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Metabolic costs of foraging and the management of O_2 and CO_2 stores in Steller sea lions

Andreas Fahlman[1,*], Caroline Svärd[1,2], David A. S. Rosen[1], David R. Jones[3] and Andrew W. Trites[1]

[1]Marine Mammal Research Unit, Fisheries Centre, Room 247, Aquatic Ecosystems Research Laboratory, University of British Columbia, Vancouver, BC, V6T 1Z4 Canada, [2]Department of Biomedical Engineering, Linköpings Universitet, 581 85 Linköping, Sweden and [3]Zoology Animal Care, 6199 South Campus Road, University of British Columbia, Vancouver, BC, V6T 1Z4 Canada

*Author for correspondence (e-mail: andreas_fahlman@yahoo.com)

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SUMMARY

The metabolic costs of foraging and the management of O_2 and CO_2 stores during breath-hold diving was investigated in three female Steller sea lions (*Eumetopias jubatus*) trained to dive between 10 and 50 m (*N*=1142 dives). Each trial consisted of two to eight dives separated by surface intervals that were determined by the sea lion (spontaneous trials) or by the researcher (conditioned trials). During conditioned trials, surface intervals were long enough for O_2 to return to pre-dive levels between each dive. The metabolic cost of each dive event (dive+surface interval; DMR) was measured using flow-through respirometry. The respiratory exchange ratio ($\dot{V}_{O_2}/\dot{V}_{CO_2}$) was significantly lower during spontaneous trials compared with conditioned trials. DMR was significantly higher during spontaneous trials and decreased exponentially with dive duration. A similar decrease in DMR was not as evident during conditioned trials. DMR could not be accurately estimated from the surface interval (SI) following individual dives that had short SIs (<50 s), but could be estimated on a dive by dive basis for longer SIs (>50 s). DMR decreased by 15%, but did not differ significantly from surface metabolic rates (MR_S) when dive duration increased from 1 to 7 min. Overall, these data suggest that DMR is almost the same as MR_S, and that Steller sea lions incur an O_2 debt during spontaneous diving that is not repaid until the end of the dive bout. This has important consequences in differentiating between the actual and 'apparent' metabolic rate during diving, and may explain some of the differences in metabolic rates reported in pinniped species.

Key words: field metabolic rate, diving physiology, marine mammal, hypometabolism, O_2 debt, CO_2 exchange, physiological plasticity.

INTRODUCTION

The metabolic cost associated with foraging is a critical component of the energy budget of animals and may significantly affect the amount of prey they must obtain to survive. It is therefore important to assess this metabolic cost and understand how it may be affected by changes in the environment and the physiology of individual animals. Consequently, accurately determining metabolic costs is of paramount importance to understanding the natural history of diving animals, and for predicting their food requirements.

Considerable attention has been given over the past three decades to measuring field metabolic rates of foraging animals using two techniques – the heart rate technique and use of stable isotopes (doubly labelled water) (Sparling et al., 2008). Recently Fahlman et al. (Fahlman et al., 2008b) showed that activity was also a reliable proxy for the metabolic costs of Steller sea lions (*Eumetopias jubatus* Schreber 1776) during diving and while at the surface. One disadvantage with both the heart rate technique and the activity method for estimating field metabolic rate is that they need lengthy calibration studies to establish the relationship between metabolic cost and heart rate or activity for different species and for different activities (e.g. swimming, walking and diving).

Respirometry is the 'gold standard' for measuring energetic costs in animals performing a variety of activities and is necessary for validation of indirect methods, e.g. doubly labelled water, heart rate technique and activity. However, it has been particularly challenging to measure metabolic rates of animals while diving under realistic conditions. Several different approaches have been used to overcome these logistical difficulties. One approach has been to record gas exchange for animals contained in a body of water that is covered naturally by ice (Kooyman et al., 1973; Ponganis et al., 1993; Williams, 2001) or artificially with grates (Sparling and Fedak, 2004), forcing the animal to surface inside a respirometer at a pre-determined place. Another approach has been to use trained animals in a captive (Hurley, 1996) or open ocean setting (Fahlman et al., 2008b). This latter method has been used in a series of recent studies to directly measure the O_2 uptake and CO_2 production rates following dives (Fahlman et al., 2008a; Fahlman et al., 2008b; Hastie et al., 2006a; Hastie et al., 2006b).

