming or rolling which potentially involved stronger sway components. As such, the Little Leonardo accelerometers would not have measured these parts of the daily activity budget.

The use of a harness for logger attachment could also explain some of the differences between accelerometers within and between animals. In 3-dimensional loggers, minor logger orientation and placement changes associated with a harness attachment would be non-significant as reductions in one axis will be accounted for in the other axes that is not the case in

a 2-dimensional logger (Halsey *et al.* 2011). Differences may also be attributable to how the static acceleration is accounted for in the two activity measures (the Little Leonardo accelerometer data required a 2 second smoothing function, whereas the Philips Actiwatch set the static acceleration as a threshold value that needed to be exceeded).

Regardless of whether accelerometer data can or cannot be used to estimate daily energy expenditure, acceleration data loggers still have great utility for estimating activity levels in a range of species. For example, Fossette et al. (2012) found that the ectothermic loggerhead sea turtle (*Caretta caretta*) use a form of active thermoregulation (thermal substitution) at the beginning of their reproductive season to maximize the reproductive output.

The future application of the accelerometry method for the measurement of the daily energy expenditure in wild northern fur seals requires further studies to complete the calibration against a full range of activities. For extended studies seeking to quantify energy expenditures (such as the 8 mo required for deployment during the northern fur seals' complete annual pelagic migration), our study indicates there is great potential for the Philips' Actiwatch based on its relative simplicity, deployment length and accuracy. However, if a more thorough understanding of the activities being performed throughout the day were desired, then quantitative data on the body posture (static acceleration) and motion (dynamic acceleration) in each axis would be required; information only attained through more advanced accelerometers (such as the Little Leonardo bi-axial accelerometers used in this study) which provide acceleration data in each individual axis. In general, $PDBA_{xy}$ has shown potential with regards to its predictive accuracy, but significantly greater memory length and battery power are required for deployment on wild northern fur seals outside of the breeding season.

3.6 Conclusions

The DLW method and accelerometer activity monitor are both good means to accurately estimate rates of energy expenditure in free-ranging northern fur seals, although on different time scales. The DLW method has reasonable accuracy, but is limited by expense, logistical challenges, and a narrow window of time (on a scale of days) with which it can be deployed. The DLW method also needs to take into account seasonal inaccuracies that potentially result from seasonal changes in the respiratory quotient (RQ) or temperature and physical fractionation, data that can be best gathered from captive animal studies. In contrast, the relatively simple and inexpensive accelerometers (such as the Philip's Actiwatch), with comparable accuracy, can be

deployed for extended periods (on a scale of months). Accelerometers are thus potentially more beneficial for measuring energy expenditure in free-ranging animals, compared to the DLW. However, measures of accelerometry could only predict rates of oxygen consumption on a short-term basis. Their use also requires additional calibration studies that encompass a fuller range of activities to ensure their predictive power remains high. Energy expenditure for any given activity is not constant, but appears to be influenced by season and time of day. These two variables must thus be taken into account when estimating the energy expenditure of free-ranging animals from measures of acceleration.

Chapter 4: Resting metabolic rate and activity: Key components of variation in daily energy expenditure for the northern fur seal

4.1 Summary

Seasonal estimates of daily energy expenditure (DEE) are required to estimate food requirements, while an understanding of the underlying components of DEE elucidates the bioenergetic priorities and physiological flexibility of individuals. We quantified seasonal DEE and four key components of the energy budgets (costs of resting metabolic rate, thermoregulation, activity and growth) of six captive northern fur seals (Callorhinus ursinus). This group of young females had an average DEE of 527.8 ± 65.7 kJ kg^{-1} d^{-1} that fluctuated seasonally (being ~ 20% greater in the fall than in the winter). Resting metabolism was the largest component of DEE (~ 80% on average) and also changed significantly with season (being higher in the fall; potentially due to molting or anticipated migratory activity). However, resting metabolism did not follow the same seasonal trend as DEE. Cost of activity was the second major component of DEE and may explain the seasonal variations in DEE. Energetic costs associated with thermoregulation and growth appeared to be negligible. The elevated innate cost of resting metabolism in the fall and higher growth rates in the summer suggests inadequate nutrition could have greater negative effects on female fur seals during these seasons than at other times of year.

4.2 Introduction

Alaska's Pribilof Islands, St. Paul and St. George, are currently home to ~ 50% of the world's northern fur seals (*Callorhinus ursinus*; Testa 2012). This population declined significantly from 1956 to 1980, and began declining again in 1998 at ~ 5% per yr (Towell *et al.* 2006, Testa 2012). The initial decline (1956 – 1980) has been linked to the commercial harvest of females, scientific pelagic collections and higher than normal juvenile mortality (Trites & Larkin 1989, Towell *et al.* 2006). However, explanations for the lack of population recovery following the cessation of these activities and for the current population decline remain elusive.

Decreases in the number of young females returning to the rookeries on St. Paul Island is among the evidence that suggests that nutritional stress may be contributing to the current decline of the Pribilof Islands' northern fur seal population (Spraker & Lander 2010). Nutritional stress has similarly been implicated as a potential explanation for the decline of numerous marine mammal and seabird populations in the Bering Sea and North Pacific Ocean (Trites & Donnelly

2003, DeMaster *et al.* 2006, Jodice *et al.* 2006, Rosen 2009). In general terms, the nutritional stress hypothesis suggests that an inability to secure adequate food (due to changes in the quality or quantity of available prey) to meet nutritional or energetic requirements can impact marine mammal populations (Trites & Donnelly 2003, Rosen 2009).

In theory, determining the likelihood that members of a population are suffering from nutritional stress should be relatively straightforward. By definition, nutritional stress occurs when there is a mismatch between daily energetic requirements and nutritional intake (King & Murphy 1985). However, like many mammals, northern fur seals have seasonal life cycles that likely result in highly seasonal energy expenditures and nutritional requirements (which may be temporally offset from each other) that become more pronounced with age (Robeck *et al.* 2001, Rosen *et al.* 2012, Rosen & Trites 2014). As a result of these seasonal bioenergetic cycles, season-specific estimates of energetic requirements and expenditures are required to assess potential conditions for nutritional stress.

Unfortunately, the daily energetic expenditures (DEE) of wild northern fur seals are virtually impossible to measure from the late fall to early summer due to their lengthy pelagic migration (Kenyon & Wilke 1953, Bigg 1990, Gentry 1998, Ream *et al.* 2005). Our study sought to fill this data gap by investigating the pattern of seasonal changes in DEE of a group of captive northern fur seals.

In tandem with seasonal variation in overall energy expenditure, there can be seasonal changes in the proportion of energy dedicated to different components of an animal's energy budget (Rosen & Kumagai 2008). This variation in energetic priorities can result in differing consequences in response to an episode of nutritional stress, depending on the time of year during which it occurs (Jeanniard du Dot *et al.* 2008b, Rosen & Kumagai 2008). Ultimately, varying energetic priorities can result in nutritional deficiencies having a greater impact during some seasons compared to others.

To investigate the varying energetic priorities of the northern fur seal and potentially identify critical seasons throughout the year, we seasonally measured four key components of the energy budget — the costs of resting metabolic rate, growth, thermoregulation, and activity — of the group of captive females. Our aim was to identify the major components of their energy budgets, quantify how they vary throughout the year, and infer the importance of each in the event of a potential nutritional stress event.

The pattern and costs of seasonal changes in total energy use and the major components making up the energy budget of northern fur seals can be quantified from individuals held in captivity to provide insight into the same processes that occur in wild populations. This reflects the fact that intrinsic physiological changes occur within the energy budget of a pinniped (*i.e.*, resting metabolic rate, growth, thermoregulation) regardless of whether it is in captivity or living in the wild (Rosen & Renouf 1998, Donohue *et al.* 2000, Sparling *et al.* 2006, Liwanag 2010, Rosen *et al.* 2012, Rosen & Trites 2014). Thus, captive studies permit identifying innate seasonality in the energy requirements and energetic priorities of northern fur seals and can be used to infer the potential effects of seasonal biotic and abiotic environmental changes on the nutritional status of wild fur seals.

The DEE of northern fur seals and other mammals should change significantly throughout the year as a cumulative response to seasonal variations in individual components of their energy budgets. Resting metabolic rate (a standard measure of baseline energy expenditure) has been shown to change seasonally, independent of other direct bioenergetic concerns (such as the costs of growth and thermoregulation) in both young northern fur seals (Rosen & Trites 2014) and in mature individuals of other pinniped species (Rosen & Renouf 1998, Sparling *et al.* 2006). Increased energy expenditures associated with the cost of growth are also predicted to occur seasonally, being highest in the summer (June to August) when immature northern fur seals exhibit increases in mass and length (Trites & Bigg 1996). In the wild, fur seals are most likely to encounter sea surface temperatures outside of their thermal neutral zone (TNZ) during their annual pelagic migration (late fall to early summer; November to June). In the captive environment, colder ambient air and water temperatures will also occur during the fall and winter periods. Hence, additional thermoregulatory costs should elevate DEE during these times of year as part of the physiological response to maintain core body temperatures (Bryden & Molyneux 1978, Williams & Worthy 2002, Liwanag 2010). Increased energy expenditures associated with higher levels of activity are also predicted in the late fall and early winter (November to January) when rapid migration to the wintering grounds would occur in the wild (Kenyon & Wilke 1953, Bigg 1990, Gentry 1998, Ream *et al.* 2005). Conversely, lower activity levels and associated metabolic rates are predicted during the late winter (January/February) and summer, when comparatively localized foraging would naturally occur (Kenyon & Wilke 1953, Bigg 1990, Gentry 1998, Ream *et al.* 2005).

