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Environment and feeding change the ability of heart rate to predict metabolism in resting Steller sea lions (*Eumetopias jubatus*)

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Abstract The ability to use heart rate (fh) to predict oxygen consumption rates ($\dot{V}_{O_2}$) in Steller sea lions and other pinnipeds has been investigated in fasting animals. However, it is unknown whether established fh:$\dot{V}_{O_2}$ relationships hold under more complex physiological situations, such as when animals are feeding or digesting. We assessed whether fh could accurately predict $\dot{V}_{O_2}$ in trained Steller sea lions while fasting and after being fed. Using linear mixed-effects models, we derived unique equations to describe the fh:$\dot{V}_{O_2}$ relationship for fasted sea lions resting on land and in water. Feeding did not significantly change the fh:$\dot{V}_{O_2}$ relationship on land. However, Steller sea lions in water displayed a different fh:$\dot{V}_{O_2}$ relationship after consuming a 4-kg meal compared with the fasting condition. Incorporating comparable published fh:$\dot{V}_{O_2}$ data from Steller sea lions showed a distinct effect of feeding after a 6-kg meal. Ultimately, our study illustrated that both feeding and physical environment are statistically relevant when deriving $\dot{V}_{O_2}$ from telemetered fh, but that only environment affects the practical ability to predict metabolism from fh. Updating current bioenergetic models with data gathered using these predictive fh:$\dot{V}_{O_2}$ equations will yield more accurate estimates of metabolic rates of free-ranging Steller sea lions under a variety of physiological, behavioral, and environmental states.

Keywords Steller sea lion · Heart rate · Oxygen consumption · Heat increment of feeding · Energetics · Otariid

Abbreviations

M_b	Body mass (kg)
V_{O_2}	Oxygen consumption
$\dot{V}_{O_2}$	Oxygen consumption rate (ml O_2 min^{-1})
$s\dot{V}_{O_2}$	Mass-corrected oxygen consumption rate (ml O_2 min^{-1} $kg^{-0.75}$)
fh	Heart rate (beats min^{-1})
fh_{inst}	Instantaneous heart rate (beats min^{-1})
Dry_{fasted}	Fasted, resting in dry metabolic chamber
$Dry_{4kg/6kg}$	Fed 4 or 6 kg in metabolic chamber
$Water_{fasted}$	Fasted, resting in swim mill
$Water_{4kg/6kg}$	Fed 4 or 6 kg in swim mill
$Water_{ow}$	Resting at the surface in open water (fed ≤ 0.36 kg)
$Water_{comp}$	Composite baseline for water trials (water$_{ow}$ + water$_{fasted}$)

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Introduction

Estimates of activity-specific energy expenditure are critical parameters for determining the overall ecological impact of predators, as well as quantifying the effects of altered behavior on energy or prey requirements. Behavior-specific bioenergetic models have been used to understand the potential role of ecosystem and behavioral changes on

marine mammal populations (Mohn and Bowen 1996; Olesiuk 1993; Stenson et al. 1997; Winship et al. 2002). However, few empirical behavior-specific energy estimates are available to parameterize such models.

Estimates of energy expenditure in free-ranging marine homeotherms have been traditionally measured using two methods. The first, doubly labeled water provides a mean estimate of metabolism over a few days (4–6 days in marine mammals, Costa 1987; Kam and Degen 1997; Roberts 1989; Speakman and Krol 2005), but presents logistical and financial challenges. Although error estimates for doubly labeled water vary among species, research on otariids suggests that doubly labeled water may overestimate field metabolic rate by 3–36% (Boyd et al. 1995; Costa and Trillmich 1988).

A second technique—the heart rate (fh) method—uses predictive equations to estimate energy expenditure from instantaneous measures of heart rate. This method can provide estimates of energy expenditure for specific activities on a much finer time scale and for longer periods of time than doubly labeled water (Boyd et al. 1995, 2004; Butler et al. 2004; Ponganis 2007; Woakes et al. 1995), but does require retrieval of fh dataloggers and derivation of species-specific predictive equations. Heart rate has been used to estimate energy expenditure in several aquatic homeotherms including penguins, seals, and Steller sea lions, *Eumetopias jubatus* (Boyd et al. 1995; Fahlman et al. 2004; Froget et al. 2002; McPhee et al. 2003; Williams et al. 1991) and has the potential to provide estimates of activity-specific energy expenditure in free-ranging animals.

The concept that recorded heart rate (fh) can be used to estimate rates of oxygen consumption ($\dot{V}_{O_2}$, an accepted proxy for energy expenditure) is based upon Fick's (1870) relationship: $\dot{V}_{O_2} = (Ca_{O_2} - C\bar{v}_{O_2}) \times V_s \times$ fh, where V_s is stroke volume, Ca_{O_2} is the arterial oxygen content, $C\bar{v}_{O_2}$ is the oxygen content of the mixed venous blood, and the function $Ca_{O_2} - C\bar{v}_{O_2}$ represents the amount of oxygen extracted from the tissues. The application of this technique relies on the assumption that an increase in fh is the primary method that animals employ to respond to increased oxygen consumption rate, and that $Ca_{O_2} - C\bar{v}_{O_2}$ and V_s stay constant or vary proportionally to heart rate.

Field application of the heart rate method requires prior species-specific calibrations defining the relationship between fh and $\dot{V}_{O_2}$ (e.g., Butler et al. 1992). Traditionally, these initial fh:$\dot{V}_{O_2}$ studies have been conducted under uniform physiological and environmental conditions to limit experimental and statistical variation. For example, calibration studies have been conducted on marine mammals that were fasting to eliminate the possible confounding effect of digestion (Butler 1993; Fahlman et al. 2004; Hurley and Costa 2001; McPhee et al. 2003;

Williams et al. 1991). However, the relationships between fh and $\dot{V}_{O_2}$ in marine mammals and birds can potentially be affected by such variables as environment (land or water) and digestive state (fed or fasted). Specifically, V_s, $Ca_{O_2} - C\bar{v}_{O_2}$, or blood flow (which could influence $Ca_{O_2} - C\bar{v}_{O_2}$) are likely affected by both feeding state (at least in dogs and primates, Vatner et al. 1970, 1974) and submergence (for a review, see Butler and Jones 1997).

Feeding has the potential to change the fh:$\dot{V}_{O_2}$ relationship via the heat increment of feeding (HIF), which represents the loss of energy during chemical and physical digestion (Blaxter 1989; Secor 2009). It is manifested as an observable increase in $\dot{V}_{O_2}$—the extent and duration of which is influenced by the composition and size of the meal as demonstrated in pinnipeds (Markussen et al. 1994; Rosen and Trites 1997). A change in $\dot{V}_{O_2}$ due to consuming a meal without a parallel rise in fh would result in different fh:$\dot{V}_{O_2}$ relationships for feeding and fasting states. While some captive studies have indirectly incorporated un-quantified feeding as a positive reinforcement training tool, none have directly compared these results with fasted data (Boyd et al. 1999; Williams et al. 1993). According to recent work with trained Steller sea lions fh can predict $\dot{V}_{O_2}$ under fasted conditions (McPhee et al. 2003), but a different fh:$\dot{V}_{O_2}$ relationship may exist when animals are feeding. However, this preliminary investigation was supplemental to the main study (i.e. it only included one animal) and therefore lacked the necessary scope to fully explore the possible effects of digestive state on metabolism.

