

# Resting metabolic rate and activity: key components of seasonal variation in daily energy expenditure for the northern fur seal (*Callorhinus ursinus*)

A.J.M. Dalton, D.A.S. Rosen, and A.W. Trites

**Abstract:** Seasonal changes in daily energy expenditure (DEE) and its key underlying components (costs of resting metabolic rate (RMR), thermoregulation, activity, and growth) were measured to determine seasonal energy requirements, bioenergetic priorities, and potential times of year when unpredicted episodes of nutritional stress would have their greatest effect on female northern fur seals (*Callorhinus ursinus* (L., 1758)). The mean ( $\pm$ SD) DEE of six captive juvenile female fur seals was  $527.8 \pm 65.7$   $\text{kJ}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$  and fluctuated seasonally (lower during summer and winter, and up to 20% greater in spring and fall). RMR also changed significantly with season and was higher in the fall (potentially due to moulting or anticipated migratory activity). However, changes in RMR did not follow the same seasonal trend as those of DEE. The largest component of DEE was RMR ( $\sim 80\%$ , on average), followed by the cost of activity (which may have driven some of the seasonal variations in DEE). In contrast, the energetic costs associated with growth and thermoregulation appeared negligible within the scope of overall energy expenditures. Elevated innate costs of RMR and higher growth rates in the fall and summer, respectively, suggest that inadequate nutrition could comparatively have greater negative effects on female fur seals during these seasons.

**Key words:** northern fur seal, *Callorhinus ursinus*, daily energy expenditure, resting metabolic rate, activity, growth, thermoregulation.

**Résumé :** Les variations saisonnières de la dépense énergétique quotidienne (DEQ) et de ses principales composantes (coûts du taux métabolisme au repos (MR), de la thermorégulation, de l'activité et de la croissance) ont été mesurées afin de déterminer les besoins énergétiques saisonniers, les priorités bioénergétiques et les moments de l'année où des épisodes imprévus de stress nutritif pourraient avoir la plus grande incidence sur les otaries à fourrure du Nord (*Callorhinus ursinus* (L., 1758)) femelles. La DEQ moyenne ( $\pm$  écart type) de six femelles juvéniles captives était de  $527,8 \pm 65,7$   $\text{kJ}\cdot\text{kg}^{-1}\cdot\text{j}^{-1}$  et variait selon la saison (plus faible en été et en hiver, et jusqu'à 20 % plus élevée au printemps et à l'automne). Le MR variait également de manière significative selon la saison, étant plus élevé à l'automne (possiblement en raison de la mue ou de l'activité migratoire anticipée). Les variations du MR ne suivaient toutefois pas la même tendance saisonnière que celles de la DEQ. La composante la plus importante de la DEQ était le MR ( $\sim 80\%$ , en moyenne), suivi du coût de l'activité (qui était peut-être à l'origine d'une partie des variations saisonnières de la DEQ). En revanche, les coûts énergétiques associés à la croissance et à la thermorégulation semblaient négligeables dans le contexte des dépenses énergétiques globales. Les coûts innés élevés du MR en automne et les taux de croissance plus importants en été donnent à penser qu'une nutrition inadéquate pourrait avoir des effets négatifs relativement plus importants sur les otaries à fourrure femelles durant ces saisons. [Traduit par la Rédaction]

**Mots-clés :** otarie à fourrure du Nord, *Callorhinus ursinus*, dépense énergétique quotidienne, métabolisme au repos, activité, croissance, thermorégulation.

## Introduction

Nutritional stress may be contributing to the decline of northern fur seals (*Callorhinus ursinus* (L., 1758)) breeding on the Pribilof Islands, Alaska (Spraker and Lander 2010). Nutritional stress has similarly been implicated as a potential explanation for the decline of other marine mammal and seabird populations in the Bering Sea and North Pacific Ocean (Trites and 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 negatively impact marine mammal populations (Trites and 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, although longer term episodes of weeks to months are usually of concern within the context of the ecology of large mammals (King and 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 and 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 and to understand their underlying interactions.

Daily energy expenditure (DEE) is a measure of the total energetic requirements of an individual. Unfortunately, DEE of wild northern fur seals is virtually impossible to measure from the late

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fall to early summer due to the duration of their annual pelagic migration (Kenyon and Wilke 1953; Bigg 1990; Gentry 1998; Ream et al. 2005). We measured the DEE of a group of young captive northern fur seals, not as a direct quantitative comparison with their wild counterparts but as a first step to documenting the extent and root cause of seasonal changes in their DEE.

DEE represents the sum of the requirements of the individual components of an animal's energy budget. However, the proportion of energy dedicated to these different constituents can change seasonally in tandem with seasonal variation in overall energy expenditure (Rosen and Renouf 1998; Rosen and 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. 2008; Rosen and Kumagai 2008). Ultimately, varying energetic priorities can result in nutritional deficiencies having a greater impact during some seasons compared with others.

To investigate the varying energetic priorities of young northern fur seals and potentially identify critical seasons throughout the year, we seasonally measured four key components of the energy budget—the costs of resting metabolic rate (RMR), growth, thermoregulation, and activity—of a group of captive female juvenile fur seals. 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 episode.

