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Season and time of day affect the ability of accelerometry and the doubly labeled water methods to measure energy expenditure in northern fur seals (*Callorhinus ursinus*)

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ABSTRACT

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 ($\dot{V}O_{2DEE}$) 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.

1. 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 (Boyd, 2002; Fahlman et al., 2008a; Halsey et al., 2009b; Minetti et al., 1999; Williams et al., 1993). 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 must instead be estimated using more indirect measures such as the doubly labeled water (DLW) turnover and accelerometry methods (Halsey et al., 2011; Nagy et al., 1999). However, these alternative methods come with their own logistical constraints and predictive limitations that are often species-specific (Butler et al., 2004; Halsey et al., 2011; Speakman, 1997). 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 (Halsey et al., 2011; Wilson et al., 2006). 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; Sparling et al., 2008; Speakman et al., 2001). This isotope washout method estimates an individual's CO_2 production using the differential elimination of

Abbreviations: 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; 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; RMR, resting metabolic rate; RQ, respiratory quotient; $\dot{V}CO_2$, rate of carbon dioxide production; $\dot{V}O_2$, rate of oxygen consumption; $\dot{V}O_{2\,DEE}$, average rate of oxygen consumption over 5-day trials; $\dot{V}CO_{2\,DEE}$, average rate of carbon dioxide production over 5-day trials; $\dot{V}O_{2\,RMR}$, resting rate of oxygen consumption.

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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 that 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 (Boyd et al., 1995; Costa, 1987; 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 (Enstipp et al., 2011; Green et al., 2009; Halsey et al., 2009a; Wilson et al., 2006). 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 (Halsey et al., 2009a; Wilson et al., 2006). Increased use of accelerometry to estimate energy expenditure in animals has been facilitated by advancements in the miniaturization of data loggers (Enstipp et al., 2011; Green et al., 2009; Wilson et al., 2006).

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 (Steller sea lions — *Eumetopias jubatus*; Fahlman et al., 2008b; and Weddell seals — *Leptonychotes weddellii*; Williams et al., 2004) 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 labor 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 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 methods 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; 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).

2. Methods

2.1. Animals

Six female northern fur seals were studied from March 2011 to January 2012. The animals were collected from a rookery on St. Paul Island, Alaska, in October 2008, following weaning at approximately 4 months 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 behaviors, 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 (7–16 °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.

2.2. Timing and general protocol

We conducted four seasonal sets of trials: 1) Mar/Apr 2011 ("Spring"; age 2.75 years old), 2) Jun/Jul ("Summer"; age 3 years old), 3) Sept/Oct ("Fall"; age 3.25 years old) and 4) Dec 2011/Jan 2012 ("Winter"; age 3.5 years old). Each set of trials took ~7 weeks 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 Sections 2.3 to 2.5). In addition, their activity was also measured using two types of accelerometers (see Section 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 (Boyd et al., 1995; Nagy, 1980). 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 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.

2.3. Metabolic chamber

The metabolic chamber consisted of a large, airtight dome, constructed of welded aluminum and Lexan™, placed over a holding pool and its associated haul out space (Fig. 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

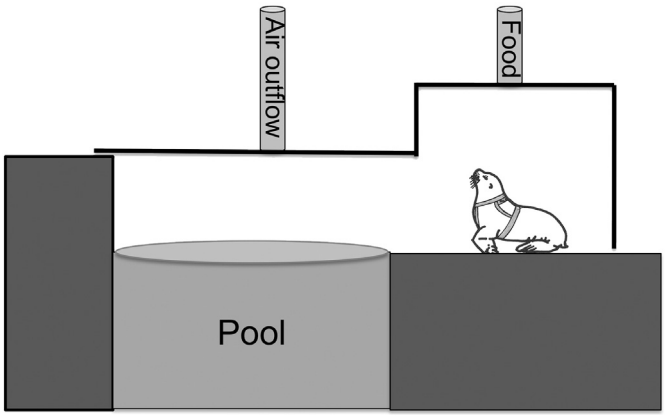

Fig. 1. Metabolic chamber schematic for measuring daily energy expenditure (DEE) of northern fur seals via respirometry. Schematic of the metabolic dome (including airlock feeding tube and excurrent air tube) constructed over one of the holding pools for measurements of oxygen consumption rates during five-day metabolic measurement trials. The metabolic chamber consisted of a circular pool (8500 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.