Trained Steller sea lions in a previous open ocean study performed repeated foraging dives on their own volition and remained at the surface in the respirometry dome for as long as they wished between dives (Fahlman et al., 2008a). Each of the animals remained within the dome following the last dive in a series of dives (bout) until the O_2 level had returned to the pre-dive level. The estimated metabolic rate of a dive event (dive + surface interval; DMR) was found to decay exponentially with dive duration suggesting that longer dives cost proportionally less energy (Fahlman et al., 2008b). In addition, the first dive in a series was also found to have the lowest metabolic cost compared with the last dive that had the highest DMR and had a longer recovery period. This indicated that perhaps the animals terminated their surface intervals before their O_2 stores were fully restored.

The diving studies suggested that Steller sea lions were diving with a slight O_2 debt throughout their dive bouts that was only paid back during the prolonged recovery period at the end of the trial. Similar results were reported for Weddell seals (*Leptonychotes*

weddelli) that took between 3 and 5 min for expired O_2 to return to pre-dive levels (Kooyman et al., 1973; Ponganis et al., 1993). Kooyman et al. (Kooyman et al., 1973) suggested their results indicated that the animals incurred an O_2 debt during a dive bout that was not completely repaid during the short surface intervals between dives but remained outstanding until the animal incurred an extended recovery period at the end of the bout. Consequently, estimating DMR by respirometry is complicated by the fact that the apparent rate of oxygen uptake ($\dot{V}_{O_2}$) during individual repeated dives is lower than the actual metabolic rate.

The dive behaviours observed for Steller sea lions and Weddell seals are consistent with certain optimal foraging models that assume that the length of the surface interval is determined by the need to replenish depleted oxygen stores (Kramer, 1988). However, recent empirical research suggests that removal of CO_2 is the main driving force regulating surface interval duration (Boutilier et al., 2001). Although insufficient uptake of O_2 would reduce the aerobic dive duration, incomplete removal of CO_2 leads to a continuous increase in blood and tissue CO_2 partial pressure (P_{CO_2}) (Fahlman et al., 2008c). The surface interval is therefore a dynamic state determined by the need for sufficient gas exchange, food availability and predator avoidance.

Physiological plasticity enables diving animals to enhance gas exchange during a surface interval and maximizes time underwater, thereby enhancing foraging efficiency. However, such adaptations complicate the ability to estimate the energetic cost of foraging since repeated dives in a dive bout may not be independent samples but may have to be considered collectively. Our study therefore aimed to improve understanding of how pinnipeds manage the O_2 debt during a dive bout while seeking to develop a method that accounts for the O_2 debt and accurately estimates the true metabolic costs of foraging.

MATERIALS AND METHODS

Animals

Experiments were conducted between April 2006 and August 2007 with three female Steller sea lions housed in a specially designed floating pen located in a coastal inlet in British Columbia, Canada. The pen allowed access to seawater and provided a base for research in the surrounding waters. The sea lions cooperated during data collection and were never restrained during any of the trials. Two of the sea lions (F97HA and F97SI) were 9 years old at the beginning of the study, and the third was 6 years old (F00BO). Body mass (M_b) of each sea lion was measured by weighing the animal before each trial. The average M_b (±s.d.) was 164.7±4.5 kg for F97HA (N=53), 215.5±4.8 kg for F97SI (N=75) and 140.9±5.2 kg for F00BO (N=64), where N is the number of days on which each sea lion took part in a trial.

Respirometry studies

All trials were performed in the morning and the sea lions were between 16 and 20 h postprandial. Before trials, each sea lion was weighed (±0.5 kg) and fitted with a webbed body harness. A VHF transmitter was attached to the harness and was used to locate the sea lion in the event of it swimming out of the trial area.

The animals were transported in a specially modified boat from their holding pen to the trial area, where they could dive from a floating respiratory dome to an underwater feeding tube placed at depth. Another research boat carrying the respirometry equipment and towing a floating barge was anchored in the trial area. The barge contained a rectangular hole through which a large wire cage (152 cm×152 cm) was placed in the water. The cage floor could be opened to allow the sea lion to enter and breathe in the dome. Closing the cage door made it possible to voluntarily contain the sea lion for short periods of time to measure metabolic rate while the animals were at the surface. Animals were seldom quiescent when in the cage. Thus, surface metabolic rate (MR_S) varied and was not resting or basal metabolic rate.