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. Although the magnitude of some costs may be specific to their environment, intrinsic physiological changes are known to occur within the energy budget of a pinniped (i.e., RMR, growth, and thermoregulation) regardless of whether it is in captivity or living in the wild (Rosen and Renouf 1998; Donohue et al. 2000; Sparling et al. 2006; Liwanag 2010; Rosen et al. 2012; Dalton et al. 2014a; Rosen and Trites 2014). Thus, captive studies permit identification of innate seasonality in the energy requirements and energetic priorities of northern fur seals that may be difficult to discern in wild counterparts. This information 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 young northern fur seals are predicted to change significantly throughout the year as a cumulative response to seasonal variations in individual components of their energy budgets. For example, RMR (a standard measure of baseline energy expenditure) has been shown to change seasonally in captive (and temporarily captive) individuals, independent of other direct bioenergetic concerns both in young northern fur seals (Dalton et al. 2014a; Rosen and Trites 2014) and in mature individuals of other pinniped species (Rosen and 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–August) when immature northern fur seals exhibit increases in mass and length (Trites and Bigg 1996). Increased energy expenditures associated with higher levels of activity are also predicted in the late fall and early winter (November–January) when rapid migration to the wintering grounds would occur in the wild (Kenyon and 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 and Wilke 1953; Bigg 1990; Gentry 1998; Ream et al. 2005). In contrast, given the northern fur seal's impressively wide zone of thermal neutrality (Dalton et al. 2014a), changes in the costs of thermoregulation required to maintain core body temperatures are likely to be minor and, therefore, have minimal impact on the DEE throughout the year (Williams and Worthy 2002).

While seasonal shifts in the separate energetic costs of the major components of an animal's energy budget 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. Therefore, information on both the total daily energy requirements and the underlying components is required to understand their physiological interaction and to identify times of the year when unpredicted episodes of nutritional stress would have their greatest negative impact on northern fur seals.

## Materials and methods

### Animals

Six female northern fur seals (Table 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 months of age (postweaning). They were raised at The University of British Columbia (UBC) Marine Mammal Energetics and Nutrition Laboratory, located within the Vancouver Aquarium (British Columbia, Canada), and trained to be familiar with all necessary husbandry behaviours, research protocols, and scientific equipment using positive reinforcement. All individuals were considered juveniles at the time of this study (sexual maturity in northern fur seals occurs between the ages of 3 and 7; COSEWIC 2010). The fur seals were normally housed in seawater pools with water temperatures that reflected conditions of the local ocean (7–16 °C). A daily diet consisting of (~90%) herring and (~10%) squid supplemented with vitamins was fed to the individuals in two separate feeds over the course of the day: two-thirds of the daily diet in the morning and one-third 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 within working and training requirements (on average,  $8.7\% \pm 1.3\%$  of the total body mass;  $1.4\text{--}1.8 \text{ kg}\cdot\text{d}^{-1}$  of prey, across all individuals and all seasons). Animal Care committees of both UBC (permit #A10-0342) and the Vancouver Aquarium approved all research protocols and animal use described.

### Timing

Our study consisted of four seasonal sets of trials, each of which took ~7 weeks to complete: (1) March–April 2011 (spring; age 2.75 years old), (2) June–July (summer; age 3 years), (3) September–October (fall; age 3.25 years), and (4) December 2011–January 2012 (winter; age 3.5 years). The order in which the individual animals were tested within each seasonal set of trials was determined randomly. Individual trials within a seasonal set were separated to permit adequate cleaning of equipment and depended on veterinary availability.

### DEE

DEE of the northern fur seals was quantified via respirometry, using a large metabolic chamber (see below) to continuously measure the rates of oxygen consumption ( $\dot{V}_{O_2}$ ) and carbon dioxide production ( $\dot{V}_{CO_2}$ ) of each individual throughout a period of nearly 5 d. 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 individual northern fur seal only interacted briefly with the training staff twice daily. The individual received a quick physical health assessment (including measuring body mass) in tandem with its first feed each morning outside of the metabolic chamber ( $8.6 \pm 4.0$  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 placed over a holding pool and the associated haul-out space and was constructed of welded aluminum and Lexan™ (detailed in Dalton et al. 2014b). Briefly, air was drawn through the metabolic chamber into a gas analysis system at  $125 \text{ L}\cdot\text{min}^{-1}$  via an excurrent

**Table 1.** Body mass (kg; mean  $\pm$  SD) during four seasonal trials (spring, summer, fall, and winter) for each of the six female northern fur seals (*Callorhinus ursinus*) from March 2011 to January 2012.

| Season | Spring          | Summer         | Fall            | Winter         |
|--------|-----------------|----------------|-----------------|----------------|
| AN08   | 19.8 $\pm$ 0.6  | 21.2 $\pm$ 0.9 | 22.2 $\pm$ 0.5  | 21.2 $\pm$ 0.3 |
| AY08   | 14.2 $\pm$ 0.08 | 17.0 $\pm$ 0.9 | 18.4 $\pm$ 0.09 | 17.7 $\pm$ 0.3 |
| KY08   | 15.3 $\pm$ 0.05 | 17.6 $\pm$ 0.8 | 19.5 $\pm$ 0.1  | 15.6 $\pm$ 0.2 |
| ME08   | 15.6 $\pm$ 0.2  | 17.1 $\pm$ 0.4 | 19.8 $\pm$ 0.3  | 19.3 $\pm$ 0.1 |
| TI08   | 18.9 $\pm$ 0.2  | 22.6 $\pm$ 0.6 | 26.6 $\pm$ 0.8  | 25.1 $\pm$ 0.1 |
| TU08   | 15.2 $\pm$ 0.1  | 18.6 $\pm$ 0.8 | 21.4 $\pm$ 0.3  | 20.3 $\pm$ 0.4 |
| Mean   | 16.5 $\pm$ 2.3  | 19.0 $\pm$ 2.3 | 21.4 $\pm$ 2.9  | 20.5 $\pm$ 2.5 |

airflow pipe located above the pool, generating a 50% air turnover rate of  $\sim$ 19 min. Proper air mixing within the metabolic chamber was attained via an internal air circulation system. The entire metabolic chamber was tested for leaks and proper air circulation prior to use (Fig. 1).