While seasonal shifts in the energetic costs associated with the different major components of an animal's energy budgets (*i.e.*, resting metabolic rate, thermoregulation, activity, and growth) are relatively straightforward to predict, the cumulative effect of simultaneous changes in these individual bioenergetic costs on total energy expenditure is much more difficult to foresee. Such information on both the total energy requirements (DEE) and the underlying components is required to identify times of the year when unpredicted episodes of nutritional stress would have their greatest negative impact on northern fur seals.

4.3 Methods

4.3.1 Animals

Six female northern fur seals (Table 2.1) participated in our study from March 2011 to January 2012. The individuals came from a rookery on St. Paul Island, Alaska, in October 2008 at ~ 4 mo of age (post-weaning). They were raised at the University of British Columbia's Marine Mammal Energetics and Nutrition Laboratory, located within the Vancouver Aquarium (British Columbia, Canada), and trained with positive reinforcement to be familiar with all necessary husbandry behaviours, research protocols and scientific equipment. The fur seals were normally housed in seawater pools with water temperatures that reflected the local ocean conditions (7 °C to 16 °C). A daily diet consisting of (~ 90 – 95%) herring and (~ 5 – 10%) squid supplemented with vitamins was fed to the individuals in two separate feeds over the course of the day: 2/3 of the daily diet in the morning and 1/3 in the afternoon. The quantity of each prey species in the daily diet was determined by training and veterinary staff, designed to provide sufficient levels of energy intake (on average: 8.7 ± 1.3% of the total body mass; 1.4 – 1.8 kg d^{-1} of prey, across all individuals and all seasons) within working/training requirements. Animal Care Committees of both the Vancouver Aquarium and the University of British Columbia (Permit #A10-0342) approved all animal use and research protocols described.

4.3.2 Timing

Our study consisted of four seasonal sets of trials each of which took ~ 7 wk to complete: 1) Mar/Apr 2011 ("Spring"; Age 2.75 yr old), 2) Jun/Jul ("Summer"; Age 3 yr), 3) Sept/Oct ("Fall"; Age 3.25 yr) and 4) Dec 2011/Jan 2012 ("Winter"; Age 3.5 yr). The order of the individuals tested within a seasonal set of trials was determined randomly.

4.3.3 Daily energy expenditure (DEE)

The daily energy expenditure was quantified *via* respirometry using a large metabolic chamber (see below) to continuously measure the rates of oxygen consumption and carbon dioxide production of each individual throughout a period of nearly five days. Once the fur seal entered the metabolic chamber (voluntarily under trainer control), it was free to undertake its normal daily activities either on land or in water. During these DEE trials, the test individual only interacted briefly with staff twice daily. Each morning, the individual received its first feed and a quick physical health assessment (including measuring body mass) outside of the metabolic chamber (8.6 ± 4.0 min). Each afternoon, the individual received its second feed within the metabolic chamber *via* a sealed access tube.

The metabolic chamber consisted of a large, airtight dome, constructed of welded aluminum and Lexan^{TM}, placed over a holding pool and the associated haulout space (Fig. 3.1). The approximate air space volume of the metabolic chamber was 3500 L. An internal air circulation system ensured proper air mixing within the chamber and the access tube over the haul-out space permitted feeding directly into the chamber without compromising the integrity of the metabolic measurements. Air was drawn through the metabolic chamber at 125 L min^{-1} into a gas analysis system *via* an excurrent airflow pipe located above the pool. This process generated a 50% air turnover rate of ~ 19 min. A door in the chamber (located on the haul out space) permitted the animal controlled entrance/egress from the metabolic chamber. The entire metabolic chamber was tested for leaks and proper air circulation prior to use. A closed circuit camera (mounted above the chamber) and a digital video surveillance system (CONTI Electronics Ltd., Vancouver, British Columbia, Canada) recorded the movement of the fur seals over the duration, of the experimental trials to check animal behaviour in the event of an unusual metabolic event.

Metabolic rates inside of the metabolic chamber were determined using open flow respirometry to calculate rates of oxygen consumption and carbon dioxide production. Briefly, measurements were made using one of two systems incorporating Sable Systems (Las Vegas, NV) mass flow pumps and oxygen and carbon dioxide analyzers to ascertain the appropriate gas concentrations of a dried subsample of the excurrent air stream (as detailed in Chapter 2). The entire gas analysis systems were calibrated with dry ambient air at the start and end of each 5-day trial, in addition to each morning, such that changes in gas concentrations were determined

against baseline (ambient) measures to account for system drift. The systems were also periodically calibrated against gases of known concentrations.

Rates of oxygen consumption were calculated from measured changes in gas concentration using LabAnalystX software (M. Chappell, UC Riverside, Riverside, California) and incorporating the appropriate equations from Withers (1977). A malfunctioning CA-1B analyzer (CO_2 sensor) was detected, in one of two gas analysis systems, in a portion of the first two seasonal sets of trials of our study. For trials with an average RQ value outside of the reasonable expected physiological range (0.65 - 1.05), the $\dot{V}O_2$ was therefore calculated using a fixed RQ value of 0.8 rather than an RQ based on the erroneous measured rates of expired CO_2. Rates of oxygen consumption were converted to estimates of energy expenditure using the energy equivalents of $\dot{V}O_2$ for different RQ values as determined by Brody (1945).

4.3.4 Cost of activity

During the DEE trials, an Actiwatch tri-axial acceleration data logger (length = 29 mm, width = 37 mm, height = 11 mm, mass = 16 g; recording range ± 2 g; Philips Healthcare, Bend, Oregon, USA) was used to record the body acceleration (as a proxy for activity) of the northern fur seals. The Actiwatch logger provided data in the form of a single unit (count) of the number of times the test subject exceeded the threshold acceleration in any dimension (surge, heave or sway) during each 15-sec interval. A previous study (Chapter 3) demonstrated a strong relationship between the resultant Actiwatch score and rates of oxygen consumption over the course of entire DEE trials throughout the year and within each season on a fine scale; indicating that this logger provided a good estimate of the physical activity and energy expenditure that would contribute to DEE.

The logger was secured inside of a Velcro-sealed pocket attached to a custom-made harness worn by the fur seal and lay dorsal to the pectoral flippers. The harness consisted of an adjustable fabric collar, anterior to the pectoral flippers, which fit closely to the individual without restricting movement. In addition to, an elastic bellyband that encircled the animal posterior to the pectoral flippers that kept the logger stationary on the fur seal (Fig. 3.2).

4.3.5 Cost of growth

Body mass measurements (± 0.02 kg) of each fur seal were collected daily prior to the individual's first feeding (at least 16 hr postprandial) by having the fur seal stand stationary on a platform scale. Measurements of body length (nose to tail; ± 1 cm) were obtained bi-weekly by

having the animal lie ventral side down on a ruler. Body mass measurements were used to calculate mass-specific metabolic rates, as well as to calculate changes in mass (*i.e.*, growth rates) over the course of each 7 wk seasonal trial. Body length measurements are inherently not as precise as mass measurements due to variation associated with body position. Therefore, multiple body length measurements were obtained within each seasonal trial, and the data were averaged to ascertain changes in length between seasonal trials.

Changes in body mass could be further partitioned into changes in body composition between seasons by incorporating single point estimates of body composition. Immediately preceding each DEE trial, the deuterium (D_2O) dilution technique was used to determine the total body water of the fur seals (Reilly & Fedak 1990). Total body water content was then used to estimate the fur seal's body composition (total body lipid and total body protein) using the "all animal – adult and pup" regression equation validated by Arnould *et al.* (1996a) for Antarctic fur seals (*Arctocephalus gazella*).

The procedure for the deuterium (D_2O) dilution technique followed the method described in Costa (1987) and Reilly and Fedak (1990). Briefly, all blood samples were collected from the caudal gluteal vein and were obtained with the animals under veterinary supervised anaesthetic (maximum 5% Isoflurane). An initial background blood sample was drawn into a serum separator tube prior to the administration of the D_2O. The injection of 99.9% D_2O water was then administered intramuscularly at a measured dosage of approximately 0.16 g kg^{-1} of animal. A second blood sample was drawn 2 hr post-injection (permitting equilibration with the body water pool; Costa 1987) to assess the increase in the concentration of ^2H. Animals were awake and kept in a holding run with a circular wading pool and running water during the 2 hr equilibration period; this did not confound the calculation of total body water, as these individuals have never been observed to drink water.

Blood samples were centrifuged and the collected serum was stored at -70 °C until analysis. Metabolic Solutions Inc. (Nashua, New Hampshire, USA) conducted the isotope analysis of the serum and dose samples using a Europa Hydra continuous flow isotope ratio mass spectrometer and the methodology described by Scrimgeour *et al.* (1993).

Changes in body condition between seasons permitted calculation of the cost of tissue deposition in northern fur seals. The cost of deposition includes the costs of digestion, transporting the dietary precursors, and the increased rate of protein turnover required for a net

protein deposition. Cost of deposition has been experimentally determined to be 1.38 kJ kJ^{-1} of protein deposited (*i.e.* for every 1 kJ of energy deposited as protein, an additional 1.38 kJ is expended for the deposition) and 0.17 kJ kJ^{-1} of lipid deposited (Roberts & Young 1988). Standard biochemical estimates of the energy content per gram of protein and lipid are 18 kJ and 39.3 kJ, respectively (Kleiber 1975, Schmidt-Nielsen 1997). Growth costs associated with the deposition of new bone tissue could not be identified in our study but are believed to be negligible (Jeanniard du Dot *et al.* 2008a).