The fh:$\dot{V}_{O_2}$ relationship may also be affected by the physical environment. One notable difference between water and air is their respective thermal properties. Water has a greater specific heat capacity relative to air and could therefore have a greater impact on thermoregulatory costs that would, in turn, influence metabolic rate. Marine mammals also physiologically respond to submersion and diving in water with a suite of adaptations that include a decrease in heart rate (bradycardia), apnea, and vasoconstriction. Further, V_s (Blix et al. 1983; Ponganis et al. 1990; Sinnett et al. 1978; Zapol et al. 1979), O_2 depletion rate (Meir et al. 2009), and blood flow (Davis et al. 1983b; Stone et al. 1973) may change during diving, during shallow submersion, or in anticipation of diving. A change in any of these parameters under any of the above conditions could affect the individual components of Fick's equation, and therefore the predictive relationship between fh and $\dot{V}_{O_2}$.

Despite the current use of fh to predict $\dot{V}_{O_2}$ of marine endotherms in the wild, it is still not clear whether the heart rate method works for otariids across different feeding states and environmental conditions. We therefore sought to investigate the effect of the physical environment (land

or water) and feeding (fasting, 4 or 6 kg meals) on the fh:$\dot{V}_{O_2}$ relationship in resting, trained Steller sea lions.

Materials and methods

Data collection

Seven female Steller sea lions ranging in age from 4 to 11 years participated in our study from April to September 2008 (Table 1). Prior to the experiments, all animals were fed a diet of herring (*Clupea pallassi*) supplemented with vitamin tablets. Animals were fasted overnight, and then weighed each morning on a platform scale (±0.5 kg). All animal procedures were conducted under the authority of University of British Columbia Animal Care Protocol (A07-0208 and A07-0413), Department of Fisheries and Oceans Canada (MML 2007-0001) and the Vancouver Aquarium. All animal work was conducted voluntarily under trainer control.

The experimental design consisted of seven trial types that varied by physical environment (dry, water, or open water) and feeding state (fed or fasted). Fasted measurements were taken from sea lions resting in a dry metabolic chamber (dry$_{fasted}$), in a swim mill (water$_{fasted}$), or in a respirometry dome floating on the ocean surface (open water trials, water$_{ow}$). Fasted trials were compared with those in which animals were fed 4 or 6 kg of herring before entering either the metabolic chamber (dry$_{4kg}$, dry$_{6kg}$) or the swim mill (water$_{4kg}$, water$_{6kg}$).

Experiments were conducted on two groups of Steller sea lions raised in captivity and previously trained to use all experimental apparatus. All trials except those conducted in open water included four female sea lions (F03AS, F00ED, F03WI, F03RO) housed at the Vancouver Aquarium (BC, Canada). These animals were held in outdoor enclosures with access to seawater pools and haulout space. Each animal completed a single replicate of each of six

Table 1 Details of experimental trials with Steller sea lions

Animal ID	Age (year)	Trial (2008)	Mass		N
			(kg)	(±SD)	
F03AS	4	7 Apr–21 Jul	157	(3.6)	6
F00ED	7	8 Apr–23 Jul	165	(2.1)	4
F03WI	4	24 Apr–22 Jul	135	(2.7)	6
F03RO	4	9 Apr–15 Jul	142	(3.9)	6
F97SI	11	1 Aug–16 Sep	218	(4.4)	5
F00BO	11	7 Aug–16 Sep	145	(5.1)	5
F97HA	8	7 Aug–4 Sep	172	(0.6)	5

Age, trial dates, body mass (kg ± SD), and number of trials (*N*) per animal are presented

trial types in random order on separate days at the Vancouver Aquarium over a period of several weeks (Table 1). Due to difficulties with behavioral cooperation, animal F00ED did not complete the water$_{4kg}$ trial, and the dry$_{4kg}$ trial for this animal was not useable due to poor quality heart rate data ($n = 4$ animals, 22 trials provided useable data). The water$_{ow}$ trials were conducted with a second group of sea lions as they rested at the ocean surface at the UBC Open Water Research Laboratory (Port Moody, BC, Canada). Three female Steller sea lions (F97SI, F97HA, F00BO; Table 1) were housed in a specially designed floating pen that provided access to seawater and haulout space (for a full description see Hastie et al. 2006, 2007). Each sea lion completed six water$_{ow}$ trials on separate days ($n = 3$ animals, 15 trials provided useable data).

Measurement of heart rate

Steller sea lions were outfitted with subcutaneous heart rate electrodes while under veterinary-supervised gas anesthesia (0–5% Isoflurane). The heart rate monitoring system consisted of (1) a heart rate datalogger (HTR, Wildlife Computers, Redmond, WA, USA) that recorded the inter-beat-interval (IBI, or R–R peak intervals of the electrocardiogram, ECG) and (2) a heart rate transmitter (HRX, Wildlife Computers) with two 26-gauge wire leads, ~32 in. in length. To reduce infection risk, 30-gauge 99.9% pure silver Teflon-coated wire (Grass Technologies, Longueuil, QC, Canada) was spliced to the terminal end of the electrode leads. These were sterilized in glutaraldehyde for a minimum of 30 min prior to each procedure (Metricide 28, Metrex, VaxServ, Scranton, PA, USA).

The placement of the heart rate recording equipment was designed for single-use deployment under anesthesia that would permit recovery of all equipment (including subcutaneous electrode wires) under trainer control after each trial. The transmitter and datalogger were carried in a pocket on a custom-fit harness worn by the animals. Electrodes were inserted subcutaneously, through neoprene circles glued to the fur, by bending the terminal end of the Teflon wire and inserting the stripped end (0.5–1.0 cm) into a 20-gauge hypodermic needle. Electrodes were placed caudal to the front flippers at the level of the heart, approximately 25 cm lateral of the dorsal midline. The wires were secured to the neoprene circles and to the fur along the spine with additional small squares of neoprene (Fig. 1b, c). Animals were allowed to completely recover from anesthesia (~20 min) in a close-contact cage or dry area before commencing the trial.