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

2.4. 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, USA) 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, USA), 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 s 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 Lab Analyst X software (M. Chappell, UC Riverside, Riverside, CA, USA — http://warthog.ucr.edu/WartHogPage/LAX%20website/LAHP.html) 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 0.80 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).

2.5. 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 ^{18}O and ^{2}H 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 h 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 h 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, 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). 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 — http://www.abdn.ac.uk/energetics-research/doubly-labelled-water/program/) 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 weights of the individuals, the isotope enrichment in each of the blood samples, and using the best estimate RQ of 0.80. 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 (Coward et al., 1985; Lifson and McClintock, 1966; Nagy, 1983; Racette et al., 1994; Schoeller et al., 1986, 1995; Speakman, 1993, 1997 — two pool and single pool estimates; Speakman et al., 1993). 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 produced a single estimate of DEE_{DLW} over each DEE trial for each fur seal.

2.6. 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™ 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.

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-second 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-second intervals, such that no additional data processing was required to obtain the dynamic acceleration measure.

2.7. Resting metabolic rate (RMR)

Resting metabolic rate (RMR) was measured in a separate specially-designed 340 L metabolic chamber (dimensions: 0.92 m × 0.61 m × 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 2.4). Trials were conducted in the morning and individuals were tested only once each day. Individuals were fasted overnight (>16 h) 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 behavior and air temperature were also recorded every 5 min throughout each trial.

2.8. 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 and 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 was 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 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 h. The time between successive blocks of time was determined using the auto-correlation estimation function (stats library in R from Venables and 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. Results

3.1. Energy expenditure via respirometry

Rates of oxygen consumption and other values are presented as mean ± 1 S.D. (Tables 1, 2, and 4). 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. 2). Converted to estimates of daily energy expenditure (DEE_{resp}), the overall average DEE_{resp} was 10,238.5 ± 1647.0 kJ d^{-1} (Table 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).

Table 1
Daily energy expenditure (DEE) estimates for 3-year old female northern fur seals derived from respirometry and doubly labeled water (DLW) 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 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)	10,875 (±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)	–

The average rate of carbon dioxide production ($\dot{V}CO_{2DEE}$) 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. 2). 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.

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} 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 increase between 6 AM and 6 PM and decrease between 6 PM and 6 AM (Fig. 4). 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 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.

3.2. Energy expenditure via doubly labeled water (DLW) method

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 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 differences 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%; Table 1).

3.2.1. 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 $interval^{-1}$; $P = 0.001$; Table 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 ($P = 0.3$; Table 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 ($P = 0.02$).

Table 2
Average (± 1 S.D.) Actiwatch and $PDBA_{xy}$ activity score estimates from six, 3-year 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

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

Average Actiwatch and $PDBA_{xy}$ activity scores separately, over the entire DEE trials, across all individuals and all seasons, were 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 was significant predictors of the average mass-specific $\dot{V}O_{2DEE}$ ($P = 0.07$).

3.2.3. $\dot{V}O_2$ 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 ($P = 0.001$).

Within each of the individual seasonal trials, both 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 these significant results ($P = 0.05$) remained consistent for the majority of the subsets (>74%). The exceptions were 1) Actiwatch and $PDBA_{xy}$ activities 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. 5). 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:

$$\dot{V}O_2 = \left[(C) + 2.2\sin\left(\frac{2(Pi)T}{24}\right) + 0.98(Pi) + (\mathrm{AAR})\right](M) \quad (1a)$$

$$\dot{V}O_2 = \left[(C) + 2.3\sin\left(\frac{2(Pi)T}{24}\right) + 0.99(Pi) + (\mathrm{AAR})\right](M). \quad (1b)$$

The predictive equations 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:

$$\dot{V}O_2 = \left[(C) + 2.3\sin\left(\frac{2(Pi)T}{24}\right) + 0.95(Pi) + (\mathrm{PAR})\right](M) \quad (2a)$$

$$\dot{V}O_2 = \left[(C) + 2.3\sin\left(\frac{2(Pi)T}{24}\right) + 0.97(Pi) + (\mathrm{PAR})\right](M). \quad (2b)$$

The season-specific components for Eqs. (1a)–(1b) and (2a)–(2b) are shown in Table 3. Within both equations, C is a seasonal constant,