4.3.6 Cost of resting metabolic rate and thermoregulation

Within each seasonal trial (but exclusive from the DEE measurements), the metabolic rate of each individual was also measured both while resting in ambient air conditions and at three different water temperatures: 2 °C, 10 °C, and 18 °C (+/- 0.5 °C). The methods are fully outlined in Chapter 2, which also provides an in-depth analysis of the cost of thermoregulation for these same individuals. Briefly, rates of oxygen consumption and carbon dioxide production were continuously measured *via* respirometry within a specially designed 340 L metabolic chamber (dimensions: 0.92 m. x 0.61 m x 0.61 m). Rates of oxygen consumption and carbon dioxide production were first measured for 20 min in ambient air conditions (cost of resting metabolic rate). Immediately following, the chamber was filled with water at one of the three different treatment temperatures, and the rates of oxygen consumption and carbon dioxide production was measured for an additional 30 min. The potential costs of thermoregulation at each water temperature within each season were calculated as the difference in metabolic rate between the wet and dry (resting) trials for each individual session. This approach controlled for any variance in resting metabolism that might obscure real changes in metabolism due to the costs of thermoregulation (*i.e.*, reduced Type II error).

4.3.7 Data analysis

Seasonal (between-trial) changes in daily energy expenditure, activity, resting metabolic rate (in ambient air), metabolic rate when immersed in water at different temperatures, and growth (average seasonal body composition, mass and body length) were determined separately using linear mixed effects models (LME; NLME library in R from Pinheiro and Bates, 2000). LME models were also constructed to determine if changes in body mass (growth rates) within a season differed between trials. All LME models included the individual as the random effect to account for repeated measures.

If significant differences were detected in any LME model, a *post-hoc* Tukey contrasts simultaneous test for general linear hypotheses was used to determine between which temperatures, season or interval the significant differences occurred. All statistical analyses were conducted using the R software package (R Development Core Team, 2012).

4.4 Results

4.4.1 Daily energy expenditure

Rates of oxygen consumption and other values are presented as mean ± 1 SD. The average mass-specific rate of oxygen consumption of the northern fur seals throughout the DEE trials ($\dot{V}O_{2DEE}$), across all individuals and all seasons, was 18.1 ± 2.4 mL O_2 kg^{-1} min^{-1}, or 351.6 ± 58.8 mL O_2 min^{-1} on an absolute basis. The average mass-specific $\dot{V}O_{2DEE}$ changed significantly (P = 0.002) throughout the year, being highest in the fall (20.5 ± 1.7 mL O_2 kg^{-1} min^{-1}) and lowest in the winter seasonal trials (16.1 ± 1.6 mL O_2 kg^{-1} min^{-1}; Fig. 4.1).

The average mass-specific rate of carbon dioxide production of the northern fur seals throughout the DEE trials ($\dot{V}CO_{2DEE}$) across all individuals and all seasons (not including the summer seasonal trials when CO_2 readings were unreliable) was 14.9 ± 6.7 mL CO_2 min^{-1} kg^{-1} or 289.4 ± 133.7 mL CO_2 min^{-1} on an absolute basis. The average mass-specific $\dot{V}CO_{2DEE}$ was also significantly different between seasons (P = 0.001; Fig. 4.1). The resultant average RQ values within each season were 0.80 in the spring, 0.77 in the fall and 0.97 in the winter.

Converted to estimates of daily energy expenditure, the overall average mass-specific DEE was 527.8 ± 65.7 kJ kg^{-1} d^{-1}. The mass-specific DEE within each seasonal trial ranged from 587.8 ± 47.2 kJ kg^{-1} d^{-1} in the fall to 481.4 ± 45.1 kJ kg^{-1} d^{-1} in the winter (Table 4.1).

4.4.2 Resting metabolic rate

The average mass-specific rate of oxygen consumption of the fur seals when resting in ambient air temperatures ($\dot{V}O_{2RMR}$) across all individuals and all seasons was 17.6 ± 7.3 mL O_2 kg^{-1} min^{-1}, which is equivalent to 97.2% of the average mass-specific $\dot{V}O_{2DEE}$. However, unusually high activity was observed in one individual (ME08) during the measurements of $\dot{V}O_{2RMR}$, such that the resulting data did not reflect resting conditions. Omitting these $\dot{V}O_{2RMR}$ data, the average mass-specific $\dot{V}O_{2RMR}$ of the northern fur seals across all seasons was 15.4 ± 5.1 mL O_2 kg^{-1} min^{-1}, or 84.2% of the $\dot{V}O_{2DEE}$. The average mass-specific $\dot{V}O_{2RMR}$ (both with and without "ME08") changed significantly throughout the year (P = 0.001); and was significantly higher in the fall (19.3 ± 3.4 mL O_2 kg^{-1} min^{-1}) compared to the other three seasonal trials

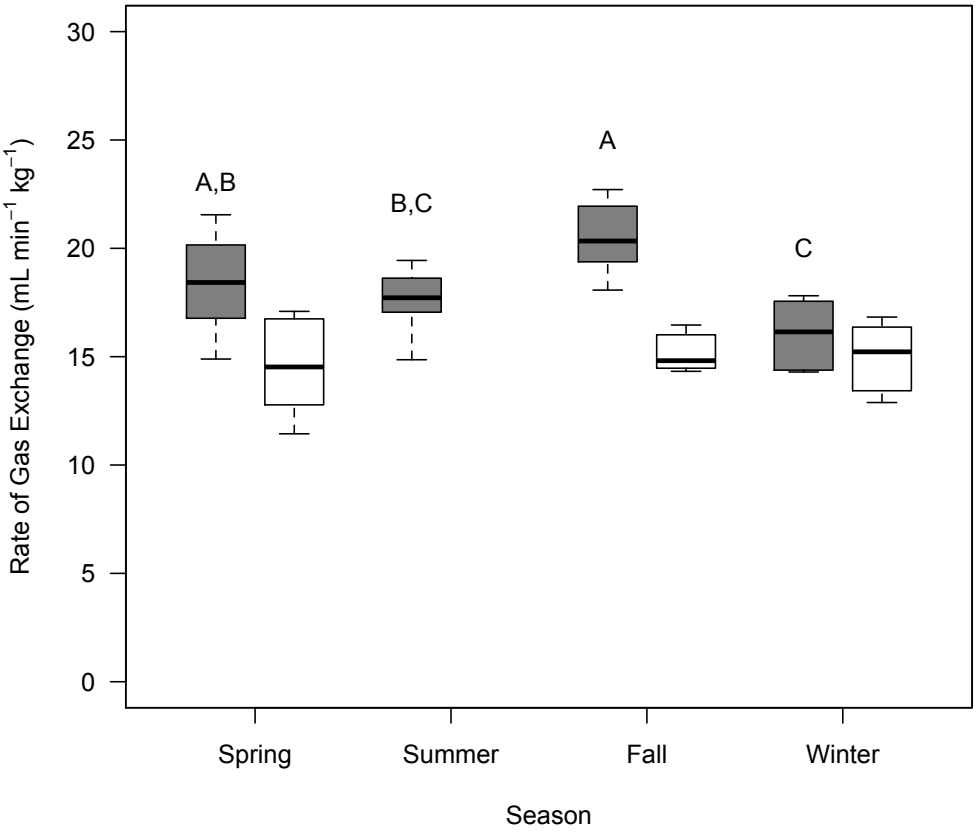

Figure 4.1 The average mass specific $\dot{V}O_2$ (gray bars) and $\dot{V}CO_2$ (white bars) of six, 3 yr old female northern fur seals throughout the nearly 5-day DEE trials measured in four seasonal sets of trials from March 2011 to January 2012. The mass-specific $\dot{V}O_2$ was highest in the fall seasonal trials, which was significantly higher than the mass-specific $\dot{V}O_{2DEE}$ in either the summer or winter seasonal trials ($p < 0.001$), which were themselves not significantly different from one another ($p = 0.25$). The mass-specific $\dot{V}O_2$ in the fall was however not significantly different than in the spring seasonal trials ($p = 0.06$). The mass-specific $\dot{V}O_2$ in the spring was also significantly higher ($p = 0.02$) than the winter but not significantly different than the summer ($p = 0.74$).

Table 4.1 Average daily energy expenditure (kJ kg^{-1} d^{-1}) and cost of tissue deposition in six 3 yr old female northern fur seals measured in four seasonal sets of trials from March 2011 to January 2012. Average changes in body mass are presented as total measured change over the course of an entire 7 wk seasonal trial, and as a daily average mass change (g d^{-1}). These daily mass changes were used to calculate the cost of deposition of new tissue, assuming that new tissue was completely lipid or protein. The cost of deposition has been experimentally determined to be 1.38 kJ kJ^{-1} of protein deposited and 0.17 kJ kJ^{-1} of lipid deposited (Roberts & Young 1988) and the standard biochemical estimates of the energy content per gram of proteins and lipids are 18 kJ and 39.3 kJ, respectively (Kleiber 1975, Jeanniard du Dot *et al.* 2008a). The costs of tissue deposition are also expressed as a percentage of the average RMR and DEE, with the first paired value based on 100% of the tissue change due to lipid and the second value assuming it is solely attributable to protein. The bracketed value is the estimated costs of tissue deposition expressed as a percentage of the average RMR and DEE incorporating measurements of body composition change between seasons that suggest that lipid and protein increased at approximately a 3:1 ratio.