Metabolic rates inside of the metabolic chamber were determined using open-flow respirometry to calculate  $\dot{V}_{O_2}$  and  $\dot{V}_{CO_2}$ , as detailed in Dalton et al. (2014a). Briefly, measurements were made using one of two systems incorporating Sable Systems' (Las Vegas, Nevada, USA) mass flow pumps, as well as oxygen and carbon dioxide analyzers, to ascertain the gas concentrations of a dried subsample of the excurrent air stream. The entire gas analysis system was calibrated with dry ambient air at the start and the end of each 5-d trial, in addition to each morning, to account for system drift such that changes in gas concentrations were determined against baseline (ambient) measures. Gases of known concentrations were also periodically used to calibrate the systems.

$\dot{V}_{O_2}$  were calculated from measured changes in gas concentration, using LabAnalystX software (M. Chappell; available from <http://warthog.ucr.edu/WarthogPage/LAX%20website/LAHP.html>, accessed 7 April 2011) and including the appropriate equations from Withers (1977). In a segment of the first two seasonal sets of trials, a malfunctioning CA-1B analyzer ( $CO_2$  sensor) was discovered in one of two of the gas analysis systems. In trials where the mean respiratory quotient (RQ) value was calculated to be outside of the reasonable expected physiological range (0.65–1.05), the  $\dot{V}_{O_2}$  was calculated using a fixed RQ value (0.8) in place of the RQ based on the erroneous measured  $\dot{V}_{CO_2}$ .  $\dot{V}_{O_2}$  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). The calories per litre of oxygen consumed range from 4.686 for an RQ of 0.7 to 5.047 for an RQ of 1.0; the difference from an RQ of 0.8 is a maximum of 5% (Brody 1945).

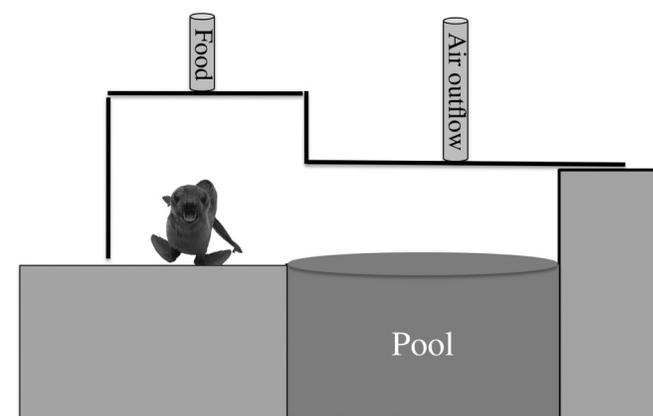
#### Activity level

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  $\pm$ 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 logger was secured inside a Velcro-sealed pocket attached to a custom-made harness worn by the fur seal and lay dorsal to the pectoral flippers. The Actiwatch logger provided a count of the number of times that the test subject exceeded the threshold acceleration in any dimension (surge, heave, or sway) during each 15 s interval. A previous study by Dalton et al. (2014b) demonstrated a strong relationship between Actiwatch score and  $\dot{V}_{O_2}$  over the course of entire DEE trials throughout the year and within each season on a fine scale, indicating that changes in this measure of activity reflected changes in the cost of physical activity.

#### Cost of growth

Body mass was measured ( $\pm$ 0.02 kg) for each fur seal each morning by having the animal stand inactive on a platform scale pre-

ceding the first feeding (at least 16 h postprandial). Measurements of body length (nose to tail;  $\pm$ 1 cm) were obtained biweekly by having the fur seal 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 an entire 7-week 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.



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Changes in body mass could be further partitioned into changes in specific body components (i.e., lipid or protein mass) between seasons by extrapolating between single-point estimates of body composition obtained in each season. Immediately preceding each DEE trial, the deuterium oxide ( $D_2O$ ) dilution technique was used to determine the total body water of the fur seals (Reilly and 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* (Peters, 1875)).

The procedure for the  $D_2O$  dilution technique followed the method described in Costa (1987) and Reilly and Fedak (1990). Briefly, all blood samples were collected with the animals under veterinary-supervised anaesthetic (maximum 5% isoflurane) and were obtained from the caudal gluteal vein. An initial background blood sample was drawn into a serum separator tube prior to the intramuscular administration of the  $D_2O$  (99.9%  $D_2O$  water) at a measured dosage of  $\sim$ 0.16 g·kg<sup>-1</sup> of animal. A second blood sample was collected 2 h after administration of the  $D_2O$  (permitting equilibration with the body water pool; Costa 1987) to determine the increase in the concentration of  $^2H$ . During the 2 h equilibration period, animals were awake and kept in a holding run with a circular wading pool and running water; this did not affect the calculation of total body water, as the drinking of water has never been observed in these individuals.