Measurement of oxygen consumption

$\dot{V}_{O_2}$ was measured using open-circuit gas respirometry as previously described for Steller sea lions (Rosen and Trites
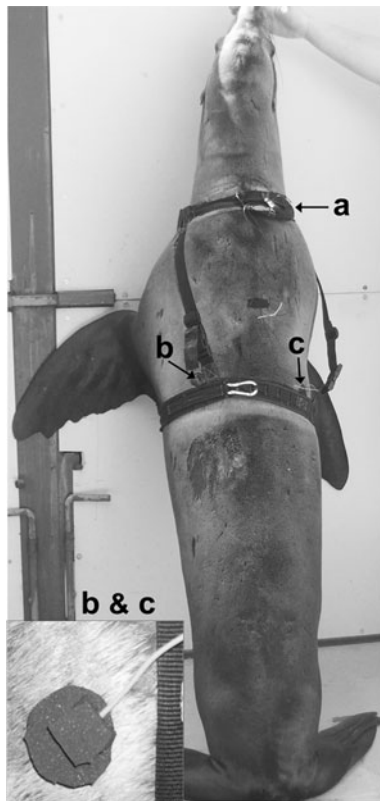
Fig. 1 Photograph of heart rate apparatus, including placement of the electrodes (**b**, **c**) and harness pocket (**a**) containing datalogger. The *insert* shows an enlarged image of the electrodes (**b**, **c**). The Steller sea lion was approximately 2-m long

1997). Fractional oxygen and carbon dioxide concentrations within a desiccated subsample of the excurrent airstream were measured using Sable System FC-1B and CA-1B analyzers, coupled to a 500H Mass Flow Generator and Controller (Sable Systems, Las Vegas, NV, USA). Fractional gas concentration readings were corrected for electronic drift against ambient air before and after each trial. Barometric pressure, relative humidity, and expired air temperature were also recorded (Airguide Instruments, Chicago, IL, USA) to correct readings to STPD.

For the 'dry' trials, $\dot{V}_{O_2}$ was measured in a metabolic chamber ($\sim$1,825 L), equipped with a camera to allow visual monitoring of behavior. Air was drawn through the chamber at a constant rate of 200 L min^{-1}. The excurrent airstream was continuously subsampled, and averaged every 3 s (water and dry trials, Sable Data Acquisition System, Sable Systems). Average air temperature inside the chamber ranged between 10.8 and 21.5°C (mean 16.0 $\pm$ 3.1°C SD, $n = 11$ trials). Animals were able to turn around inside the chamber, but not exercise.

For the water$_{fasted}$, water$_{4kg}$, and water$_{6kg}$ trials, $\dot{V}_{O_2}$ was measured in a swim mill (3.2 m $\times$ 1.8 m $\times$ 1.0 m; no

water current was applied) using the same flow meters and gas analysis system as for 'dry' trials. The animals were only able to surface under a transparent 120 L Plexiglas dome. The animals were kept in a smaller (1.60 m $\times$ 0.89 m $\times$ 0.84 m) inner cage to prevent tangling of the electrode wires, but which was large enough to allow them to turn around or rest on the bottom of the swim mill. Average water temperature inside the swim mill ranged between 9.0 and 13.6°C (mean 12.2 $\pm$ 1.3°C SD, $n = 11$ trials).

Water$_{ow}$ trials were either conducted at a dive site next to the animals' holding pen or the animals were transported to a nearby dive site in a 22-ft research boat. A second boat towing a floating barge carried the respirometry equipment to the dive site. The barge had a square hole in the middle containing a cage (1.52 m $\times$ 1.52 m $\times$ 2.5 m) and floating transparent Plexiglas respirometry dome (100 L). An open-circuit gas respirometry system similar to the one employed at the Vancouver Aquarium was used with the same respirometry calibrations and settings unless specified differently. Air was drawn through the respirometry dome at 475 L min^{-1}. The excurrent airstream was averaged every 0.5 s to capture the quick changes in $\dot{V}_{O_2}$ observed after surfacing from dives for a concurrent study (Young 2010).

Fasted trial protocol

The dry$_{fasted}$ and water$_{fasted}$ (0 kg fed) trials provided physical environment-specific fh:$\dot{V}_{O_2}$ relationships for comparison with feeding trails. Fasting trials were 90 min maximum duration, which was a reasonable time for an animal to remain calm without food reinforcement and reliably re-enter the apparatus for future trials. In one instance F00ED was fed 0.52 kg of squid (*Loligo opalescens*) to distract her while a transmitter was adjusted. It is unlikely that this event confounded results given the relatively minor and delayed metabolic rate effects of squid digestion in Steller sea lion (Rosen and Trites 1997, 1999). During the water$_{fasted}$ trial for F03AS, the heart rate electrodes became displaced and the trial was terminated early (mean duration for $n = 8$ fasted trials: 81 $\pm$ 16 min SD).

For water$_{ow}$ trials, the sea lions rested for approximately 6–10 min in the respirometry dome while fh and $\dot{V}_{O_2}$ were recorded. Animals remained at the surface in the respirometry dome longer if steady state $\dot{V}_{O_2}$ values were not observed. Animals were fed 0.02 kg herring pieces (max 0.36 kg per resting trial, mean 0.22 $\pm$ 0.09 kg SD, $n = 15$ trials) through a delivery tube in the respirometry dome to facilitate cooperation. Water$_{ow}$ trials took less than $\sim$15 min to complete; therefore it was unlikely that results were influenced by HIF (Rosen and Trites 1997).

Feeding trial protocol

A feeding trial had two phases of data collection: (1) measurement of pre-feed (fasted) $\dot{V}_{O_2}$ and fh for 20–25 min ($\dot{V}_{O_2 pre-feed}$) and (2) continuous measurement of post-feeding $\dot{V}_{O_2}$ and fh for 4–4.5 h ($\dot{V}_{O_2 digesting}$). Between the two phases, the animals were fed for 5–10 min, and the $\dot{V}_{O_2}$ equipment was paused since the integrity of the flow-through system was breached. Comparison of the $\dot{V}_{O_2 pre-feed}$ data to the fasted control (dry$_{fasted}$ or water$_{fasted}$) for each animal confirmed that any observed changes in fh or $\dot{V}_{O_2}$ were due to digestion rather than other factors (e.g., that measurements were taken on different days). The duration of the feeding phase of trials was selected to capture the peak in HIF, which occurs at approximately 4 h following a 4-kg meal of herring (Rosen and Trites 1997). Post-feed data collection period varied slightly among trials due to electrode performance (mean = 225 ± 30 min SD, $n = 14$ fed trials).

Data analysis

Heart rate

Data downloaded from the heart rate datalogger were analyzed with Microsoft Excel and R 2.9.2 (R Core Development Team 2009). First, inter-beat-intervals (IBI) were converted to instantaneous heart rate (fh$_{inst}$) using the following equation: $\text{fh}_{inst}(\text{beats min}^{-1}) = \frac{60\,\text{s}}{\text{IBI}}$.