Season	Daily Energy Expenditure (kJ kg^{-1} d^{-1})	Average Mass Change (kg)	Average Mass Change (g d^{-1})	Cost of Deposition if Lipid (kJ d^{-1})	Cost of Deposition if Protein (kJ d^{-1})	% of Average RMR	% of Average DEE
Spring	533.2 ± 77.8	0.7	14.3	95.4	354.9	1.5, (2.4), 5.6	1.1, (1.8), 4.0
Summer	508.7 ± 46.3	1.2	24.5	163.6	608.3	2.1, (3.3), 7.7	1.7, (2.5), 6.2
Fall	587.8 ± 47.2	-0.8	-15.3	-102.3	-380.2	-3.1, (-1.4), -0.9	-3.1, (-1.4), -0.8
Winter	481.4 ± 45.1	-0.3	-6.3	-42.3	-157.2	-2.3, (-0.8), -0.6	-1.6, (-0.7), -0.4

(overall mean 14.1 ± 4.9 mL O_2 kg^{-1} min^{-1}; P = 0.005), which did not differ significantly from one another (P = 0.5; Table 2.1). When comparing these measures by season, the $\dot{V}O_{2RMR}$ accounted for 71.4% of the $\dot{V}O_{2DEE}$ in the spring seasonal trials, 81.2% in the summer, 94.2% in the fall and 93.6% in the winter.

Ambient air temperatures below 2.5 °C were found to increase the mass-specific $\dot{V}O_{2RMR}$ in the winter seasonal trials, as described in the in-depth analysis of the cost of thermoregulation for these same individuals in Chapter 2. Removal of trials in which mass-specific $\dot{V}O_{2RMR}$ was measured in ambient air temperatures below 2.5 °C decreased the average mass-specific $\dot{V}O_{2RMR}$ for the winter to 11.4 ± 3.9 mL O_2 kg^{-1} min^{-1} (and 70.8% of the $\dot{V}O_{2DEE}$); however the overall seasonal trend in the mass-specific $\dot{V}O_{2RMR}$ did not change (*i.e.*, fall metabolism remained higher than all other seasons).

4.4.3 Thermoregulation

At water temperatures ranging from 2 °C to 18 °C, the northern fur seals in our study appeared to be thermally neutral in all seasons for the water temperatures tested, except during the summer when metabolic rates were higher in the 2 °C water trials. In those 2 °C water trials, the $\dot{V}O_{2Wet}$ was 19.0 ± 6.5 mL O_2 kg^{-1} min^{-1}, which was 36% greater than the $\dot{V}O_{2RMR}$ (P = 0.04).

4.4.4 Growth

Average growth rates within a season (standardized as changes in the body mass over the 7 wk trial) were not significantly different from zero during either the fall (-0.8 ± 1.0 kg; P = 0.1) or winter seasonal trials (-0.3 ± 0.6 kg; P = 0.3). However, growth rates were significantly positive during both the spring (0.7 ± 0.6 kg; P = 0.05) and summer seasonal trials (1.2 ± 0.8 kg; P = 0.01).

Average body mass of the northern fur seals increased significantly over the course of the first three phases of the study by 3.9 kg (P = 0.001), although it dropped slightly at the end. The average body mass was lowest during the spring (16.7 ± 2.5 kg), significantly higher in the subsequent summer trials (19.3 ± 2.5 kg; P = 0.001) and higher again in the fall seasonal trials (21.2 ± 2.8 kg; P = 0.01). While the average body mass was slightly lower during the winter trials (20.6 ± 2.5 kg), it was still significantly higher in the winter than in the spring (P = 0.001; Table 2.1).

Average length of the northern fur seals also significantly increased by 8.4 cm, (from 104.0 ± 6.1 to 112.4 ± 5.7 cm; P = 0.001), over the course of the study. The rate of increase was

significantly different over the course of the year (P = 0.01). Average length increased more between the spring and summer trials and between the fall and winter trials compared to the rate of increase in average length that occurred between the summer and fall trials (P = 0.01).

The average absolute amount of lipid in the fur seals' bodies increased significantly by 1.5 kg (from 0.7 ± 0.3 to 2.3 ± 0.8 kg; P = 0.001) over the course of the study. The rate of change in absolute lipid mass however did not differ between seasons (P = 0.2). When combined with changes in body mass between seasons, the % body mass comprised of lipid increased at a constant rate throughout the year. Our study animals had the lowest absolute amount and relative concentration of lipids during the spring (0.7 kg or 4.6 ± 2.1% of total body mass) and the highest absolute amount and concentration of lipids during the winter (2.3 kg or 11.0 ± 3.7% of body mass).

The average absolute amount of protein in the fur seals' bodies also increased significantly over the course of the study period by 0.5 kg (from 3.9 ± 0.7 to 4.4 ± 0.6 kg; P = 0.001). Unlike lipids, however, the rate of change in protein between the seasons did change significantly with the time of year (P = 0.01). The average absolute total body protein increased, but not significantly between the spring and summer trials by 0.4 kg (from 3.9 ± 0.7 to 4.3 ± 0.7 kg; P = 0.05). The average absolute total body protein increased again significantly between the summer and fall by 0.3 kg (from 4.3 ± 0.7 to 4.6 ± 0.6 kg; P = 0.01), before significantly decreasing between the fall and winter by 0.2 kg (from 4.6 ± 0.6 to 4.4 ± 0.6 kg; P = 0.05). As a result of absolute changes in both protein and lipid mass, the % body mass comprising protein decreased throughout the year, being highest during the spring trials (23.1 ± 0.7%), and lowest in the winter seasonal trials (21.4 ± 1.1%).

It was impossible to directly estimate the energetic cost of tissue growth within a trial or its relative contribution to DEE as we only had measurements of changes in body composition between seasons. The energetic cost of growth associated with the deposition of new tissue could be estimated to be as high as 7.7% of the average RMR and 6.2% of the DEE in the summer if all of the observed changes in body mass were attributed to protein deposition (Table 4.1). However, this is unlikely, as measurements of body composition change between seasons suggest that lipid and protein increased at approximately a 3:1 ratio. We therefore combined measured changes in total mass with this inter-season ratio of tissue growth to estimate costs of growth within a season (Table 4.1).

4.4.5 Activity level

The average Actiwatch activity score during the DEE trials of the northern fur seals across all individuals and all seasons was 76.3 ± 10.1 counts per 15 sec interval, and differed significantly between seasons ($P = 0.04$) due solely to the difference between the fall (84.3 ± 6.3 interval^{-1}) and winter (69.6 ± 9.9 interval^{-1}; $P = 0.001$) trials. The activity scores in the spring (74.5 ± 7.7 interval^{-1}) and summer (76.3 ± 11.6 interval^{-1}) were not significantly different from each other, or from the fall and winter ($P = 0.1$).

4.5 Discussion

Identifying critical times of the year when unpredicted episodes of nutritional stress would have the greatest negative effect on northern fur seals requires knowing their total energy requirements, the individual costs of the underlying components of their energy budgets, and an understanding of how these expenditures change throughout the year. In our study, the daily energy expenditure (DEE) of our female fur seals changed significantly throughout the year due largely to seasonal variation in the costs of resting metabolic rate, and activity. Less of the variation in DEE could be explained by the costs of thermoregulation, and growth. Overall, the mass-specific DEE was higher in the spring and fall, and lower during the summer and winter. Summer and fall correspond to increased costs of growth and resting metabolism, respectively, and could be times of year when inadequate nutrition could have the greatest negative effect on young female fur seals.

4.5.1 Daily energy expenditure

Animals in artificial environments should not necessarily be expected to have the same energy expenditures as their wild counterparts. In our study, the measures of daily energy expenditure (DEE) for our female northern fur seals are likely minimal compared to those experienced by wild individuals. The measured average field metabolic rates (FMR) in a variety of otariids (California sea lions; *Zalophus californianus*, northern fur seals and Antarctic fur seals) have been shown to be 3.3 to 6.7 times Kleiber's (1975) allometric prediction for the basal metabolic rate (BMR) of similarly sized terrestrial mammals (Kleiber 1975, Costa & Gentry 1986, Costa & Trillmich 1988, Costa *et al.* 1989, Boyd & Duck 1991, Costa *et al.* 1991, Arnould *et al.* 1996b). As the basal energetic costs for a variety of marine mammal species are 1.4 to 2.8 times Kleiber's prediction (Kleiber 1975, Williams *et al.* 2001, Williams *et al.* 2007), FMRs in otariids are typically ~ 2.5 times the basal energetic costs. In our study, the average DEE was

only ~ 20% higher than the resting metabolic rates (RMR). Since our measured RMR was comparable with other juvenile otariids, the lower DEE of our animals must be due to some other component of their energy budget (South *et al.* 1976, Miller 1978, Donohue *et al.* 2000, Rosen & Trites 2000).

The relatively lower DEE of our captive animals likely resulted from their restricted dive depths and lack of active foraging in comparison to their wild counterparts. The costs of physical movement can exceed any other energetic function by a factor of 10 or more (Darveau *et al.* 2002). This suggests that the added cost of activity within DEE was relatively small in our study, and did not approach the expenditures that would be required in the wild.