A transparent Plexiglas dome (either 100 or 200 l internal volume) was placed above the cage to collect respiratory gases (O_2 and CO_2). A mass flow controller (Model 500H, Sable Systems, Las Vegas, NV, USA) pulled 475 l min^{-1} of air through the dome. This mass flow meter automatically corrected flow rates to standard temperature and pressure (STP) in the case of variations in temperature and barometric pressure. A subsample of the air was pulled through a canister of anhydrous $CaSO_4$ (Drierite, W. A. Hammond, Xenia, OH, USA) to a paramagnetic O_2 (FC-1B O_2, Sable Systems, Las Vegas, NV, USA) and an infrared CO_2 analyzer (CA-10A, Sable Systems, Las Vegas, NV, USA). Air flow rate as well as O_2 and CO_2 levels were sampled at 2 Hz and saved to a laptop computer.

The gas analyzers were calibrated before and after a dive trial with ambient air (20.94% O_2) and 1.0% CO_2 in N_2 from a commercial gas mixture (Praxair, Canada). Temperature (°C) and humidity (%) of the excurrent gas was measured using a commercial sensor (Springfield Precise Temp., Springfield Precision Instruments, Wood Ridge, NJ, USA). Average respirometer temperature was 16.6±6.1°C (range: 0.8–28.5°C; N=186), humidity was 77±15% (range: 35–100%) and barometric pressure was 102.0±0.7 kPa (range: 99.5–103.9 kPa). All flows were corrected to STP dry (STPD).

The accuracy in measured $\dot{V}_{O_2}$ and rate of CO_2 production ($\dot{V}_{CO_2}$) was determined by simultaneous N_2- and CO_2-dilution tests (Fahlman et al., 2005; Fedak et al., 1981) and estimated values were within 4% of the measured values. Addition of CO_2 confirmed that minimal amounts of CO_2 were dissolved and lost in the seawater. The effective volume of the system was either 120 l or 220 l, including the volume of the respirometer (100 or 200 l) and the plastic hose to the analyzers (20 l). With a flow rate of 475 l min^{-1}, this gave time constants of 0.25 and 0.46 min for the small and large domes, respectively. The time required to reach a 95% fractional transformation to a new steady state was 3.2 times the time constant, or 48 s (small dome) and 90 s (large dome) (Fahlman et al., 2005).

A dive trial consisted of either a single dive or a bout of repeated dives (2–15 dives) to a simulated foraging patch. A tube and pump system allowed fish (previously frozen herring) to be delivered to various depths at different rates (0–12 fish min^{-1}) simulating the sea lion feeding on food patches of varying densities. Before diving, the sea lion was instructed by a trainer to enter the respirometry dome. Once inside the cage, the door was closed and the animal remained in the dome for 6–10 min while MR_S was measured. Duration was extended if steady values of O_2 and CO_2 were not recorded during the last 2 min. The sea lion was then instructed to swim to the end of the feeding tube that was placed at depths between 10 m and 50 m. The sea lion returned to the metabolic dome at the end of each dive.

Two different diving protocols were used – ‘spontaneous’ and ‘conditioned’ trials. For spontaneous dive trials, the sea lion determined the duration of the dive and the surface interval without instructions from the trainer. During conditioned dive trials, the door was closed between dives and the sea lion remained in the respirometry dome until the O_2 and CO_2 returned to pre-dive levels, usually between 5 and 8 min. For both experimental protocols, the sequences of dive and surface intervals were repeated with two to eight dives in each trial (one trial per day) and the last dive was followed by a recovery period in the respirometry dome (6–10 min).