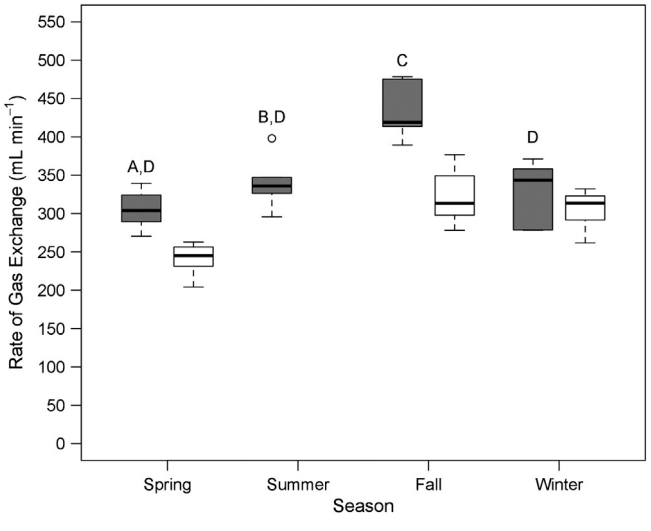

Fig. 2. Average $\dot{V}O_2$ and $\dot{V}CO_2$ throughout the daily energy expenditure trials for 3-year old female northern fur seals. The average $\dot{V}O_{2DEE}$ (gray boxes) and $\dot{V}CO_{2DEE}$ (white boxes) throughout 5-day daily energy expenditure trials are presented on a seasonal basis for 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 ($P = 0.2$).

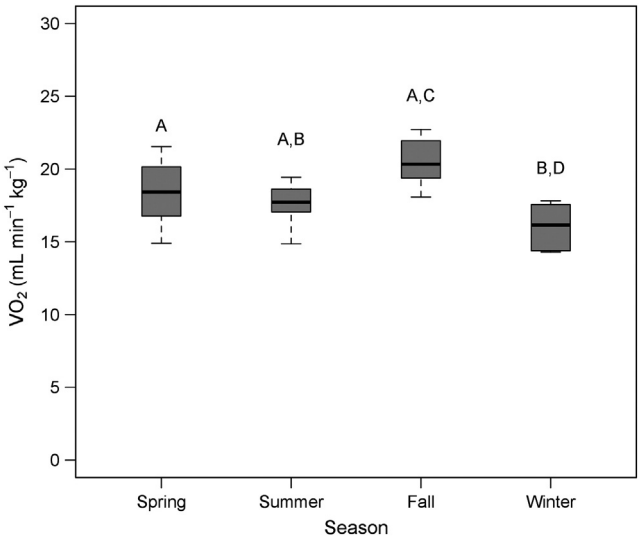

Fig. 3. Average mass-specific $\dot{V}O_2$ throughout the daily energy expenditure trials for 3-year old female northern fur seals. The average mass-specific $\dot{V}O_{2DEE}$ throughout 5-day daily energy expenditure trials is presented on a seasonal basis for six individuals. The average mass-specific $\dot{V}O_{2DEE}$ 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 ($P = 0.7$).