Nevertheless, the DEE of the northern fur seals did change significantly throughout the year. This concurs with previous studies that have demonstrated intrinsic physiological changes within a captive pinniped's energy budget (Rosen & Renouf 1998, Donohue *et al.* 2000, Sparling *et al.* 2006, Liwanag 2010, Rosen *et al.* 2012, Rosen & Trites 2014). These seasonal differences in DEE can, presumably, be traced back to underlying seasonal changes in one or more of the key components of the energy budget: the cost of thermoregulation, growth, RMR, heat increment of feeding (HIF) and/or activity.

4.5.2 Resting metabolic rate (RMR)

It would be predicted that the cost of resting metabolism is the most likely component of the northern fur seals' energy budget to account for the observed seasonal changes in DEE, given that it comprised ~ 80% of the DEE on average and is known to change seasonally in pinnipeds (Rosen & Renouf 1998, Sparling *et al.* 2006). However, while there was significant seasonal variation in RMR in our study animals, it did not follow the same seasonal pattern that was observed in the DEE.

The northern fur seals' RMR in ambient air was, on average, 2.9 times Kleiber's (1975) allometric prediction for terrestrial mammals during the spring, summer and winter seasonal trials. This is consistent with studies by Miller (1978) and Donohue *et al.* (2000) using northern fur seals that ranged from post-molt pups to 5 yr of age. Relative to the other three seasons, the RMR during the fall seasonal trials was significantly elevated (4.2 times Kleiber). This seasonal variation in RMR is unlikely to be a product of differences in absolute body size over the course of the year. First, while both RMR and body mass are highest in the fall, the differences in metabolism between the fall and other trials were greater than the observed differences in body

mass (*i.e.*, could not be accounted for by any reasonable scaling factor). Second, although body mass changed significantly from the winter to summer, the absolute $\dot{V}O_{2RMR}$ did not vary in the same manner.

While the observed changes in RMR were not attributable to body mass *per se*, it is possible that they were due to some seasonal aspect of physical growth. Physical growth in many otariids is highly seasonal (Jeanniard du Dot *et al.* 2008b, Rosen & Kumagai 2008, Rosen *et al.* 2012). While the costs of growth will only contribute to changes in DEE through measured changes in RMR, it is still important to decipher the degree to which growth contributed to the observed changes in RMR.

4.5.3 Growth

Seasonal growth was evident in our study animals during the spring and summer trials, while body mass did not change significantly within the fall and winter trials. The intra-seasonal changes in body mass correspond with the rates of change in body mass and length observed in our study animals between seasons. These trends are also reasonably consistent with growth curves constructed by Trites and Bigg (1996) for wild immature female northern fur seals, which predicted stable body mass (and body length) from early spring (March) to late May, increases in body mass from late May to the end of July, and mass loss during the remainder of the year (Trites & Bigg 1996).

The direct costs of growth in these juvenile female northern fur seals were estimated to be as high as 7.7% of the average RMR in the summer, assuming all new tissue was deposited as protein. However, measurements of body composition change suggested that lipid and protein increased at approximately a 3:1 ratio. As the cost of lipid deposition is energetically less expensive than the cost of protein deposition, the actual costs of growth are likely only making a negligible contribution to changes in the northern fur seals' RMR and DEE throughout the year (maximum ~ 3% of RMR).

It might be argued that the higher growth rates observed in the summer and, to a lesser degree, in the spring, might have indirectly contributed to energetic expenditures through an up-regulation of metabolic processes required to facilitate these higher growth rates. However, this does not fit the pattern observed in the changes in mass-specific RMR. If anything, this metabolic up-regulation would have a tendency to mask the greater RMR observed in the fall by raising measured RMR in the spring and summer trials.

4.5.4 Molting

In contrast to the costs of physical growth, we believe the costs directly associated with molting (versus any potential secondary effects on thermoregulation or activity) is the most logical explanation for the elevated RMR that was observed in the fall (Boyd *et al.* 1993). The molt of northern fur seals in their third year (such as our study animals) is centered in September (Scheffer 1962), which coincided with our study's fall seasonal trials. In Steller (*Eumetopias jubatus*) and California sea lions a 30% - 87% increase in metabolism is associated with the molt (Kumagai 2004, Williams *et al.* 2007). The 50% increase in RMR in our fall seasonal trials relative to the other seasons is thereby consistent with the metabolic increases attributed to molting in these previous studies.

Whereas molting is hypothesized to account for the seasonal changes in RMR, it does not explain the observed seasonal changes in DEE. RMR accounted for a varying proportion of DEE throughout the year, from a high of 94.2% of the northern fur seal's DEE in the fall to only 70.8% in the winter. Energetically, the amount of DEE that was in excess of that attributable to RMR was highest in the spring (152.5 kJ kg^{-1} d^{-1}), less in the winter (140.6 kJ kg^{-1} d^{-1}) and summer (95.6 kJ kg^{-1} d^{-1}), and lowest in the fall (34.1 kJ kg^{-1} d^{-1}) (Fig. 4.2).

4.5.5 Thermoregulation

It does not appear that these additional energy expenditures (or, by extension, the seasonal variation in observed DEE) are the result of changes in thermoregulatory costs. Increased metabolic rates associated with the costs of thermoregulation are anticipated during periods when environmental temperatures are outside of the northern fur seal's thermal neutral zone (TNZ). Therefore, one might expect that the fur seals were most likely to experience additional thermal costs during the months when the average air and water temperatures were lowest. The lowest average ambient air temperatures occurred during the spring trials (9.1 °C), when the discrepancy between RMR and DEE was greatest. However, as an apparent increase in metabolism was only associated with air temperatures below 2.5 °C in the winter (Chapter 2), this cannot account for the increased discrepancy between RMR and DEE in the spring, nor was it a common occurrence during the winter DEE trials. Water temperatures were on average lowest in the winter (Dec. = 9.6 °C; Jan. = 8.8 °C). However, the fur seals in our study were found to be thermally neutral throughout the year in water temperatures from 2 °C to 18 °C, with the exception of the summer seasonal trials at 2 °C (Chapter 2), which were clearly never

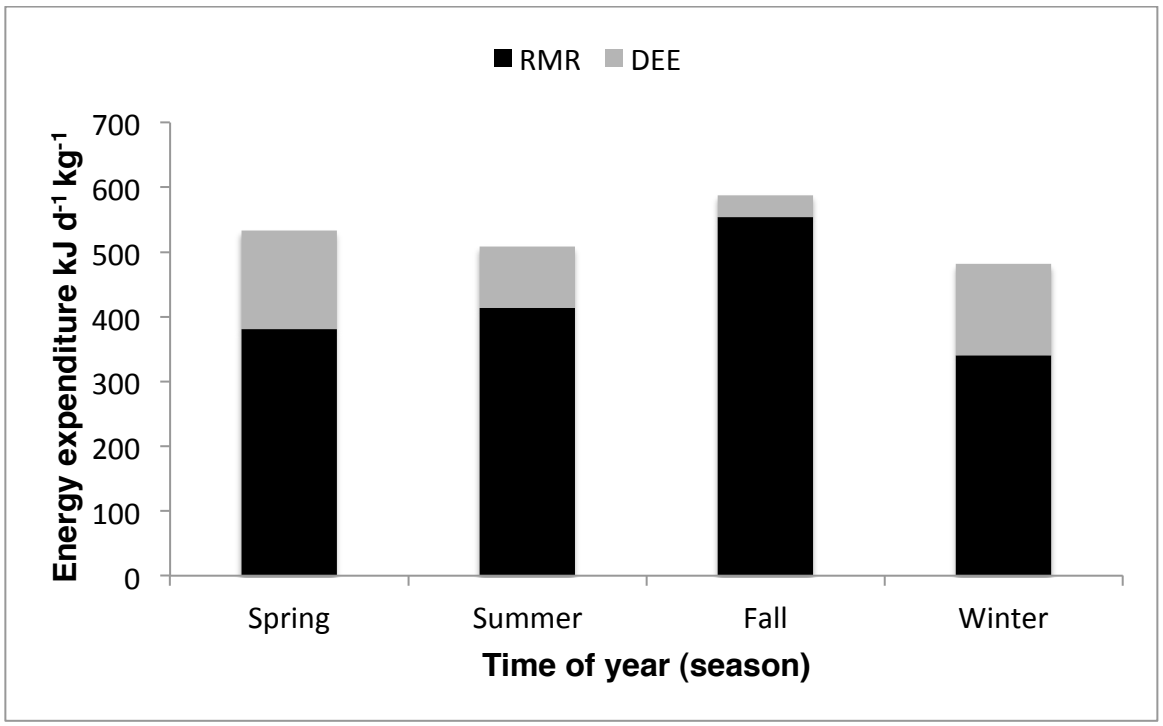

Figure 4.2 The resting metabolic rate (RMR; black bars) and daily energy expenditure (DEE; gray bars) of six, 3 yr old female northern fur seals measured in four seasonal sets of trials from March 2011 to January 2012. The RMR energy expenditure data of one individual (ME08) was omitted, as a result of unusually high activity that was determined to not be a reflection of resting conditions. RMR energy expenditure data recorded at ambient air temperatures below 2.5 °C were also omitted as Ambient air temperatures below 2.5 °C were found to increase the mass-specific $\dot{V}O_{2RMR}$. RMR and DEE are presented as Kilojoules of energy per day per kilogram of animal.

experienced during the summer DEE trials. Therefore, it is unlikely that any seasonal variation in DEE was due to thermoregulatory costs associated with seasonally changing water or air temperatures.