We applied the following algorithms to systematically remove any fh$_{inst}$ values that were artifacts of muscle or wire movement. Field comparisons of the same model heart rate datalogger to a portable ECG on harbor seals have shown that artificial beats caused by sudden movement are recorded as 206.9 beats min^{-1} or often >230 beats min^{-1} (Greaves et al. 2005). Furthermore, frequency histograms of all fh$_{inst}$ showed that 79% of all fh$_{inst}$ in our study were ≤240 beats min^{-1}; therefore, all fh$_{inst}$ values of 206.9 or >240 beats min^{-1} were eliminated. These data likely resulted from electromyographic noise or electronic noise from wire movement. Second, fh$_{inst}$ values were removed if the target cell was ≥ ±1 SD from an 11-point mean. Sections with large amounts of apparent noise were manually examined as necessary. For the fasting (∼90 min) and feeding trials (∼240 min) in the swim mill and metabolic chamber, fh$_{inst}$ values were averaged in consecutive 5-min intervals to yield mean fh (beats - min^{-1}). For the water$_{ow}$ trials fh$_{inst}$ was averaged over the entire 2-min resting period (prior to a dive for a concurrent study, Young 2010). Although empirical data from some terrestrial mammals show that fh may decrease with increasing body mass (M_b), regressions derived for otariids (California sea lions, northern fur seals) were not significant (Castellini and Zenteno-Savin 1997). Therefore, we did not mass-correct fh data.

Oxygen consumption

Oxygen consumption data was analyzed using Datacan Data Analysis software (V 1.0.24; Sable Systems Inc., Las Vegas, NV). Rates of oxygen consumption were calculated as per Withers (1977, using Eq. 3b) as previously described (Rosen and Trites 1997). For all of the trials except water$_{ow}$, $\dot{V}_{O_2}$ was averaged in Microsoft Excel over the same 5-min intervals as was fh data. The synchronization process also accounted for the 30-s lag time for air traveling between the respirometry chamber or dome and the gas analyzers. For the water$_{ow}$ trials, $\dot{V}_{O_2}$ was averaged over the same last 2 min of the resting period as was fh, after $\dot{V}_{O_2}$ had reached steady-state. $\dot{V}_{O_2}$ was mass-corrected ($s\dot{V}_{O_2}$), and presented as ml O_2 min^{-1} kg$^{-0.75}$. There has been considerable debate over whether metabolic rate scales with body mass (Brown and West 2005; Savage et al. 2007; White and Seymour 2005). We chose to mass-correct $\dot{V}_{O_2}$ using an exponent of 0.75 to account for the M_b range (134–217 kg) of the sea lions in our study, and to facilitate comparisons with other studies.

Statistical analysis

Data from each animal within a trial and data from each animal across trial types was treated as a repeated measures set using linear mixed-effects (LME) models in R 2.9.0 (nlme library from Pinheiro and Bates 2000). LME models characterize individual variation relative to the mean of the population while considering the correlation between repeated measurements within and among animals. All models were run using the maximum likelihood method, and the slope and intercept were allowed to vary for each animal during model optimization.

Animal ID was treated as a random effect for all models, which permitted applying inferences from the sample population to the free-ranging population. Fixed effects that were explored included the amount of food fed (0, 4, 6 kg) and the environment (dry, water, open water). The 5-min averages for time-series data during the trials minimized autocorrelation of measurements made on the same animal.

For each analysis, the best model in terms of fixed effect factors and homogeneity of variance corrections was determined using an ANOVA. To clarify, ANOVA serves dual purposes for LME models. An ANOVA executed on a single model generated a conditional F test to determine the significance of model slope, intercept, and fixed effects. An ANOVA performed on two nested models (the fixed effect model hierarchically nested within the model without fixed

effects) produced likelihood ratio tests (LRT) that compared the two models. All models presented only had one fixed effect applied at a time; therefore, for all LRT tests $df = 1$. Statistical significance was set at $\alpha = 0.05$.

Incorporation of McPhee et al. (2003) data

An additional fh and $\dot{V}_{O_2}$ dataset collected on Steller sea lions by McPhee et al. (2003) under similar experimental conditions was integrated into our dataset to investigate whether a larger sample size that included different animals and both genders would affect our conclusions. Only comparable data were incorporated into our dataset. Briefly, this earlier study examined the relationship between fh and $\dot{V}_{O_2}$ in four Steller sea lions aged 1.3–3 years housed at the Vancouver Aquarium (M97TI, M97KO, F97HA, F97SI, McPhee et al. 2003). Data were collected under varied environment and activity conditions (dry inactive, water active, water inactive) using an open circuit respirometry system similar to that described here, and custom subcutaneous electrodes for the measurement of fh. Trials were conducted in the same metabolic chamber and swim mill as for our study, but water current was applied in some water trials (water active). At the end of McPhee et al.'s focal study, preliminary data exploring the potential effects of feeding were conducted on a single animal (M97TI) for water trials only ($n = 6$ trials). M97TI entered the swim mill 32–73 min after eating 6 or 12 kg of herring, and $\dot{V}_{O_2}$ and fh were measured until $\sim$3.5–4.25 h after ingestion.

The statistical analysis performed by McPhee et al. produced an overall mean regression for the four test sea lions that differed from ours (partly due to a lack of repeated measures control), making direct comparisons difficult. The $\dot{V}_{O_2}$ data were therefore converted from the original units of ml O_2 h^{-1} kg$^{-0.60}$ to ml O_2 min^{-1} kg$^{-0.75}$ using raw values of total $\dot{V}_{O_2}$ (ml h^{-1}) and body mass (M_b). The log transformation of $\dot{V}_{O_2}$ was also removed as Q–Q (quantile) plots showed that data were normally distributed. The raw data from McPhee et al. was re-analyzed using the same LME models and repeated measures design in R 2.9.0, as described above. Animal was treated as a random factor, and gender, amount fed, and trial type were tested as fixed factors for each model.

Confidence intervals (95% CI) for selected regression models were calculated by bootstrapping (R Core Development Team 2009; Whitlock and Schluter 2009). The bootstrapping procedure was repeated 1,000 times per model (while conserving the structure of the original model), and the ordered 24th and 976th bootstrapped replicates were plotted to represent the 95% CI for a model.

The error associated with the predictive equations was not constant, but rather increased with distance from the mean values. However, many studies evaluating other techniques to estimate energy expenditure report a single, average error (i.e. Boyd et al. 1995). To facilitate comparison with other methods, we derived a single representative value of the error associated with the predictions, which we termed the average residual. This was calculated by dividing the mean absolute residual value of a specific model by the median predicted $\dot{V}_{O_2}$ value.

Results

Fasted relationships

Mean fh for dry$_{fasted}$ trials ranged from 74 to 123 beats min^{-1} and $s\dot{V}_{O_2}$ ranged from 20 to 77 ml O_2 min^{-1} kg$^{-0.75}$. Heart rate significantly predicted $s\dot{V}_{O_2}$ in fasted animals resting on land ($F_{1,60} = 11.03$, $P = 0.002$; Table 2: Eq. 1; Fig. 2a).