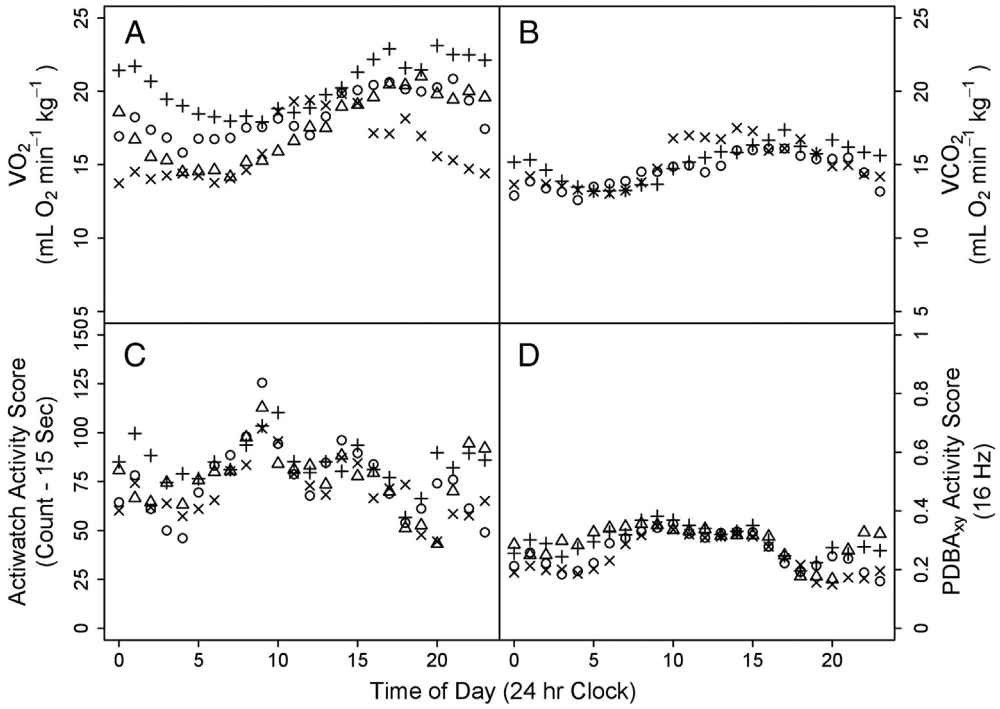

Fig. 4. Hourly changes in $\dot{V}O_2$, $\dot{V}CO_2$, and activity score (Actiwatch and $PDBA_{xy}$) throughout the daily energy expenditure trials for 3-year old female northern fur seals. Average hourly (A) $\dot{V}O_2$, (B) $\dot{V}CO_2$, (C) Actiwatch activity score and (D) $PDBA_{xy}$ activity score estimates throughout the day of six individuals from March 2011 to January 2012 during 5-day daily energy expenditure trials.

T represents the time of day on a 24 hour 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 4, Figs. 6 and 7).

4. Discussion

4.1. 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 (Nagy et al., 1999;

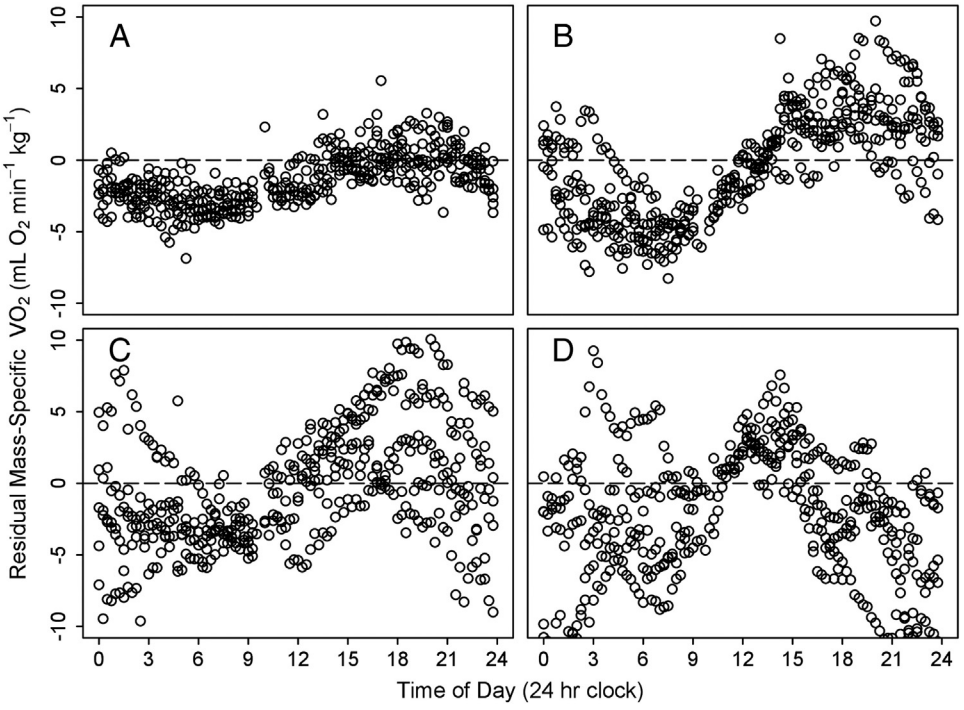

Fig. 5. Model validation, 15-minute time interval — Philips' Actiwatch, no time of day correction. The difference (residual) in the estimated mass-specific $\dot{V}O_2$ (mL O_2 min^{-1}) predicted from the Philips' tri-axial Actiwatch acceleration data logger from the measured mass-specific $\dot{V}O_2$ (mL O_2 min^{-1}) for test northern fur seal "ME08" throughout the course of the day, using the 15-minute 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.

Table 3
The season-specific components of the predictive equations (Eqs. (1a)–(1b) and (2a)–(2b)) 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 ($PDBA_{xy}$)

Speakman, 1997). However, these estimates of field metabolic rates and 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).

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%, mostly attributable to differences in the way model parameters were combined. 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).

Table 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-minute time intervals are shown in Figs. 6 and 7.