4.5.6 Heat increment of feeding

The variation observed in the DEE also does not appear to be the result of additional energy expenditures associated with the heat increment of feeding. While the average daily food intake changed significantly throughout the year, the heat increment of feeding cost would be projected to be lowest in the spring and highest in the fall, when the lowest and highest average food intake levels occurred. This is not in agreement with the observed seasonal variation in DEE. Additionally, the average amount of food fed during the trials differed by only 0.45 kg (with the composition remaining the same), and therefore the resulting changes in HIF were unlikely to make a significant impact (Rosen & Trites 1997, Rosen 2009).

4.5.7 Activity

Variation in the energetic cost of activity could explain the seasonal changes in the northern fur seals' DEE. Bioenergetically, this explanation makes sense, given the high potential costs of physical movement (Darveau *et al.* 2002). In our study we used a quantitative measure of activity, and the resulting average Actiwatch activity score over the DEE trials was a significant predictor of the average mass-specific $\dot{V}O_{2DEE}$ across all the seasons and all individuals (Chapter 3). These scores were 21% higher in the fall (84.3 ± 6.3 interval^{-1}) than in the winter (69.6 ± 9.9 interval^{-1}; P = 0.001) trials, which compares favourably to the 27% difference observed in DEE between these seasons. Further, while the relationship between Actiwatch score and DEE cannot be used to calculate the actual cost of activity, it can be used to make a rough estimate. Given that an (average) increase in Actiwatch activity score equates to an average increase in mass-specific $\dot{V}O_{2DEE}$ of 0.16 mL O_2 kg^{-1} min^{-1} (Chapter 3), the 14.7 difference in Actiwatch score between the seasons would translate roughly into a difference of 68 kJ kg^{-1} d^{-1}. This again compares favourably to the total observed seasonal difference in DEE of ~106 kJ kg^{-1} d^{-1}.

Unfortunately, the measured differences between the individuals' RMR and DEE (*i.e.*, potential added cost of activity) do not correspond directly with the differences in the measured activity levels. This discordance potentially results from the interplay of RMR with anticipated activity. In the wild, female fur seals undertake a substantial southward migration beginning in

October (late fall) and lasting between ~ 1-3 mo (Kenyon & Wilke 1953, Bigg 1990, Gentry 1998, Ream *et al.* 2005). Increased seasonal RMR may be an adaptation for the substantial metabolic machinery needed to support the high energy turnover rates associated with supplying fuels, disposing of waste and repairing tissue during migration, as well as the required high levels of muscular activity (Kersten & Piersma 1987, Lindstrom 1997). This up-regulation may actually decrease the apparent cost of activity, through increased energetic efficiencies; in other words, part of the cost of activity is exhibited in measures of RMR. This might be the case in the fall, when average activity level is highest, yet the DEE of the northern fur seal was found to be only 6% higher than the RMR. Conversely, when the wintering grounds have been reached upon the completion of the migration, that metabolic machinery may be reduced in response to the new energetic conditions (Lindstrom 1997). However, the downside is that the costs of a given level of activity might be higher in the winter than in the fall.

4.5.8 Critical seasons

While the elevated RMR in the fall might represent a time of year with improved energetic efficiencies for the costs of activity, these elevated innate costs could also indicate a critical season during which an episode of nutritional stress might have a greater impact compared to others. The suggestion is that the direct costs of locomotion during the fall migration represent ecological requirements due to changes in climate and prey distribution, and therefore cannot be scaled back. Further, the major costs during that season are related to resting metabolism (perhaps due to the molt or up-regulation to facilitate higher activity costs) suggesting a lack of flexibility in their fall energy budgets. Hence, this season may represent a critical time of year when inadequate nutrition would have the greatest potential impact.

Alternately, the summer may represent a critical nutritional period, given the high-energy requirement for growth. Growth rates are highest in this period, contributing to both overall changes in body size as well as contributing to the energetic and thermoregulatory condition required for the subsequent winter months. Although RMR is not as great as in the fall, metabolism in the summer may also be up-regulated as an adaptation to facilitate these high growth rates. Hence, restricted food intake during this period would have a long-term impact on body size and subsequent energy balance, barring any compensatory growth.

4.6 Conclusions

Overall, the DEE of the northern fur seals changed significantly throughout the year — with the cost of resting metabolism comprising the major component. The seasonal pattern of the cost of resting metabolism differed from that of the DEE, due perhaps to the costs of molting or anticipated migratory activity. In contrast to resting metabolism, the costs of growth, heat increment of feeding and thermoregulation appeared to be negligible within the scope of overall energy expenditures. Cost of activity, however, appears to be the second major component of the DEE and may have driven some of the seasonal variations observed in DEE.

Changes in the major components of the energy budgets of the northern fur seal can be used to infer critical times of year when inadequate nutrition would have a significant impact. Thus summer may be a critical nutritional period, as the normal high growth rates in combination with restricted food intake could have long-term impacts on body size and energy balance. However, the fall is likely to be more critical when resting metabolic rates are higher than at any other time of year. This elevated cost of resting metabolism may improve the energetic efficiencies of the costs of activity or be related to the costs of molting, but may come at the expense of restricting flexibility within the energy budget, leading to severe consequences during unpredicted periods of nutritional stress.

Chapter 5: Conclusion

5.1 Research summary

The study of energetics is essential to understanding the life history and fitness of organisms (Iverson *et al.* 2010). The aim of my thesis was to enhance the understanding of the seasonal variation in the daily energy expenditure (DEE) and energetic priorities of northern fur seals, using captive individuals to elucidate potential reasons for their population decline in the wild. In addition, I tested and calibrated three alternative proxies for measuring energy expenditure for potential application on wild individuals.

The research involved simultaneous measures of different aspects of energy expenditure over four seasons in six captive subadult female northern fur seals. The daily energy expenditure (DEE) of the fur seals was measured over 5-days *via* respirometry (oxygen consumption). Simultaneously, I tested three alternative proxies for the measurement of energy expenditure — specifically two methods of accelerometry (2-dimensional and 3-dimensional) and the doubly labeled water (DLW) method. In tandem with the measures of DEE, I examined shifts in energetic priorities by measuring four key components of the energy budget within each season: the costs of growth (*via* changes in body mass, length, and body composition), activity, resting metabolic rate, and thermoregulation.

Daily energy expenditure was measured in a specially designed metabolic chamber that enclosed both a pool and the associated haulout space, permitting the fur seals to undertake normal levels of activity and experience normal thermal environments. The average mass-specific DEE of the northern fur seals was 527.8 ± 65.7 kJ kg^{-1} d^{-1}, which is equivalent to 3.7 times Kleiber's allometric prediction for similarly sized adult terrestrial mammals (Kleiber 1975). However, the average mass-specific DEE changed significantly throughout the year. The DEE was highest in the fall, and lowest in the winter, with the spring and summer being intermediate. I initially presumed that this seasonal variation in DEE would be linked to a parallel change in one of the key components of the energy budget.

The largest component of the energy budget of the northern fur seals was the cost of resting metabolism and comprised ~ 80% of the DEE on average. As with the DEE, the resting metabolic rate (RMR) was found to change significantly throughout the year — however, the pattern of this seasonal variation was different than that of the DEE. The mass-specific RMR in

the fall was 50% higher on average than the remaining seasonal trials (which did not differ significantly from one another).

These changes in RMR appeared unrelated to changes in either growth or thermoregulatory costs. In fact, the direct costs of those two energy budget components of the fur seals appear negligible relative to the DEE. I found evidence of seasonal growth occurring during the spring and summer, which is consistent with the growth curves constructed by Trites and Bigg (1996) for wild immature female northern fur seals. However, combining measures of growth and body composition revealed that the costs of growth only constitute a maximum of ~ 3% of the RMR of the northern fur seal throughout the year.

My study also revealed that potential thermoregulatory costs were insignificant. The young female northern fur seals appeared to be thermal neutral in water temperatures from ~ 2 °C to 18 °C (specific upper and lower critical water temperatures were not identified). This was determined by comparing the fur seals' metabolic rates when resting in dry ambient air conditions to their metabolic rates when resting immersed at three isolated water temperatures: 2 °C, 10 °C, and 18 °C, during each of the seasonal trials. The only exception to this broad thermal capacity was an increase in metabolism when the fur seals were subjected to the 2 °C water temperatures in the summer. Similarly, the rates of oxygen consumption when resting in ambient air conditions (1 °C - 18 °C) were not related to air temperature, except below 2.5 °C in the winter. Given the water and air temperatures experienced throughout the DEE trials, it appears the individuals were always within their thermal neutral zone, and thus experienced no additional energetic expenditures associated with the costs of thermoregulation.

I contend that the elevated RMR exhibited in the fall potentially relates to energetic costs associated with molting. The elevated RMR might also be an adaptation for an increase in metabolic machinery needed to support the high energy turnover rates associated with supplying fuels, disposing of waste and repairing tissue during the high levels of muscular activity connected with the initial phase of the northern fur seal's annual pelagic migration in the wild.