Average fh and $s\dot{V}_{O_2}$ for Steller sea lions resting in water were similar regardless of type of water environment (water$_{ow}$ vs. water$_{fasted}$) and ranged from 57 to 108 beats min^{-1} and 18 to 47 ml O_2 min^{-1} kg$^{-0.75}$. The fh:$\dot{V}_{O_2}$ relationship for water$_{ow}$ trials did not differ from water$_{fasted}$ data (LRT = 0.05, $P = 0.82$; Fig. 2b). These trials were therefore combined to create a composite predictive equation for animals resting in water (water$_{comp}$, Table 2: Eq. 2). This equation encompassed a wider environmental scope and also had greater statistical power due to increased sample size.

The relationship between fh and $s\dot{V}_{O_2}$ during dry$_{fasted}$ trials was significantly different than when measured under apparently similar physiological conditions in water (LRT = 14.6, $P = 0.001$ compared to water$_{comp}$). This suggests that two separate environment-specific equations are needed to accurately predict $\dot{V}_{O_2}$ in fasted animals (Table 2: Eqs. 1, 2). These unique equations (water$_{comp}$ and dry$_{fasted}$) were employed in subsequent comparisons between fed and fasted states for particular physical environments.

Effect of feeding

Mean fh and $s\dot{V}_{O_2}$ for dry$_{4kg}$ trials ranged from 53 to 128 beats min^{-1} and from 18 to 78 ml O_2 min^{-1} kg$^{-0.75}$. Heart rate and oxygen consumption distributions for dry$_{6kg}$ were similar to dry$_{4kg}$ and ranged from 54 to 124 beats min^{-1} and from 19 to 79 ml O_2 min^{-1} kg$^{-0.75}$. Heart rate predicted $s\dot{V}_{O_2}$ of animals after consuming a 4- or 6-kg meal on land, and meal size did not affect this predictive equation (LRT = 0.39, $P = 0.53$). Furthermore, the relationship between fh and $s\dot{V}_{O_2}$ for dry$_{fasted}$ trials was not different than for either dry$_{4kg}$ or dry$_{6kg}$ trials (dry$_{6kg}$: LRT = 3.0, $P = 0.08$; dry$_{6kg}$: LRT = 1.4, $P = 0.23$), or

Table 2 Equations for selected models ($\dot{V}_{O_2} = a \cdot \text{fh} + b$) demonstrating the linear relationship between heart rate (fh, beats min^{-1}) and oxygen consumption ($\dot{V}_{O_2}$, ml O_2 min^{-1} $\text{kg}^{-0.75}$) of fasted and fed Steller sea lions (food in kg)

Eqn.	Fig.	Food (kg)	Slope (a)	(±SE)	Intercept (b)	(±SE)	Slope P value	Intercept P value	Model description
1	2a	0	0.53	(0.16)	−3.31	(18.01)	0.002	<0.001	Dry$_{\text{fasted}}$
2	2b	<0.36	0.20	(0.07)	16.7	(4.96)	0.005	<0.001	Water$_{\text{comp}}^{a}$
3	3	0, 4, 6	0.31	(0.12)	19.2	(13.91)	0.009	<0.001	Dry$_{\text{all}}$
4	4a	4	0.21	(0.08)	16.4	(6.90)	0.011	<0.001	Water$_{\text{4kg}}$
5	4b	0, 6	0.17	(0.03)	19.4	(3.11)	<0.001	<0.001	Water$_{\text{6kg}}$ + water$_{\text{comp}}$ (+food NS factor)
6	4c	0 or 6					<0.001	<0.001	Water$_{\text{6kg+McPhee}}$ + water$_{\text{comp+McPhee}}$
			0.36	(0.06)	13.9	(8.41)			Water$_{\text{6kg+McPhee}}$
			0.36	(0.06)	11.7	(6.02)			Water$_{\text{comp+McPhee}}$
							0.033	<0.001	Water$_{\text{+McPhee}}$
7a		4	0.13	(0.06)	24.5	(6.05)			4 kg
7b		6	0.13	(0.06)	23.0	(6.43)			6 kg
7c		12	0.13	(0.06)	23.6	(6.61)			12 kg
8		4, 6, 12	0.12	(0.06)	24.5	(5.54)	0.030	<0.001	Water$_{\text{+McPhee}}^{a}$ (4, 6, 12 mixed)
9		0, 4, 6	0.32	(0.10)	17.0	(11.63)	<0.001^{a}	<0.001^{a}	Dry$_{\text{all+McPhee}}^{a}$

Regressions were derived using mixed-effects linear models within a repeated measures framework. Model parameters include slope (±SE), intercept (±SE), and P values (F test). Figure references are given for equations where applicable, and model descriptions are defined in the list of abbreviations

[a] Recommended models for field application

when data from the dry$_{\text{4kg}}$ and dry$_{\text{6kg}}$ trials were combined ($F_{1,373} = 0.98$, $P = 0.32$). Ultimately, a single linear equation was generated to predict the $s\dot{V}_{O_2}$ of animals that were fasted or fed on land (dry$_{\text{all}}$, $F_{1,374} = 6.9$, $P = 0.009$; Table 2: Eq. 3; Fig. 3).

Mean fh and $s\dot{V}_{O_2}$ for water$_{\text{4kg}}$ trials ranged from 60 to 93 beats min^{-1} and from 19 to 46 ml O_2 min^{-1} $\text{kg}^{-0.75}$, and these ranges were similar for water$_{\text{6kg}}$ (60–105 beats min^{-1} and 18–44 ml O_2 min^{-1} $\text{kg}^{-0.75}$, respectively). The amount of food fed (0 vs. 4 kg) while resting in water was a highly significant factor in the linear model. There was also an interaction between meal size and fh for the water$_{\text{4kg}}$ data ($F_{1,195} = 10.16$, $P = 0.002$), suggesting different predictive relationships are needed to describe fh of fasted versus fed (4 kg meals) animals in water (Table 2: Eq. 4; Fig. 4a). In contrast, fh did not predict $s\dot{V}_{O_2}$ in water$_{\text{6kg}}$ trials ($F_{1,138} = 1.42$, $P = 0.24$). In fact, none of the models examined for water$_{\text{6kg}}$ (alone or as a combined dataset with water$_{\text{4kg}}$) were linear; therefore, we were unable to compare water$_{\text{4kg}}$ and water$_{\text{6kg}}$ trials against each other using LME models. Although the water$_{\text{6kg}}$ data were not significantly linear on its own, it became so when combined with the water$_{\text{comp}}$ data ($F_{1,206} = 30.3$, $P < 0.001$, Fig. 4b). The model that included food as a fixed effect was not significantly improved compared with the model with 0 and 6 kg data mixed, suggesting that the fh:$s\dot{V}_{O_2}$ relationship for water$_{\text{6kg}}$ trials did not differ from water$_{\text{comp}}$ trials (LRT = 19.7, $P = 0.002$, Fig. 4b).