Blood samples were centrifuged and the collected serum was stored at  $-70^\circ C$  until analysis. Isotope analysis of the serum and dose samples was conducted, following the methodology described by Scrimgeour et al. (1993), by Metabolic Solutions Inc.

(Nashua, New Hampshire, USA) using a Europa Hydra continuous-flow isotope-ratio mass spectrometer.

Changes in body composition between seasons permitted calculation of the cost of tissue deposition, which 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<sup>-1</sup> of protein deposited and 0.17 kJ·kJ<sup>-1</sup> of lipid deposited (Roberts and Young 1988). Standard biochemical estimates of the energy content per gram of protein and lipid are 18 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. 2009).

### Cost of RMR 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 ± 0.5, 10 ± 0.5, and 18 ± 0.5 °C. The methods are fully outlined in Dalton et al. (2014a), which also provides an in-depth analysis of the cost of thermoregulation for these same individuals. Briefly,  $\dot{V}_{O_2}$  and  $\dot{V}_{CO_2}$  were continuously measured via respirometry within a specially designed 340 L metabolic chamber (dimensions: 0.92 m × 0.61 m × 0.61 m). First,  $\dot{V}_{O_2}$  and  $\dot{V}_{CO_2}$  were measured in ambient air conditions for 20 min (cost of RMR). Immediately following, the chamber was partly filled with water at one of the three different experimental treatment temperatures and the  $\dot{V}_{O_2}$  and  $\dot{V}_{CO_2}$  were measured for an additional 30 min. The individuals were trained to remain calm, with minimal activity, within the chamber. To control for any variance in resting metabolism that might obscure changes in the costs of thermoregulation, the potential costs of thermoregulation at each water temperature within each season were calculated as the difference in metabolic rate between wet and dry (resting) trials for each individual session (i.e., reduced type II error).

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

### Data analysis

Separate linear mixed-effects (LME) models were used to determine seasonal (between-trial) changes in DEE, activity, RMR (in ambient air), and growth (mean seasonal body composition, body mass, and body length; NLME library in R from Pinheiro and Bates 2000). All LME models included the individual as the random effect to account for repeated measures. Post hoc Tukey contrasts simultaneous tests for general linear hypotheses were used to determine between which of the seasons significant differences occurred (when significant differences were detected in any LME model).

LME models were also separately constructed to determine if the rate of change between seasons in growth (mean seasonal body composition or body length) were significantly different over the course of the study. Again, all LME models included the individual as the random effect to account for repeated measures. Post hoc Tukey contrasts simultaneous tests for general linear hypotheses were again used to determine between which interval significant differences occurred, if significant differences were detected in any LME model.

To determine if mass was a significant predictor of mass-specific DEE or RMR, we separately constructed LME models of the mass-specific DEE and RMR against mass. Again, individual was included as the random effect to account for repeated measures.

One sample Student's *t* tests were also constructed to determine if changes in body mass (growth rates) within a season were significantly different from zero. The R version 3.0.1 software package was used to conduct all statistical analyses (R Development Core Team 2012).

## Results

### DEE

$\dot{V}_{O_2}$  and other values are presented as means ± 1 SD. The mean mass-specific rate of oxygen consumption of the northern fur seals throughout the DEE trials ( $s\dot{V}_{O_2,DEE}$ ) across all individuals and all seasons was 18.1 ± 2.4 mL O<sub>2</sub>·kg<sup>-1</sup>·min<sup>-1</sup>, or 351.6 ± 58.8 mL O<sub>2</sub>·min<sup>-1</sup> on an absolute basis. Both the mean  $\dot{V}_{O_2,DEE}$  ( $P = 0.0001$ ) and mean  $s\dot{V}_{O_2,DEE}$  ( $P = 0.002$ ) changed significantly throughout the year. The mean  $s\dot{V}_{O_2,DEE}$  was highest in the fall trials (20.5 ± 1.7 mL O<sub>2</sub>·kg<sup>-1</sup>·min<sup>-1</sup>) and lowest in the winter trials (16.1 ± 1.6 mL O<sub>2</sub>·kg<sup>-1</sup>·min<sup>-1</sup>; Fig. 2). Mass was not found to be a significant predictor of  $s\dot{V}_{O_2,DEE}$  ( $P = 0.1$ ). Seasonal absolute  $\dot{V}_{O_2,DEE}$  results are detailed in Dalton et al. (2014b).

Converted to estimates of DEE, the overall mean mass-specific DEE was 527.8 ± 65.7 kJ·kg<sup>-1</sup>·d<sup>-1</sup>. The mass-specific DEE within each seasonal trial ranged from 587.8 ± 47.2 kJ·kg<sup>-1</sup>·d<sup>-1</sup> in the fall to 481.4 ± 45.1 kJ·kg<sup>-1</sup>·d<sup>-1</sup> in the winter (Table 2).