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%

In our study, the three estimates of the DEE_{DLW} that corresponded most closely to the measured rates of oxygen consumption (DEE_{resp}) of our fur seals 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 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 gray 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 that 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%) isotopes (compared to an isotope depletion of >35% in our 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.

It is important to note that, no matter which model was chosen to calculate the DEE_{DLW}, the accuracy of our estimates was 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, significant 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. There is no reason to believe that this was the result of a violation to any of the 6 major assumptions of the DLW method, as described thoroughly by Speakman (1997), and certainly not on a seasonal basis.

A seasonal change in the respiratory quotient (RQ) appears to have contributed to some of the observed seasonal differences between 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 (equilibrium and kinetic) and the

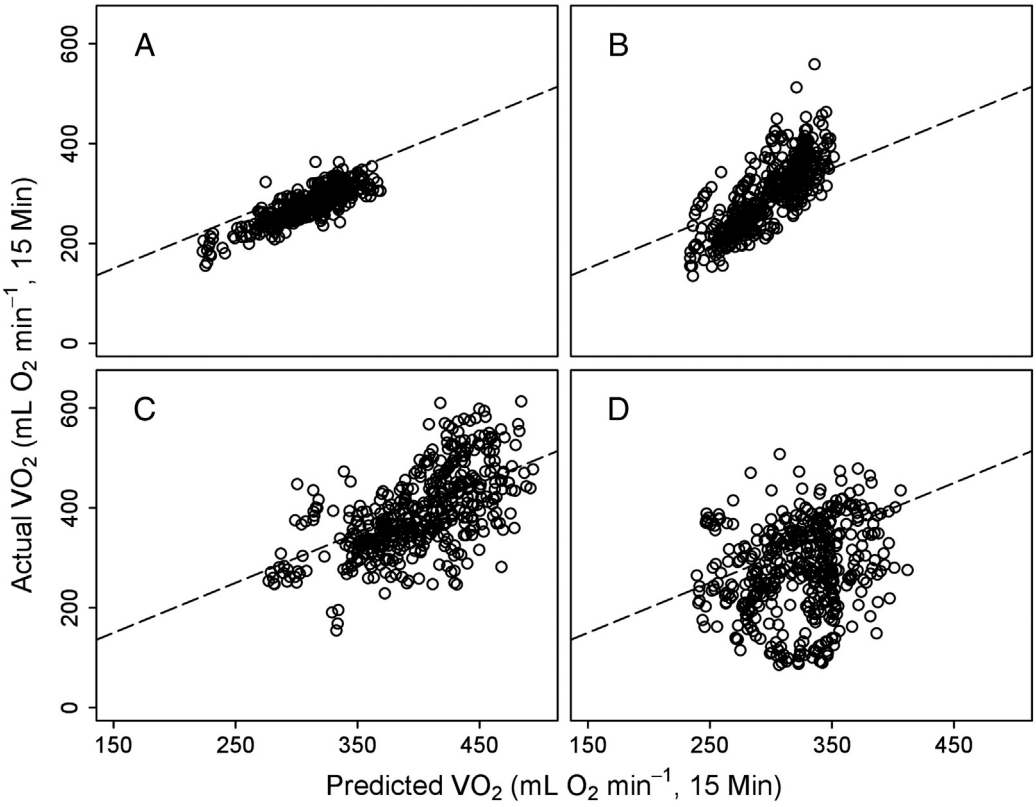

Fig. 6. Model validation, 15-minute time interval — Philips' Actiwatch, with time of day correction. The measured $\dot{V}O_2$ (mL O_2 min^{-1}) for test northern fur seal "ME08" compared against the estimated $\dot{V}O_2$ (mL O_2 min^{-1}) predicted from the Philips' tri-axial Actiwatch acceleration data logger using the 15-minute time interval (see Eq. (1a) and Table 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 $\dot{V}O_2$.