The average residual error of the dry$_{\text{fasted}}$ model (Eq. 1) was 14%, and was 19% for water$_{\text{comp}}$ (Eq. 2, Fig. 2a, b). Average residual error for the dry$_{\text{all}}$ model (fasted and fed) was 27% (Eq. 3, Fig. 3), and 15% for water$_{\text{4kg}}$ (Eq. 4, Fig. 4a; calculations were not made for water$_{\text{6kg}}$ as it did not differ from water$_{\text{comp}}$).

Integration of McPhee et al. (2003) data

We also evaluated our results using LME models with the inclusion of data collected for McPhee et al. (2003). Since only trial type (but not gender) was a significant factor, we pooled the data with respect to gender (trial type: $F_{1,230} = 4.48$, $P = 0.01$; gender: $F_{1,2} = 7.91$, $P = 0.12$). Combining the data from the dry$_{\text{fasted}}$, dry$_{\text{4kg}}$, and dry$_{\text{6kg}}$ trials with comparable data from McPhee et al. (dry$_{\text{fasted}}$) confirmed that food was not a significant factor affecting the fh:$s\dot{V}_{O_2}$ relationship for Steller sea lions on land ($F_{1,389} = 0.86$, $P = 0.36$), although the combined data set provided a refined predictive equation (dry$_{\text{all+McPhee}}$, Table 2: Eq. 9).

In contrast to our initial results, the incorporation of comparable water trials from McPhee et al. demonstrated that fh can predict $s\dot{V}_{O_2}$ after a 6-kg meal, and that this relationship differed from that for sea lions fasting in water (LRT = 13.32, $P < 0.001$, Table 2: Eq. 6; Fig. 4c). Incorporating a wider range of meal sizes in water (0, 4, 6, 12 kg) further demonstrated that each meal size significantly
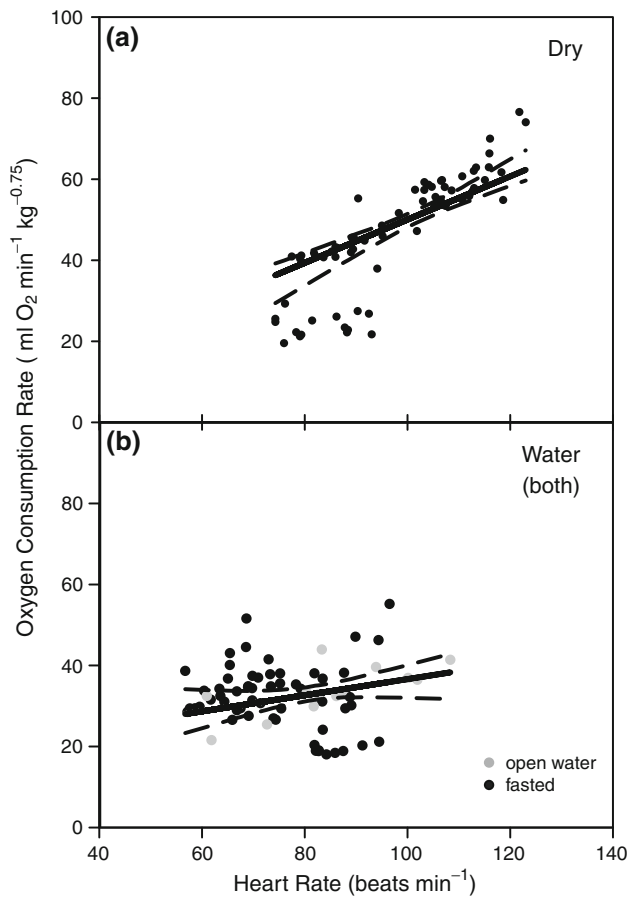
Fig. 2 The relationship between heart rate (fh) and oxygen consumption ($\dot{V}_{O_2}$) in Steller sea lions differed when fasted on land (**a**) and in water (**b**). There was no difference in the relationship between trials conducted in the swim mill (*dark grey circles*) and while resting at the surface in open water (*light grey circles*, **b**). Regressions were derived using mixed-effects linear models within a repeated measures framework. The predictive lines are indicated with *solid lines*, and the 95% bootstrap confidence intervals are indicated with *dashed lines*

Fig. 3 The relationship between heart rate (fh) and oxygen consumption ($\dot{V}_{O_2}$) did not differ among Steller sea lions that were fasting on land (*black triangle*), or digesting 4 kg (*open triangles*) and 6 kg (*gray triangles*). A single equation (*solid line*) was therefore derived to predict $\dot{V}_{O_2}$ on land. The 95% bootstrap confidence intervals are indicated with *dashed lines*

changed the fh:$\dot{sV}_{O_2}$ relationship in this environment (LRT = 17.77, P < 0.001, Table 2: Eqs. 7a–c).

Incorporating data from McPhee et al. showed similar average residual errors relative to error estimates derived from only our data. The average residual error of the water$_{+McPhee}$ model when all meal sizes were combined (11%, Eq. 8) was the same as when meal sizes were separated (11%, Eqs. 7a–c), but less than the model error for dry$_{all+McPhee}$ ($\sim$28%, Eq. 9).

Discussion

Influence of physical environment on the fh:$\dot{V}_{O_2}$ relationship

Our results were consistent with previous studies demonstrating a linear relationship between fh and $\dot{V}_{O_2}$ in fasted
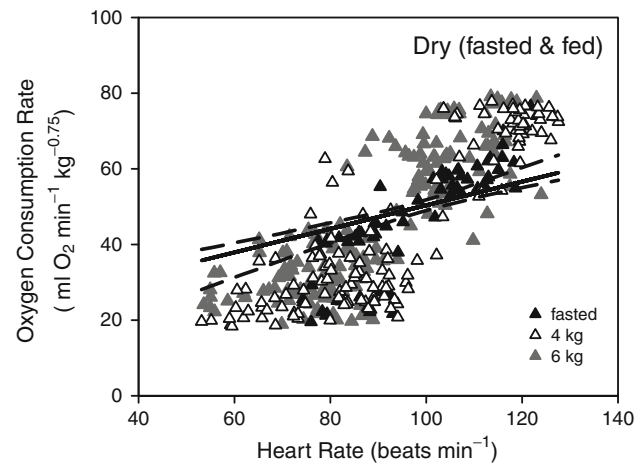
pinnipeds (Boyd et al. 1995; Butler 1993; Hindell and Lea 1998; McPhee et al. 2003), both when animals were resting on land and in the water. Of significance, however, was our finding that the relationship between fh and $\dot{V}_{O_2}$ in fasted animals differed between these two physical environments (Fig. 2c).