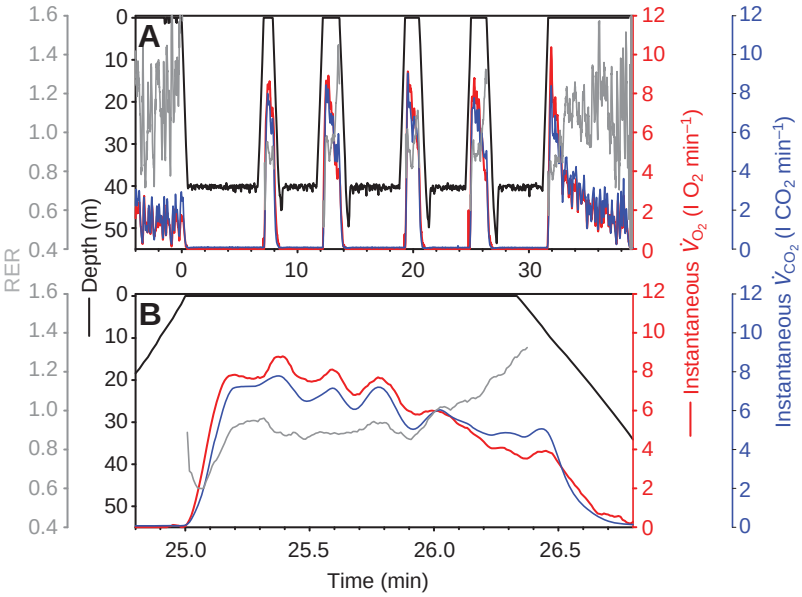

Fig. 1. Instantaneous oxygen consumption ($\dot{V}_{O_2}$; red line), instantaneous carbon dioxide production ($\dot{V}_{CO_2}$; blue line) and respiratory exchange ratio (RER, $\dot{V}_{CO_2}/\dot{V}_{O_2}$; grey line) during a representative series of five spontaneous dives by a Steller sea lion to 40 m (A), and during a single surface interval after the fourth dive in the series (B). Dive depth is shown by the black line, and O_2 and CO_2 have been corrected for delay in response of gas analyzer and flow through the system.

Fish were delivered down the tube at a constant rate that was determined before the start of each trial. To ensure that the sea lions were not naïve to the required dive parameters during a trial, each animal was trained at each depth and duration for 5–7 days before any data were collected.

Average $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ was calculated by integrating the instantaneous oxygen consumption rate over the entire post-dive surface interval, and dividing this by the dive event duration (Fahlman et al., 2008b). All surface intervals were >60 s in a conditioned dive series and >5 s in a spontaneous dive series.

Water temperatures at the surface and at the end of the feeding tube were monitored during each trial using remote temperature loggers (Onset Computer, Pocasset, MA, USA). Surface temperature ranged from 4.3 to 18.9°C (mean 12.4±4.0°C, N=159), and the range at depth was between 5.7°C and 17.3°C (mean 11.1±2.3°C, N=153). Water current at the surface and at depth was scored by eye as low, medium, or high.

Data assessment and statistical analysis

Mixed models regression was used to determine which model best described the relationship between MR_S or DMR and the independent variables. Independent variables included ten experimental variables (dive duration, depth, M_b, surface interval, water current at depth, water temperature at the surface or at depth, respirometer temperature, the dive number in a series of dives and respirometer humidity) as independent fixed covariates and three factors (current, with or without harness, and spontaneous or conditioned trial). Initially, a univariate analysis on each independent variable was performed, and only those variables with P<0.20 (Wald's tests) were considered in the multivariate analysis. Stepwise techniques were used to search for the best model. Nested regressions were compared with each other using the Akaike Information Criterion (AIC) (Akaike, 1974) and log-likelihood ratio testing. The models were analyzed and corrected for departures from normality, outliers, and linearity as detailed by Neter et al. (Neter et al., 1996). Statistical analyses were performed using R (version 2.5.1, The R foundation for Statistical Computing). Acceptance of significance was set at P<0.05, and 0.05<P<0.1 was considered significant for trends. All values were reported as means ± s.d., unless otherwise specified.

RESULTS

Dive characteristics

DMR was measured for 1142 dives with confirmed depths for 1133 of those dives. Of these, 896 dives, or 78% of the total, were to 10 m. The remaining dives were split between depths of 30 m (92; 8%), 50 m (64; 6%), 20 m (38; 3%), 15 m (25, 2%), 40 m (13, 1%), 25 m (3, <1%) and 35 m (2, <1%).

Average dive durations and depths for conditioned dives (2.28±1.53 min and 20.1±14.3 m, respectively; N=479) were significantly longer and deeper than spontaneous dives (0.97±0.83 min and 11.5±6.3 m; N=717; P<0.01, mixed model ANOVA). Dive duration increased with depth (1.2±1.0 min at 10 m and 4.0±1.5 min at 50 m, P<0.01, mixed model ANOVA).

Rate of oxygen consumption ($\dot{V}_{O_2}$)

Fig. 1 shows a representative spontaneous dive bout of five repeated dives to 40 m, and six surface intervals during which $\dot{V}_{O_2}$ and the respiratory exchange ratio (RER, $\dot{V}_{CO_2}/\dot{V}_{O_2}$) were measured. Average surface metabolic rate for all animals was 1.45±0.44 l O_2 min^{-1} (N=191; Table 1) and did not differ significantly between spontaneous (1.41±0.38 l O_2 min^{-1}, N=70) and conditioned (1.48±0.48 l O_2 min^{-1}, N=121) trials (mixed model ANOVA, LL-ratio test=1.90, P>0.1, 1 d.f.).