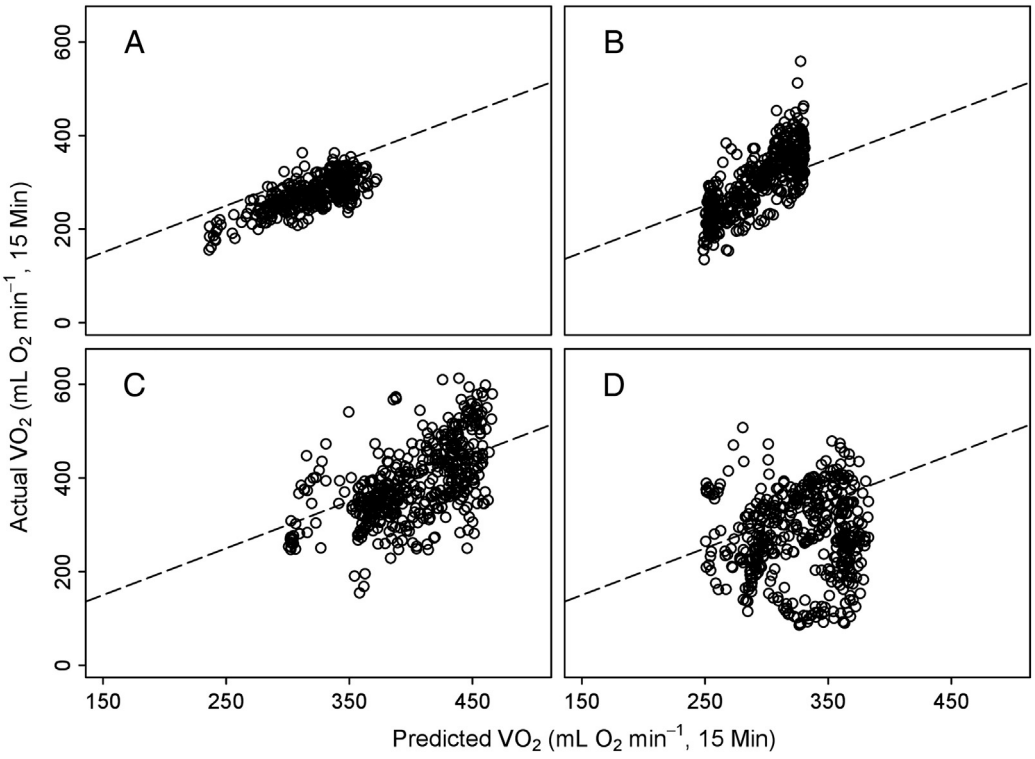

Fig. 7. Model validation, 15-minute time interval — Little Leonardo bi-axial acceleration data logger ($PDBA_{xy}$), with time of day correction. The measured rates of oxygen consumption (mL O_2 min^{-1}) for test northern fur seal "ME08" compared against the estimated rates of oxygen consumption (mL O_2 min^{-1}) predicted from Eq. (2a) and Table 3 using the $PDBA_{xy}$ activity scores and the 15-minute 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 $\dot{V}O_2$.

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). 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% (LeFebvre, 1964; Lifson et al., 1955; Speakman, 1997; Tiebout and Nagy, 1991). However, the largest and smallest average difference between the DEE_{DLW} and DEE_{resp} estimates occurred during the summer (air = 12.6 °C; water = 16.3 °C) and fall (12.8; 15.2 °C) 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 given that 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 from October to June (Bigg, 1990; Gentry, 1998; Kenyon and Wilke, 1953; Nagy, 1983; 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).

4.2. 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 other energetic functions by a factor of 10 or more (Darveau et al., 2002; Wilson et al., 2006). However, the lack of significance in our study may have been due to the fact that the relationship between activity and $\dot{V}O_2$ may be non-linear (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 over an extended period, 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). Overall, significant predictive relationships between activity and energy expenditure (averaged over both 15 and 60 min intervals) were found and were significantly improved when season, time of day and RMR were incorporated into the 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 relatively short periods of time on a treadmill, during specific times of year (Green et al., 2009; Halsey et al., 2009a, 2009b; Wilson et al., 2006). Additionally, none of these validation studies appear to have accounted for circadian oscillators that regulate the day–night cycle of metabolic and behavioral processes, or the effects of photoperiod on the annual rhythm of energy metabolism (Warner et al., 2010).

In essence, incorporating both season (with an independent constant) and time of day into our predictive models corrected for changes in zero-activity metabolism that were 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 "non-active" metabolic processes such as thermoregulation and 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 sea 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. The improved accuracy that resulted from lengthening the sampling interval is likely due to the averaging or smoothing of fine scale peaks in the activity level 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 suggest 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 (Enstipp et al., 2011; Halsey et al., 2009a), but we purposely designed our study to incorporate as little human 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 was 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), which may have improved our predictive ability (although see Halsey et al., 2009a).

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*) uses 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 measuring 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 expenditure (such as during the 8 months required for northern fur seals to complete their annual pelagic migration), our study indicates that 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 was 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 our study), which provide acceleration data in each axis. In general, $PDBA_{xy}$ has shown potential with regard 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.

5. 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 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.

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