This difference could be due to physical properties of air compared to water—for example, water provides greater buoyancy which affects postural muscle tone. The thermal properties of the two mediums are also distinct. Water conducts heat $\sim$25 times better than air, possibly impacting thermoregulation. Among homeotherms, thermoregulation should only be a confounding influence in ambient conditions beyond the thermoneutral zone, where animals employ active (affecting $\dot{V}_{O_2}$) and passive (affecting tissue perfusion) thermoregulatory mechanisms. The thermoneutral zone has not been directly measured on Steller sea lions, but estimates for California sea lions range from 6.4 to 22.4°C (Liwanag et al. 2009). Although temperature could have impacted our results, it is unlikely that our animals were outside their thermoneutral zone for extended periods during trials conducted while in water or on land (ambient temperature ranged from was 9.0 to 19.0°C although 19.0°C only occurred briefly during summer months). We therefore concluded that ambient temperature was not a likely confounding effect.

Submergence can invoke a suite of physiological changes that could cause fasting fh:$\dot{V}_{O_2}$ relationships in resting animals to differ between physical environments. The diving response includes apnea, peripheral vasoconstriction, bradycardia, and a variety of hematological changes (Butler and Jones 1997), any of which could alter individual
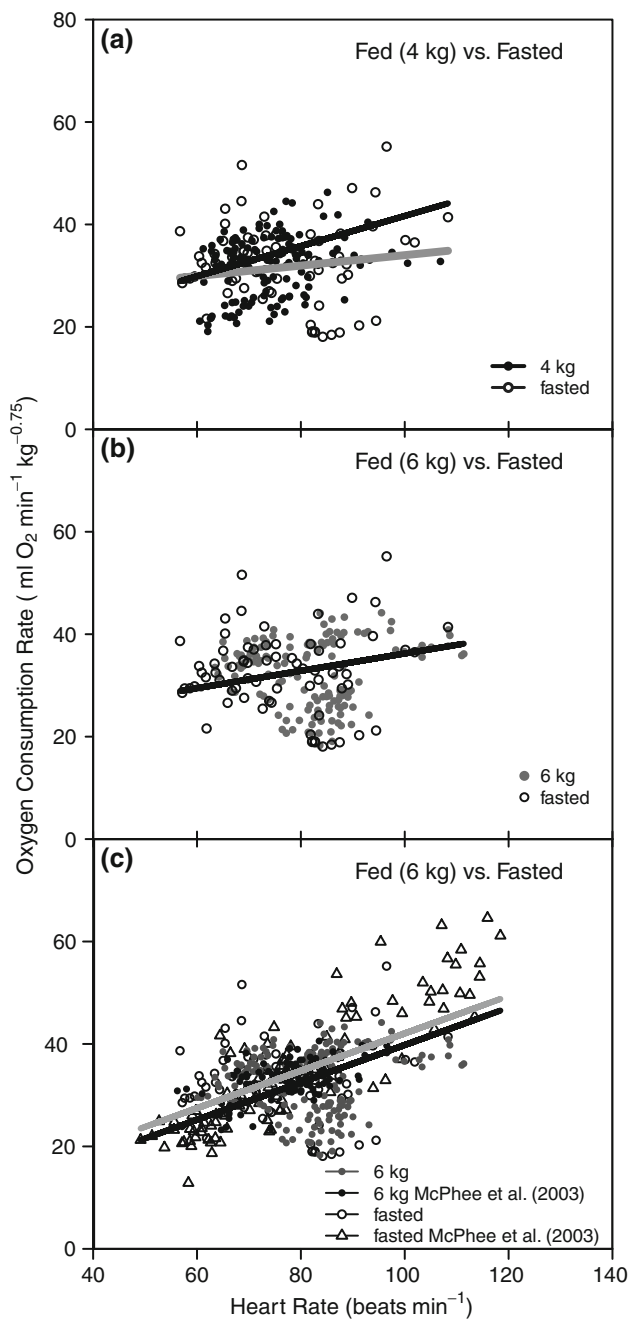
Fig. 4 The relationship between heart rate (fh) and oxygen consumption ($\dot{V}_{O_2}$) differed among Steller sea lions that were fasting in water (*open circles*, **a**, **b**), or digesting 4 kg (*black circles*, **b**). Initially, the relationship did not differ between when sea lions were fasting (*open circles*, **b**) or fed 6 kg (*dark gray circles*, **b**), but combining similar trials from McPhee et al. with the present study showed that feeding changed the relationship in water for all meal sizes (**c**). Data from McPhee et al. (2003) were included in **c** with permission. Confidence intervals are not plotted to preserve clarity of figures but are described in the discussion

components of Fick's equation, and therefore affect the resulting fh:$\dot{V}_{O_2}$ relationship. Altered stroke volume, peripheral resistance, and heart rate will have the clearest

and most direct impact on Fick's equation. It is not clear as to what extent stroke volume changes during shallow submergence, but research suggests that it may be constant in otariids during voluntary head submergence (for a review see Elsner et al. 1964). Altered tissue perfusion (Stone et al. 1973) during diving (usually demonstrated as peripheral vasoconstriction) produces physiologically significant changes in blood flow, but it is unknown whether this occurs during shallow submergence for sea lions. Heart rate was the only component of the dive response that we measured directly, and instantaneous fh traces during water_fasted trials revealed some evidence for bradycardia (when animals submerged for 5–20 s), typical of diving homeotherms (Ponganis et al. 1997). Trials in water also had generally lower ranges for both fh and $\dot{V}_{O_2}$. The same fh value predicted a lower $\dot{V}_{O_2}$ in all of the water-based trials compared with similar trials on land, suggesting an overall reduction in metabolic rate (perhaps mediated by vasoconstriction) that outpaced the decline in heart rate.

Influence of feeding on the fh:$\dot{V}_{O_2}$ relationship

Our study is the first to simultaneously measure changes in both fh and $\dot{V}_{O_2}$ during digestion of known food amounts in marine mammals. We predicted that the fh:$\dot{V}_{O_2}$ relationship would change in response to feeding due to increased $\dot{V}_{O_2}$ (associated with HIF) not accompanied by a parallel increase in fh (Vatner et al. 1970, 1974). It has been suggested that pinnipeds defer digestion when diving or swimming until at the surface or on land (Markussen et al. 1994; Rosen 2007; Sparling et al. 2007), although support for this theory is not universal (Davis et al. 1983a; McConnell et al. 1992; Rosen and Trites 2003; Svärd et al. 2009). However, we found no evidence of deferred digestion due to submergence, given that average $\dot{V}_{O_2}$ increased in water during the feeding trials (particularly towards the end of the trial) and a greater $\dot{V}_{O_2}$ increase was noted for 6 kg compared to 4 kg meals.

In water, we noted that linear fh:$\dot{V}_{O_2}$ relationships for fed Steller sea lions differed significantly from relationships for fasting animals. While feeding resulted in increased fh and $\dot{V}_{O_2}$, these parameters changed at different rates, resulting in a greater $\dot{V}_{O_2}$ predicted from a given fh for animals fed 4 kg relative to those that were fasted (Fig. 4a). Using only our dataset, the difference between the fasted fh:$\dot{V}_{O_2}$ relationship and the postprandial fh:$\dot{V}_{O_2}$ relationship was apparent after a 4 kg-eal but not a 6-kg meal. By analyzing a larger dataset (i.e. by integrating comparable data from McPhee et al. 2003), the difference between fasted and 6 kg trials was also significant (Fig. 4c), confirming that digestion sufficiently affected physiological state to result in a new fh:$\dot{V}_{O_2}$ relationship for animals in water. Furthermore, meal size was relevant, as

evidenced by distinct $fh:\dot{V}_{O_2}$ relationships for 4, 6, and 12 kg meals.