The average metabolic rate during a dive event was 1.65±0.66 l O_2 min^{-1} (N=1142), and was significantly higher for spontaneous dive events (DMR_{Spon}) than for conditioned dives (DMR_{Cond}; mixed model ANOVA, LL-ratio test=15.9, 1 d.f., P<0.01) (Table 1). RER was higher for conditioned trials (normally falling within a range of 0.7–1.0, but occasionally reaching values as low as 0.6 and as high as 1.05). During spontaneous trials, RER was within the range 1.82 to 0.34. RER was also more variable during spontaneous trials compared with conditioned trials (Table 1). RER was low after a sea lion surfaced and began breathing, usually less than 0.7, and increased as the surface interval progressed (Fig. 1B). RER increased immediately before diving, which is suggestive of pre-dive hyperventilation (Fig. 1B). There was a positive correlation between RER and the surface interval (SI) for both conditioned and spontaneous trials (P<0.01). However, there was no relationship between RER and SI for SIs >1.7 min (P>0.05).

Table 1. Metabolic measurements

Animal	M_b (kg)	($l\ O_2\ min^{-1}$) BMR	MR_S	MR_{Smin}	MR_{Smax}	DMR	DMR_{Cond}	DMR_{Spon}	RER_{MRS}	RER_{DMR}	$RER_{DMRCond}$	$RER_{DMRSpon}$
F97SI	215.5±4.8 (75)	0.56±0.03	1.76±0.34 (72)	1.31±0.20	2.53±0.317	1.89±0.71 (432)	1.83±0.30 (204)	2.00±0.78 (228)	0.92±0.10 (48)	0.71±0.23 (316)	0.83±0.09 (147)	0.62±0.27 (169)
F97HA	164.7±4.5 (53)	0.46±0.03	1.52±0.42 (55)	1.08±0.16	2.33±0.328	1.71±0.56 (335)	1.60±0.37 (124)	1.80±0.59 (211)	0.86±0.17 (45)	0.71±0.18 (292)	0.85±0.13 (110)	0.63±0.16 (182)
F00BO	140.9±5.2 (64)	0.41±0.034	1.04±0.18 (64)	0.78±0.06	1.38±0.13	1.32±0.53 (375)	1.21±0.22 (151)	1.41±0.55 (224)	0.90±0.10 (43)	0.73±0.16 (299)	0.85±0.11 (112)	0.66±0.14 (187)
Grand mean	176.6±32.9 (192)	0.48±0.03	1.45±0.44 (191)	1.06±0.26	2.08±0.58	1.65±0.66 (1142)	1.57±0.40* (479)	1.74±0.70* (663)	0.89±0.14 (136)	0.72±0.19 (907)	0.84±0.11* (369)	0.64±0.19* (538)

M_b, average body mass; BMR, estimated basal metabolic rate; MR_S, surface metabolic rate (before diving); RER_{MRS}, respiratory exchange ratio at the surface before diving; DMR, metabolic rate of a dive event; RER_{DMR}, RER during a dive event for conditioned (DMR_{Cond}, $RER_{DMRCond}$) and spontaneous dives (DMR_{Spon}, $RER_{DMRSpon}$).
The average of the 10 lowest (MR_{Smin}) and highest (MR_{Smax}) measured MR_S values are reported for each sea lion. Values are means ± s.d. (*N*).
*Significant difference between spontaneous and conditioned trials ($P<0.05$).
BMR was estimated from Kleiber's (Kleiber, 1961) equation: BMR=70 $M_b^{0.75}$, where B is in kcal day^{-1} or converted to $l\ O_2\ min^{-1}$, assuming a mixed diet in which 1 $l\ O_2$=4.9 kcal; BMR=0.00993 $M_b^{0.75}$.