The cost of activity is another major component in the energy budget of the northern fur seal and potentially constitutes the influential factor in the seasonal variation of the DEE. The level of physical activity was quantified over the 5-day DEE trials by means of an Actiwatch tri-axial acceleration data logger attached to the fur seal *via* a specially designed harness. The Actiwatch provided a non-dimensional activity score over user-defined time periods. The

average Actiwatch activity score throughout the DEE trials was found to be a significant predictor of the average mass-specific DEE, across all seasons and individuals. The difference between the lowest and highest average seasonal activity score was 21%. Comparatively, this was mirrored by seasonal differences of ~ 27% in the average seasonal DEE of the northern fur seals. While the actual cost of activity could not be calculated from the relationship between Actiwatch score and DEE, the data could be used to make the rough estimate that the 14.7 difference in Actiwatch score between the seasons translates roughly into a difference of 68 kJ kg^{-1} d^{-1}, which compares favourably to the total observed seasonal difference in DEE of ~ 106 kJ kg^{-1} d^{-1}. Curiously, while the variation in activity was closely related to seasonal differences in DEE, it was not closely associated with the "extra" energetic expenditure above RMR. I propose that this might be the result of physiological interactions between direct locomotion costs and maintenance costs that facilitate different normal levels of activity during the year.

I quantified the DEE through respirometry, which is considered a relatively direct measure of energy expenditure. However, alternate means of estimating energy expenditure need to be employed in the field. I tested and calibrated three potential proxies for field energy expenditures — two devices for measuring accelerometry and the doubly labeled water (DLW) turnover method — by comparing their results to measured rates of oxygen consumption over the 5-day trials. One accelerometry device was a Little Leonardo bi-axial acceleration data logger (M190L-D2GT) that measured the raw acceleration, which can be converted to measures of Partial Dynamic Body Acceleration (PDBA). The other device was the previously described Philips Healthcare Actiwatch tri-axial acceleration data logger, which provided a score of the number of times the threshold acceleration was exceeded in any dimension over a user-defined time period.

The DLW method is commonly employed in studies of wild marine mammals, but rarely validated for use in this group of animals. I found that the best DLW model overestimated measured rates of oxygen consumption by 13.1 ± 16.5%. In addition, results from the DLW method were found to be highly dependent on the technique (sets of equations and assumptions) used to convert the raw isotope data into estimates of carbon dioxide production. Hence, part of any observed "difference" between study results may simply be the result of variances in the equations used. Additionally, I found that the time of year must also be considered when

applying the DLW method, as seasonal changes in the respiratory quotient (RQ) or environmental temperatures appear to produce additional seasonal biases.

In comparison, accelerometry failed to predict the average mass-specific rate of oxygen consumption, within each individual season over the entirety of the DEE trials, regardless of the type of accelerometer used. Accelerometers, however, store high-resolution data that can be used to quantify activity-specific and fine scale estimates of energy expenditure. My study indicated that on the finer time scales of 15 and 60 min, accelerometry was able to estimate the energy expenditure of the northern fur seals. The best accelerometry estimates of energy expenditure had an average difference of 13.8 ± 39.5% (15 min intervals) and 5.4 ± 29.3% (60 min intervals) from measures derived from respirometry. Similar to the DLW method, the application of accelerometry for estimating energy expenditure must also account for season. Additionally, my study found that time of day must also be accounted for in order to derive accurate estimates of energy expenditure from measures of accelerometry. Of further interest was the fact that the Actiwatch activity measures generally provided more accurate predictions of energy expenditure compared to those made by the Little Leonardo $PDBA_{xy}$ activity measures. I believe the difference is primarily related to the number of axis in which acceleration was measured, in partnership with the diversity of the daily activities of the northern fur seal.

5.2 Ecological impacts

A central goal of my thesis was to place my findings into a broader context related to the ecology and conservation of the northern fur seal. In Chapter 2, I compared the measured zone of thermal neutrality for the young female northern fur seals to the average sea surface temperatures encountered during the described annual migration of wild, juvenile female individuals. This comparison indicated that these opportunistic foragers have the ability to exploit a large portion of the Bering Sea and North Pacific Ocean without added metabolic costs for thermoregulation. Thus, individuals have the thermal capacity to alter their migration pattern in response to changes in prey availability and distribution (Sigler *et al.* 2009).

In Chapter 4, changes in the relative energetic costs of individual components of the energy budget were used to infer energetic priorities as well as critical seasons during which episodes of nutritional stress might have a greater impact compared to others. The high rates of growth of immature female northern fur seals in the summer (and to a lesser degree the spring) likely represents a critical nutritional period for them, as restricted food intake could produce

long-term impacts on body size and energy balance. The elevated costs of resting metabolism in the fall, however, likely make the fall the most critical time of year. Regardless of the degree to which the elevated fall RMR may improve the energetic efficiencies of the costs of activity or be related to the costs of molting, the fact that RMR constitutes such a high proportion of DEE during this season may severely limit flexibility within the energy budget of the northern fur seal in the face of decreased food intake.

The results obtained in my thesis can further be used to explore the hypothesis that the decline of the northern fur seal on the Pribilof Islands is related to an inability to acquire and digest sufficient prey necessary to meet energetic requirements. By combining the data obtained on the seasonal patterns of energy use in juvenile female northern fur seals with estimates of field metabolic rates, physiological digestive capacity, and prey composition, it is possible to investigate the timing and conditions that could result in prey quality-induced nutritional stress among wild fur seals.

While accurate measures of DEE were made with our captive fur seals, these estimates are clearly minimal compared to those experienced by wild northern fur seals. This is based on the assumption that activity levels — including those associated with foraging — are going to be higher in the latter group. Increasing the DEE to 2.5 times the measured RMR would approximate a realistic cost of activity (due to foraging and migration), and mirror the relationship between FMR and RMR found in most otariids in the wild (Kleiber 1975, Costa & Gentry 1986, Costa & Trillmich 1988, Costa *et al.* 1989, Boyd & Duck 1991, Costa *et al.* 1991, Arnould *et al.* 1996b, Williams *et al.* 2001, Williams *et al.* 2007).

These seasonal estimates of DEE permit the approximation of the food intake mass of different dietary prey items required to meet those energy demands, based on each item's energetic content and taking into account appropriate factors of digestibility. The former requires knowing the proximate composition (or gross energy) of different prey items, while the latter accounts for losses through the processes of digestion, including the heat increment of feeding (HIF), urinary energy loss (UEL) and fecal energy loss (FEL) (Ronald *et al.* 1984, Worthy 2001, Rosen 2009). For example, the biologically useful energy (net energy) that northern fur seals obtain from Pacific herring, a representative high quality prey species, is ~ 7.6 kJ g^{-1} (wet mass), as compared to ~ 4.2 kJ g^{-1} (wet mass) for juvenile walleye pollock, a representative low quality prey species (Table 5.1).

Table 5.1 Average net (biologically useful) energy (per g wet mass) available to the northern fur seal for maintenance or activity obtained through the consumption of a representative high quality prey item Pacific herring (*Clupea pallasii*) and a representative low quality prey item, juvenile walleye pollock (*Theragra chalcogramma*). The average energetic density (per g wet mass) of Pacific herring was determined by Bigg *et al.* (1978), Sidwell (1981), and Perez (1994) and for the juvenile walleye pollock by Miller (1978) and Perez (1994). The assimilation efficiency (aka percent energy absorptive uptake) of the northern fur for Pacific herring was determined by Miller (1978) and Fadely *et al.* (1990) and for walleye pollock by Miller (1978). Urinary energy loss directly correlates with the levels of nitrogen absorbed and is calculated as a percentage of the digestive energy (in this study 8.4%; consistent with the UEL measured in juvenile harp seals (6.9 to 9.5%; Keiver *et al.* 1984), ringed seals (8.6%; Parsons 1977) and harbour seals (~ 5% the gross energy; Ashwell-Erikson & Elsner 1981).

Prey Species:	Energetic density ($kJ\ g^{-1}$):	Assimilation Efficiency (%):	Urinary Losses ($kJ\ g^{-1}$):	Net Energy ($kJ\ g^{-1}$):
Pacific Herring (*Clupea pallasii*)	8.9	92.3	0.7	7.6
Walleye Pollock (*Theragra chalcogramma*)	5.2	88.3	0.4	4.2

Food intake requirements must also take into account the metabolic cost of growth as well as the cost of tissue storage (or conversely, the energy released from tissue catabolism). The former is evident as changes in RMR (as measured in Chapter 4), while the latter is an additional cost that is dependent on whether the tissue catabolism or storage is comprised of lipid or protein (Table 5.2).

The end product of these calculations is an estimate of the mass of prey that must be consumed to fulfill the needs of a 20 kg juvenile female northern fur seal (the average mass of the individuals in my study) throughout the year consuming a diet consisting solely of either herring or pollock at different times of the year. These estimates of consumption range from a low of 2.1 kg per day of Pacific herring in the winter to as high as 6.8 kg per day of juvenile walleye pollock in the fall (Table 5.2).

Although this exercise of estimating food requirements cannot reveal whether fur seals are able to find and acquire sufficient prey to meet their energy demands, it does shed light on whether they can feasibly process the required amount of prey. Specifically, these estimates of required daily prey mass can be compared to estimates of digestive capacity, which is related to physiological and anatomical limitations (Weiner 1992). Although no specific estimates currently exist for northern fur seals of this age group, the maximum digestive capacity has been estimated to be between 14 – 16% of the body mass in adult Steller sea lions and 27% in younger northern fur seals (Rosen & Trites 2004, Rosen *et al.* 2012). For a 20 kg individual the maximum digestive capacity would be estimated to be between 2.8 and 5.4 kg. In comparison to the calculated food intake levels, this suggests that the northern fur seal's digestive capacity is capable of meeting their daily energetic requirements throughout the year given a diet consisting solely of Pacific herring, a high quality prey item. However, there is a higher likelihood throughout the year that it is more difficult for fur seals to thrive if their diet consists solely of a low quality prey item, such as juvenile walleye pollock.