### RMR

The mean mass-specific rate of oxygen consumption of the fur seals when resting in ambient air temperatures ( $s\dot{V}_{O_2,RMR}$ ) across all individuals and all seasons was 17.6 ± 7.3 mL O<sub>2</sub>·kg<sup>-1</sup>·min<sup>-1</sup>, which is equivalent to 97.2% of the mean  $s\dot{V}_{O_2,DEE}$ . However, unusually high activity was observed in one individual (ME08) during the measurements of  $\dot{V}_{O_2,RMR}$  such that the resulting data did not reflect resting conditions. The measured  $\dot{V}_{O_2,RMR}$  of ME08 was approximately twice that of other individuals and of her own  $\dot{V}_{O_2}$  when immersed in water. The mean  $s\dot{V}_{O_2,RMR}$  of the northern fur seals across all seasons was 15.4 ± 5.1 mL O<sub>2</sub>·kg<sup>-1</sup>·min<sup>-1</sup>, or 84.2% of the  $s\dot{V}_{O_2,DEE}$  when the  $s\dot{V}_{O_2,RMR}$  data of ME08 were omitted. The mean  $s\dot{V}_{O_2,RMR}$  (both with and without ME08) changed significantly throughout the year ( $P = 0.001$ ), and was significantly elevated in the fall trials (19.3 ± 3.4 mL O<sub>2</sub>·kg<sup>-1</sup>·min<sup>-1</sup>) compared with the three remaining seasonal trials (overall mean 14.1 ± 4.9 mL O<sub>2</sub>·kg<sup>-1</sup>·min<sup>-1</sup>;  $P = 0.005$ ) that were not significantly different from one another ( $P = 0.5$ ; Table 1). Mass was a significant predictor of  $s\dot{V}_{O_2,RMR}$  ( $P = 0.008$ ). When comparing these measures by season, the  $s\dot{V}_{O_2,RMR}$  accounted for 71.4% of the  $s\dot{V}_{O_2,DEE}$  in the spring trials, 81.2% in the summer trials, 94.2% in the fall trials, and 93.6% in the winter trials.

Ambient air temperatures below 2.5 °C were found to increase the  $s\dot{V}_{O_2,RMR}$  in the winter trials, as described in the in-depth analysis of the cost of thermoregulation for these same individuals in Dalton et al. (2014a). Removal of trials in which  $s\dot{V}_{O_2,RMR}$  were measured in ambient air temperatures below 2.5 °C decreased the mean  $s\dot{V}_{O_2,RMR}$  for the winter trials to 11.4 ± 3.9 mL O<sub>2</sub>·kg<sup>-1</sup>·min<sup>-1</sup> (and 70.8% of the  $s\dot{V}_{O_2,DEE}$ ); the overall seasonal trend in the  $s\dot{V}_{O_2,RMR}$ , however, did not change (i.e., the  $s\dot{V}_{O_2,RMR}$  in the fall remained higher than the three other seasons).

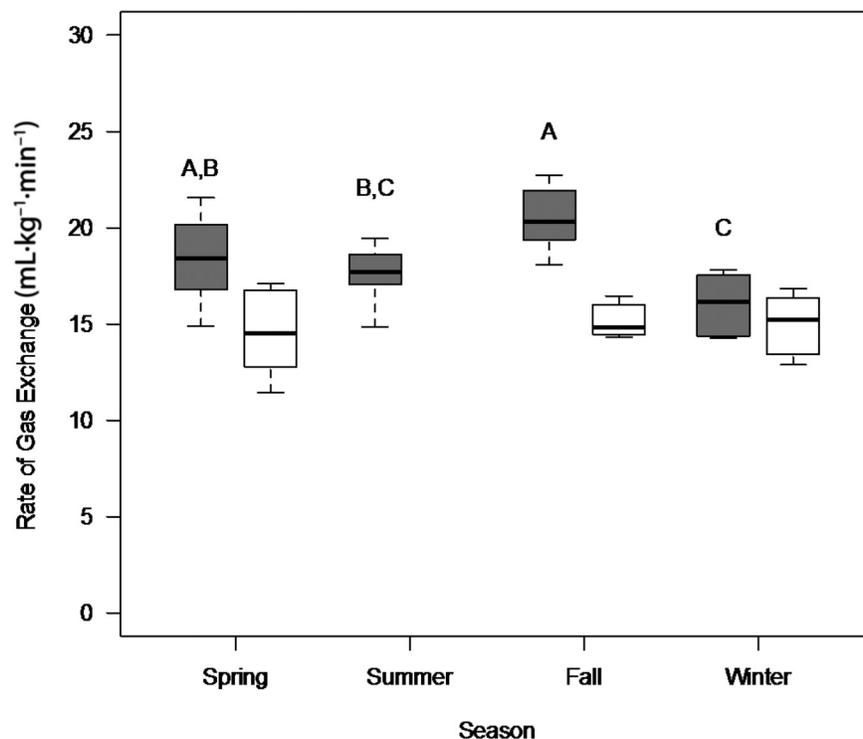
### Thermoregulation in water

The northern fur seals in our study appeared to be thermally neutral in all seasons for the water temperatures tested (from 2 to 18 °C), except during the summer when metabolic rates were higher in the 2 °C water trials (Dalton et al. 2014a). In those 2 °C water trials, the  $s\dot{V}_{O_2}$  was 19.0 ± 6.5 mL O<sub>2</sub>·kg<sup>-1</sup>·min<sup>-1</sup>, which was 36% greater than the  $s\dot{V}_{O_2,RMR}$  ( $P = 0.04$ ; Dalton et al. 2014a).