In contrast to both our predictions and the results from the trials in water, we concluded that digestion on land did not affect the relationship between fh and $\dot{V}_{O_2}$ (Fig. 3). This conclusion held when additional dry_{fasted} trials from McPhee et al. (2003) were analyzed (Table 2: Eq. 9). This could suggest that HIF contributes to comparably higher average $\dot{V}_{O_2}$ and fh that fall within the same relationship observed in fasted animals, unlike trials in water. However, data ranges for $\dot{V}_{O_2}$ and fh from all dry trial types overlap thoroughly (Fig. 3). In other words, despite existing data describing a postprandial HIF response in Steller sea lions (Rosen and Trites 1997), we did not observe a consistent $\dot{V}_{O_2}$ elevation following feeding. This was despite the fact that our 4-h trial duration was intended to capture the HIF peak (Rosen and Trites 1997). We therefore suggest that the HIF response was obscured by variation in animal activity and our 5-min data averaging. Analyzing the timecourses of fh and $\dot{V}_{O_2}$ relative to the fasted baseline detected some evidence of HIF onset on land (Fig. 5). In most feeding trials, $\dot{V}_{O_2}$ was initially below the fasted baseline, but tended to increase 10–15 min into the trial and remain elevated above the equivalent fasted values until about 10 min before the end of the trial. Regardless of the reason for the lack of distinct HIF peak in oxygen consumption, the end result was that a single predictive equation can be used to accurately estimate $\dot{V}_{O_2}$ from fh without having to determine whether the animal is fasted or fed (see "Field applications" below).

Field applications

Practical limitations must be considered before applying the equations in Table 2 in the field. The predictive models presented are species-specific and age-specific for adult,
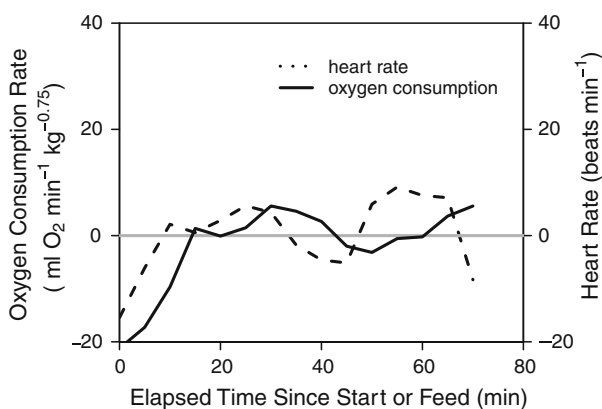

Fig. 5 Representative trial showing heart rate (fh, *dashed line*) and oxygen consumption rate ($\dot{V}_{O_2}$, *solid line*) of a Steller sea lion relative to baseline values (fed 0 kg, *grey line* at zero) following a single meal of 4 kg on land (Animal F03AS)

non-reproductive female Steller sea lions within the body mass range of our sample population (Table 1). Also, $water_{comp}$ equations are specific to sea lions resting in water, and further research should explore the $fh:\dot{V}_{O_2}$ relationship when animals are diving (see Young 2010). As the magnitude and duration of HIF is known to vary by meal size and composition (Rosen and Trites 1997), the predictive equations may be specific for Steller sea lions digesting 0–12 kg Pacific herring. Despite these limitations, the equations generated by our study should help refine determinations of field metabolic rate in pinnipeds.

Error of the fh method

The average residual errors of our models ranged from 11 to 28%. The error estimates noted here were greater than the error estimates for the doubly labeled water method in fur seals (~3%, Trillmich and Kooyman 2001), but less than the doubly labeled water method error noted for California sea lions (~36%, Boyd et al. 1995). However, our predictive equations are based on specific environmental and physiological conditions, and errors will naturally increase if the wrong predictive equation is applied to the data. For the following exercise, percent error for each of the predictive equations was calculated relative to the fasted ($water_{comp}$) baseline using an average fh of 100 beats min^{-1}.

Incorrectly using the $dry_{all+McPhee}$ equation (Eq. 9) to estimate $s\dot{V}_{O_2}$ of a free-ranging Steller sea lion fasting in water (Eq. 2) would overestimate $s\dot{V}_{O_2}$ by approximately 34% (similar to the 36% error for the doubly labeled water method, Boyd et al. 1999). Bio-logging can be employed to measure fh and to determine when an animal is on land, resting in water, or diving (Ponganis 2007). Therefore, these types of data can be used to choose an environment and behavior-appropriate equation. Specifically, Eq. 9 should be used when animals are on land (fed or fasted), and Eq. 2 should be used when fasted animals are resting at the water surface (Table 2).

Statistical analyses clearly demonstrate that predictive equations are also specific to feeding state. Recent developments in animal-mounted cameras and stomach temperature pills also allow the occurrence (and perhaps size) of feeding events to be determined (Davis et al. 1999). However, consideration must be given as to whether the increased financial and logistical efforts required to obtain this additional information are warranted. Applying the equations derived for animals feeding 4–12 kg in water (Eqs. 7a–c) to a fasted Steller sea lion in water resulted in <2% error in $s\dot{V}_{O_2}$. Considering the small error associated with applying the incorrect predictive equation, we recommend estimating $\dot{V}_{O_2}$ of Steller sea lions resting in water using the predictive model that encompasses the widest

range of data (0–12 kg meals) but does not distinguish among digestive states (Eq. 8).

Given the lack of statistical distinction between predictive equations for fed versus fasted sea lions on land, we recommend using the composite equation developed from all fasted and fed trials on land ($dry_{all+McPhee}$, Eq. 9) to estimate $s\dot{V}_{O_2}$ of Steller sea lions on land, regardless of the amount of food consumed. As a demonstration, a fh of 100 beats min^{-1} produces similar estimations of $s\dot{V}_{O_2}$ for fasted or fed animals on land (50, and 49 ml O_2 min^{-1} $kg^{-0.75}$). This simplifies estimates of energy expenditure by removing the need to determine food intake in the field. Thus, our findings demonstrate that separate equations should be used to predict $\dot{V}_{O_2}$ on land and in water, and that the effect of digestion on the fh:$\dot{V}_{O_2}$ relationship in water is not significant enough to warrant determining the food intake of free-ranging animals.

Using the recommended equations in the appropriate circumstances will allow reasonably accurate estimates of behavior-specific metabolic rates for Steller sea lions in the wild. These estimates can then be used to update current bioenergetic models that help scientists and managers elucidate the interaction between Steller sea lions and their environment.

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