Of particular concern is the fall period, when it appears that juvenile female northern fur seals are living on the "nutritional edge" even on a diet consisting solely of high quality prey items and cannot physically meet the required daily prey mass with a diet consisting solely of low quality prey items. Part of this high-energy requirement may be a construct of the fact that our DEE correction was based on a higher RMR observed in that season. However, it is also the time of year when activity costs due to migration movement may truly be higher.

Table 5.2 Estimated prey consumption requirements by season for representative 20 kg subadult female northern fur seals consuming a diet solely of either Pacific herring or juvenile walleye pollock. The measured resting metabolic rate (RMR; mL O_2 kg^{-1} min^{-1}) of the northern fur seal, throughout the year, is used to estimate the Daily Energy Expenditure at 250% of the RMR, which approximates a realistic cost of activity for animals in the wild. The DEE (kJ d^{-1}) is also calculated for a representative 20 kg female northern fur seal (the average mass of this study's individuals). Also included in the prey consumption requirements is additional energy attributable to energy storage during periods of mass gain (growth) or energy released during periods of mass loss (tissue catabolism) based on the average change in body mass (g d^{-1}) and composition per season. The paired values in this and subsequent calculations are the result of whether the cost of tissue storage (or conversely, the energy released from tissue catabolism) is comprised of lipid or protein. The sum of the costs of DEE and tissue growth are subsequently used to estimate the prey consumption requirements by season separately for animals consuming a diet solely of either Pacific herring or juvenile walleye pollock. The estimates include costs of digestive inefficiency resulting in fecal and urinary energy loss (as per Table 5.1). However, costs due to heat increment of feeding (HIF) were already included in the DEE I measured as individuals were fed (90 - 95% frozen Pacific herring and 5 - 10% squid) throughout the trials (Harris 1966, Rosen 2009). HIF, is not a fixed value but varies with the composition, and the size of a meal (Blaxter 1989, Rosen 2009). A HIF correction factor of 3.3% is applied to the pollock consumption to account for the difference between prey items (herring vs pollock) based on studies by Rosen and Trites (1997, 2000) and Rosen (2009).

Season	RMR (kJ kg^{-1} d^{-1})	DEE (kJ kg^{-1} d^{-1})	DEE - 20 kg Animal (kJ d^{-1})	Average Mass Change (g d^{-1})	Raw Energy Costs of Growth (kJ d^{-1})	Mass of Pacific herring (kg d^{-1})	Mass of walleye pollock (kg d^{-1})
Spring	380.7 ± 40.7	951.8	18949.2	14.3	257.1, 561.4	2.5, 2.6	4.7, 4.8
Summer	413.1 ± 124.2	1032.8	20685.0	24.5	440.8, 962.5	2.8, 2.9	5.2, 5.3
Fall	553.7 ± 97.5	1384.3	27917.5	-15.3	-601.5, -275.5	3.6, 3.6	6.7, 6.8
Winter	340.8 ± 116.6	852.0	16490.1	-6.3	-113.9, -248.6	2.1, 2.2	4.0, 4.0

Information on the diet of northern fur seal suggests they use their annual migration to take advantage of seasonal prey pulses (Perez & Bigg 1986, Newsome *et al.* 2007, COSEWIC 2010). They exploit abundant and spatially aggregated species such that their diet greatly reflects the spatial and temporal variability of available prey (Perez & Bigg 1986, Lowry *et al.* 1991, COSEWIC 2010). However, most of our current knowledge of northern fur seal diet is limited to that acquired at rookeries during the breeding period. Data of the fur seal diet at sea is sparse and problematic, since much of this information was collected for the North Pacific Fur Seal Commission from 1958 to 1974, prior to the lack of population recovery and the current population decline (Perez & Bigg 1986, Towell *et al.* 2006). It is not known whether the diet of the northern fur seal during the pelagic phase of their life cycle has changed since this collection period. If the diet has shifted to primarily low quality prey items as seen in other North Pacific seabirds and marine mammals (Merrick *et al.* 1997, Trites & Donnelly 2003, Osterblom *et al.* 2008), there is a strong probability that nutritional stress is occurring at certain times of the year.

5.3 Future research

The conclusion that some level of nutritional stress is likely to occur if only low quality prey items are available to northern fur seals throughout the year lends credence to the hypothesis that nutritional stress is a significant causal factor in the decline of the northern fur seal on the Pribilof Islands. Nevertheless, future research is still required on both sides of the nutritional balance equation — the daily energetic intake and expenditures of individuals — to substantiate nutritional stress as a potential cause.

The inability to acquire current dietary information outside of the breeding season leaves a large data gap from October to June on what prey the northern fur seal are consuming during the annual pelagic migration. Traditional methods, such as at-sea captures paired with fecal sample collection or gastric lavage, are one option to fill this data gap, but are impractical due to financial costs and logistics. Obtaining such dietary information therefore requires new methods. For example, a video recording tag that is both minimal in size and has the capability to record/store information throughout the entire migration or activate/detach at a set point in time could be used to record information on prey types, as well as provide data on foraging strategies and energetics.

Additional information is also required on the daily energy expenditure of wild northern fur seals throughout the year, particularly during the non-breeding period. Use of the DLW

method for measurements of DEE throughout the year is impractical and expensive, as the effective time frame and quick turnover of the DLW requires at least one, if not repeated, at-sea captures. However, such data could be obtained *via* one of the alternative methods calibrated in my thesis or other potential novel methods. The use of accelerometry seems to have great potential. However, further calibration at a broader range of activities, without inducing physiological stress, is still required prior to any meaningfully application of accelerometry for the prediction of energy expenditure in wild individuals. The Philips' Actiwatch acceleration data logger showed the greatest potential for quantifying the energy expenditure of northern fur seals throughout the annual pelagic migration based on its relative simplicity, deployment length and accuracy. More advanced accelerometers (such as the Little Leonardo bi-axial accelerometers I used), are currently limited by memory and battery power. These advanced accelerometers nevertheless possess great potential to not only measure energy expenditure, but also deliver a more detailed understanding of the activities being performed by the seals throughout the day, *via* the quantitative data recorded on the body posture (static acceleration) and motion (dynamic acceleration) in each axis.

An additional area for future research is to understand how the northern fur seal manages to have such a broad thermal neutral zone (TNZ). I hypothesized that the fur seals' broad TNZ resulted from a higher density of guard hairs and, in particular, their accompanying sebaceous glands. However an increased number of sebaceous glands may not necessarily equate with a greater volume of sebaceous secretions (sebum), and sebum composition is extremely species-specific. Testing this hypothesis across a range of species against their associated thermal capabilities and quantifying the volume and composition of sebaceous secretion could provide greater insight into the thermal physiology of mammals. In tandem to these physiological studies of thermal capacity, it is also important to better document the complete range of water temperatures that wild individuals encounter throughout the year, particularly during the annual migration (as opposed to relying on oceanographic extrapolations). Instrumenting wild fur seals throughout the year with thermal sensors would permit quantification of the water temperatures they encounter, and reveal when and for how long individuals may be experiencing environmental temperatures outside of their TNZ. The technology for this already exists and has been applied during shorter term (commonly breeding season) studies (Ream *et al.* 2005,

Nordstrom *et al.* 2013); however technological advancements are still required for long-term studies.

5.4 Captive studies

Overall, the use of captive individuals is both the greatest strength and weakness of my thesis. Captive studies, such as this one, are limited in their ability to simulate natural conditions. For example, studies with marine mammals are unable to incorporate the costs associated with prey capture, prey selection and deep dives. Despite these inherent shortcomings, captive pinnipeds demonstrate intrinsic physiological changes within their energy budgets, including seasonal shifts in the costs of resting metabolic rate, growth, thermoregulation and, to a degree, activity. Therefore, the pattern and cost of seasonal changes in both total energy use and major energy budget components can be experimentally quantified under controlled conditions that would be impossible to acquire from animals in the wild.

The use of captive individuals also limits the age, gender and number of individuals examined. My study used only six individuals, which inherently limits statistical power. However, the statistical analysis used (linear mixed effects models with the individual included as a random effect to account for repeated measures) increases the strength of the analysis and permits reputable conclusions. The age and gender of the study individuals inescapably meant that I could only draw conclusions for young female northern fur seals. Fortunately, females are the more critical gender of northern fur seal due to the species' polygynous mating system. Further, fewer young female fur seals have been observed returning to the rookeries (Spraker & Lander 2010), indicating a particular role of females in the overall population decline.

Finally, it must be emphasized that research with captive animals in a laboratory setting provides information about a species that currently would be virtually unobtainable from wild individuals. None of the parameters I obtained could be readily measured from wild fur seals. The length of their pelagic migration means that the repetitive measurements necessary to quantify the DEE, resting metabolic rate and growth are essentially impossible to obtain throughout the year. Physiological field studies do not permit the controlled experimental manipulations required for the identification of the broad thermal neutral zone. Calibrating the DLW and accelerometry methods against respirometry is also impossible in the wild for northern fur seals since their surfacing locations are vast and unpredictable. Finally, all of my controlled condition measurements were undertaken on non-stressed individuals, which could not have

been accomplished with wild individuals. Ultimately, the information I obtained on captive northern fur seals, which might never be attainable on wild individuals, enhances understanding of the energetics of the species, and provides a better understanding of their life history and fitness.

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