### Growth

Mean growth rates within a season (standardized as changes in the body mass over the 7-week trial) were significantly positive during both the spring trials (0.7 ± 0.7 kg;  $P = 0.05$ ) and the summer

**Fig. 2.** Mean ( $\pm$ SD) mass-specific rate of oxygen consumption ( $s\dot{V}O_2$ ; grey boxes) and mean mass-specific rate of carbon dioxide production ( $s\dot{V}CO_2$ ; white boxes) of six 3-year-old female northern fur seals (*Callorhinus ursinus*) throughout the nearly 5-d daily energy expenditure (DEE) trials measured in four seasonal sets of trials from March 2011 to January 2012. The  $s\dot{V}O_2$  was highest in the fall trials, which was significantly higher than the  $s\dot{V}O_{2,DEE}$  in either the summer or the winter trials ( $P < 0.001$ ), which were themselves not significantly different from one another ( $P = 0.25$ ). The  $s\dot{V}O_2$  in the fall trials, however, was not significantly different than in the spring trials ( $P = 0.06$ ). The  $s\dot{V}O_2$  in the spring was also significantly higher ( $P = 0.02$ ) than in the winter, but was not significantly different than in the summer ( $P = 0.74$ ).



**Table 2.** Mean ( $\pm$ SD) daily energy expenditure (DEE) and cost of tissue deposition in six 3-year-old female northern fur seals (*Callorhinus ursinus*) measured in four seasonal sets of trials from March 2011 to January 2012.

| Season | DEE<br>(kJ·kg <sup>-1</sup> ·d <sup>-1</sup> ) | Mean body<br>mass change |                   | Cost of deposition<br>(kJ·d <sup>-1</sup> ) | Percentage<br>of mean |      |
|--------|--|--------------------------|-------------------|---|-----------------------|------|
|        |  | kg                       | g·d <sup>-1</sup> |   | RMR                   | DEE  |
| Spring | 533.2 $\pm$ 77.8                               | 0.7                      | 14.3              | 160.3                                       | 2.4                   | 1.8  |
| Summer | 508.7 $\pm$ 46.3                               | 1.2                      | 24.5              | 274.8                                       | 3.3                   | 2.5  |
| Fall   | 587.8 $\pm$ 47.2                               | -0.8                     | -15.3             | -171.8                                      | -1.4                  | -1.4 |
| Winter | 481.4 $\pm$ 45.1                               | -0.3                     | -6.3              | -71.0                                       | -0.8                  | -0.7 |

**Note:** Mean changes in body mass are presented as total measured change over the course of an entire 7-week seasonal trial (kg) and as a daily mean body mass change (g·d<sup>-1</sup>). These daily mass changes were used to calculate the cost of deposition of new tissue, assuming that new tissue was 3 g lipid·(g protein)<sup>-1</sup>. The costs of tissue deposition are also expressed as a percentage of the mean resting metabolic rate (RMR) and mean DEE.

trials ( $1.2 \pm 0$  kg;  $P = 0.01$ ). Growth rates were not significantly different from zero during either the fall trials ( $-0.8 \pm 1.0$  kg;  $P = 0.1$ ) or the winter trials ( $-0.3 \pm 0.6$  kg;  $P = 0.3$ ).

Mean body mass (the mean body mass during the 7-week trial) of the northern fur seals differed significantly between trials ( $P = 0.001$ ); it increased over the course of the first three phases of the study by 4.9 kg and then dropped slightly between the fall and the winter trials. As a result, the mean body mass was lowest during the spring trials ( $16.5 \pm 2.3$  kg) and highest in the fall trials ( $21.4 \pm 2.9$  kg; Table 1).

Mean length of the northern fur seals also significantly increased by 8.4 cm (from  $104.0 \pm 6.1$  to  $112.4 \pm 5.7$  cm;  $P = 0.001$ ) over the course of the study. The rate of increase was significantly different over the course of the study ( $P = 0.01$ ). Mean length

increased more between the spring and the summer trials and between the fall and the winter trials compared with the rate of increase in mean length that occurred between the summer and the fall trials ( $P = 0.01$ ).

The mean absolute amount of lipid in the bodies of the northern fur seals significantly increased over the course of the study by 1.5 kg (from  $0.7 \pm 0.3$  to  $2.3 \pm 0.8$  kg;  $P = 0.001$ ). The rate of change in absolute lipid mass, however, did not differ significantly between seasons ( $P = 0.2$ ). When combined with changes in body mass between seasons, the percent body mass composed of lipids increased at a constant rate throughout the study. As a result, the lowest absolute amount and relative concentration of lipids occurred during the first trial, the spring ( $0.7$  kg or  $4.6\% \pm 2.1\%$  of total body mass), and the highest absolute amount and concentra-

tion of lipids was found during the final trial, the winter ( $2.3 \text{ kg}$  or  $11.0\% \pm 3.7\%$  of body mass).

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

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 mean RMR and  $6.2\%$  of the DEE in the summer if all of the observed changes in body mass were attributed to protein deposition. However, this is unlikely because 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 interseasonal ratio of tissue growth to estimate costs of growth within a season. As we assumed a constant cost per gram for body mass changes, the statistical results for differences in cost of growth within a season are identical to those presented for body mass. Specifically, the costs of growth were not greater than zero during the fall and winter trials, but were significantly greater during the spring and summer trials (Table 2).

### Activity level

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

### 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 how these expenditures change throughout the year. In our study, the DEE of our juvenile female northern fur seals changed significantly throughout the year due largely to seasonal variation in the costs of RMR 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 corresponds to increased costs of growth, while fall corresponds with increased costs of resting metabolism. These could be times of year when inadequate nutrition could have the greatest negative effect on young female northern fur seals.

### DEE

Animals in controlled environments should not necessarily be expected to have the same energy expenditures as their wild counterparts. In our study, the measures of DEE for our juvenile female northern fur seals are likely minimal compared with those experienced by wild individuals. The measured mean field metabolic rates in a variety of otariids (California sea lions (*Zalophus californianus* (Lesson, 1828)), northern fur seals, and Antarctic fur seals) have been shown to be 3.3–6.7 times Kleiber's (1975) allometric prediction for the basal metabolic rate of similarly sized terrestrial mammals (Costa and Gentry 1986; Costa and Trillmich 1988; Costa et al. 1989; Boyd and Duck 1991; Costa et al. 1991; Arnould et al. 1996b). The basal energetic costs for a variety of marine mammal species range from 1.4 to 2.8 times Kleiber's prediction (Williams et al. 2001; Williams et al. 2007); field metabolic rates in otariids are typically  $\sim 2.5$  times these basal energetic costs. In our study, the mean DEE was only  $\sim 20\%$  higher than the 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 and Trites 2000).

The relatively lower DEE of our captive animals likely resulted from their restricted dive depths and lack of active foraging compared with their wild counterparts. The costs of physical movement can surpass, by a factor of 10 or more, any other energetic function (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. On the other hand, the lower costs of activity may have enabled us to more clearly identify seasonal changes in other energetic parameters.

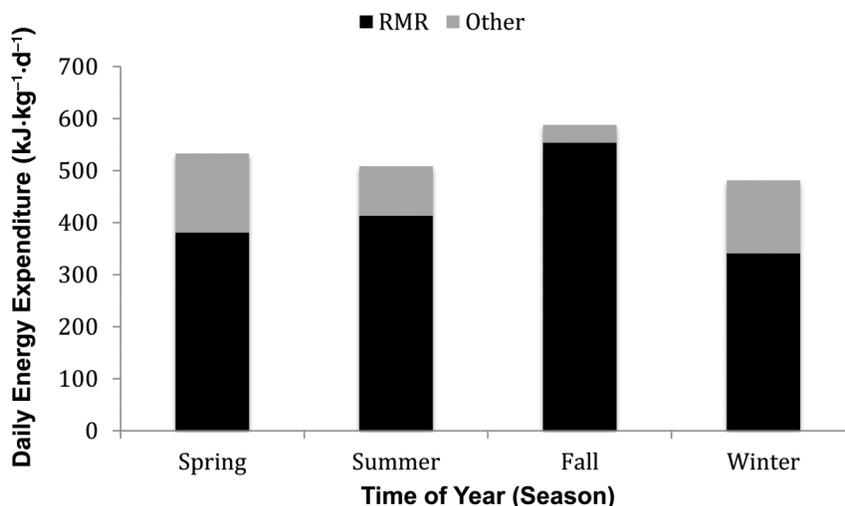
Despite or because of the lack of high activity costs, the DEE of these northern fur seals changed significantly throughout the year. This concurs with previous studies that have demonstrated intrinsic physiological changes within a captive pinniped's energy budget (Rosen and Renouf 1998; Donohue et al. 2000; Sparling et al. 2006; Liwanag 2010; Rosen et al. 2012; Dalton et al. 2014a; Rosen and 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, such as the cost of thermoregulation, growth, RMR, heat increment of feeding (HIF), and (or) activity.

### 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  $\sim 80\%$  of the DEE, on average, and is known to change seasonally in pinnipeds (Rosen and 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 trials. This is consistent with studies by Miller (1978) and Donohue et al. (2000) using northern fur seals that ranged from postmoult pups to 5-year-old juveniles. Relative to the other three seasons, the RMR during the fall trials was significantly elevated (4.2 times Kleiber's predictions). This seasonal variation in RMR was not a product of differences in absolute body size over the course of the year. First, although both RMR and body mass are highest in the fall, the differences in metabolism between the fall and the 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 the summer trials, the  $s\dot{V}O_{2\text{RMR}}$  did not vary in the same manner.

**Fig. 3.** The daily energy expenditure (DEE) of six 3-year-old female northern fur seals (*Callorhinus ursinus*) measured in four seasonal sets of trials from March 2011 to January 2012 with the portion of the DEE that is accounted for by the resting metabolic rate (RMR; black bars) and other components (grey bars). 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 RMR mass-specific rate of oxygen consumption ( $\dot{V}O_{2,RMR}$ ).



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. 2008; Rosen and 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.

### 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 intraseasonal 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.

The direct costs of growth to RMR (and indirectly to DEE) in the juvenile female northern fur seals were negligible (maximum ~3% of RMR assuming a reasonable ratio of lipid to protein deposition). 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.

### Moulting

In contrast to the costs of physical growth, we believe the most logical explanation for the elevated RMR observed in the fall is the costs directly associated with moulting (versus any potential secondary effects on thermoregulation or activity; Boyd et al. 1993). The moult 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 trials. In Steller sea lions (*Eumetopias jubatus* (Schreber, 1776)) and California sea lions, a 30%–87% increase in metabolism is associated with the moult (Kumagai 2004; Williams et al. 2007). The 50% increase in RMR in our fall trials relative to the other seasons is thereby consistent with the metabolic increases attributed to moulting in these previous studies.

Whereas moulting is hypothesized to account for the seasonal changes in RMR, it does not explain the observed seasonal changes in the DEE. RMR accounted for a varying proportion of the 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<sup>-1</sup>·d<sup>-1</sup>), less in the winter (140.6 kJ·kg<sup>-1</sup>·d<sup>-1</sup>) and summer (95.6 kJ·kg<sup>-1</sup>·d<sup>-1</sup>), and lowest in the fall (34.1 kJ·kg<sup>-1</sup>·d<sup>-1</sup>) (Fig. 3).

### 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. Therefore, one might expect that the fur seals were most likely to experience additional thermal costs during the months when the mean air and water temperatures were lowest. The lowest mean 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 (Dalton et al. 2014a), 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 (December = 9.6 °C; January = 8.8 °C). However, the northern fur seals in our study were found to be thermally neutral throughout the year in water temperatures from 2 to 18 °C, with the exception of the summer trials at 2 °C (Dalton et al. 2014a), which were clearly never experienced through ambient water conditions 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.

### HIF

The variation observed in the DEE also does not appear to be the result of additional energy expenditures associated with HIF (which was not measured in this study). While the mean daily food intake changed significantly throughout the year, the HIF cost would be projected to be lowest in the spring and highest in the fall, paralleling mean food intake levels. This is not in agreement with the observed seasonal variation in DEE. Additionally, the mean 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 and Trites 1997; Rosen 2009).

### Activity

Variation in the cost of activity could explain the seasonal changes in the northern fur seals' DEE. This explanation makes sense bioenergetically given the high potential costs of physical movement (Darveau et al. 2002). The quantitative measure of activity that we used in this study, Actiwatch activity score, has previously been found to be a significant predictor of the mean  $\dot{V}O_{2,DEE}$  across all the seasons and all individuals (Dalton et al. 2014b). In our study, the Actiwatch activity scores were 21% higher in the fall ( $84.3 \pm 6.3$  counts  $\cdot$  (15 s interval) $^{-1}$ ) than in the winter ( $69.6 \pm 9.9$  counts  $\cdot$  (15 s interval) $^{-1}$ ;  $P = 0.001$ ) trials, which compares favourably to the 27% difference observed in DEE between these seasons. Furthermore, 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 (mean) increase in Actiwatch activity score equates to a mean increase in  $\dot{V}O_{2,DEE}$  of  $0.16$  mL  $O_2 \cdot$  kg $^{-1} \cdot$  min $^{-1}$  (Dalton et al. 2014b), the  $14.7$  counts  $\cdot$  (15 s interval) $^{-1}$  difference in Actiwatch score between the seasons would translate roughly into a difference of  $68$  kJ  $\cdot$  kg $^{-1} \cdot$  d $^{-1}$ . This again compares with the total observed seasonal difference in DEE of  $\sim 106$  kJ  $\cdot$  kg $^{-1} \cdot$  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 seasonal differences in the measured activity levels. This discordance potentially results from the interplay of RMR with anticipated activity. In the wild, female northern fur seals undertake a substantial southward migration beginning in October (late fall) and lasting between  $\sim 1$  and 3 months (Kenyon and 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 and 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 mean 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 of this seasonal physiological adaptation is that the costs of a given level of activity might be higher in the winter than in the fall.

### Critical seasons

There are different ways to evaluate which seasons might be "critical" for wild northern fur seals to receive adequate nutrition.

Alternate hypotheses suggest that critical seasons for nutritional stress might be those with the highest total energy requirements, those with the greatest innate energetic demands, or those with high-energy requirements for critical systems.

Hence, the fall period might be considered the most critical due to the high direct costs of locomotion during the fall migration, which represent ecological requirements due to changes in climate and prey distribution, and therefore cannot be scaled back. Although the northern fur seals in our study were not undertaking similar levels of activity, we hypothesize that some of the seasonal changes in RMR that we did observe reflect innate physiological changes that facilitate changes in activity. These innate changes may indicate that the fall period is critical for adequate nutrition, not because of the total energetic requirements but because of a lack of flexibility in their fall energy budgets. Regardless of whether the observed increases in RMR were due to the moult or up-regulation to facilitate higher activity costs, the observation that these changes appear to be intrinsic even in an artificial environment suggests that this season may represent a critical time of year when inadequate nutrition would have the greatest potential impact on this age class.

Alternately, the summer may represent a critical nutritional period, given the energy requirement for growth (as high as 7.7% of the mean RMR; likely  $\sim 3\%$  of RMR assuming a reasonable ratio of lipid to protein deposition). Growth rates are highest in this period, contributing both to overall changes in body size and to the energetic and thermoregulatory conditions 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, a lack of flexibility in their summer energy budgets coupled with restricted food intake during this period would have a long-term impact on body size and subsequent energy balance, barring any compensatory growth.

### Conclusions

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

Changes in the major components of the energy budgets of these captive juvenile northern fur seals 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 RMR 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 moulting, but may come at the expense of restricting flexibility within the energy budget, leading to severe consequences during unpredicted periods of nutritional stress.

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