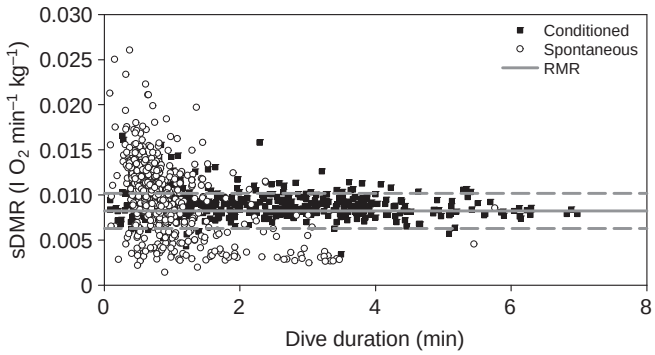

Fig. 2. Relationship between mass-corrected metabolism during a dive event (dive+surface interval; sDMR in $l\ O_2\ min^{-1}\ kg^{-1}$) and dive duration for three Steller sea lions diving repeatedly to 10–50 m depths. Each dive was separated by a surface interval determined by the sea lion (spontaneous trials; open circles) or by the researcher (conditioned trials; filled squares). Surface intervals during conditioned trials were long enough for $\dot{V}_{O_2}$ to return to pre-dive levels between each dive. sDMR was estimated using a mass exponent of 1.0. The solid grey line is pre-diving resting metabolism (±1 s.d., *N*=191; grey dashed line) for all sea lions.

The mixed model ANOVA analysis suggested that $\log(M_b)$ and water surface temperature (T_s) significantly affected $\log(MR_S)$ and that the best model was:

$$\log(MR_S) = -2.21 + 1.08\log(M_b) - 8.32\times10^{-3}\,T_s. \quad (1)$$

For all dive data, the best model predicting DMR included $\log(M_b)$, depth (D), dive duration (DD), duration of SI and the order of repeated dives (DiveNo). This was:

$$\log(DMR) = -1.70 + 0.83\log(M_b) + 1.22\times10^{-3}\,D - 4.99\times10^{-2}\,DD + 1.09\times10^{-2}\,SI + 5.07\times10^{-3}\,DiveNo. \quad (2)$$

For conditioned dives (dives with an SI >50 s), however, the best model included only DD, $\log(M_b)$ and the water temperature at depth (T_{depth}):

$$\log(DMR) = -2.58 + 1.26\log(M_b) - 7.18\times10^{-3}\,DD + 4.59\times10^{-3}\,T_{depth}. \quad (3)$$

In all analyses, $\log_{10}$-transformed M_b was significantly correlated with $\log_{10}$-transformed MR_S and DMR. The mass exponents for Eqns 1 and 3 were not significantly different from 1. Mass-specific surface metabolic rates (sMR_S) and mass-specific dive event metabolic rates (sDMR) were thus estimated as $MR_S M_b^{-1}$ and $DMR\,M_b^{-1}$, respectively (Figs 2 and 3).

The first dive in a series of spontaneous dives had the lowest estimated $\dot{V}_{O_2}$ and the last dive had the highest. There was also a positive relationship between estimated $\dot{V}_{O_2}$ and the SI. sDMR for all dives decreased exponentially towards sMR_S with increasing dive duration, and at times fell below sMR_S (Fig. 2). However, separating the data into conditioned and spontaneous dives showed only $sDMR_{Spon}$ decreased exponentially whereas there was only a weak indication of a decrease in $sDMR_{Cond}$ with dive duration (Fig. 2). For dives longer than 1 min, $sDMR_{Spon}$ was significantly lower than $sDMR_{Cond}$ ($P<0.01$, *t*-test) and $sDMR_{Spon}$ commonly decreased below sMR_S (Fig. 2). Variability in $sDMR_{Spon}$ was larger than $sDMR_{Cond}$, although variability decreased for $sDMR_{Spon}$ as the SI increased (Fig. 3).

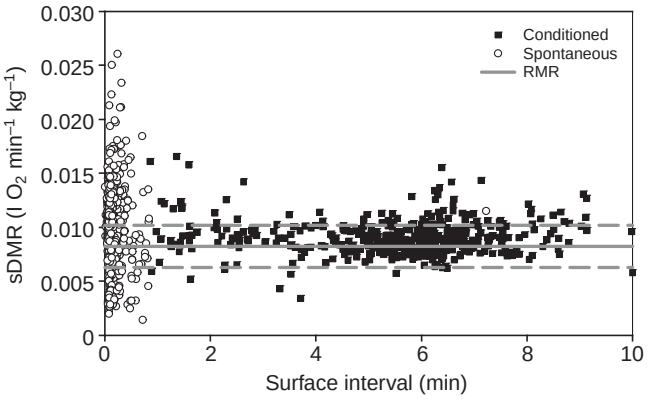

Fig. 3. Relationship between mass-corrected metabolism of a dive event ($l\,O_2\,min^{-1}\,kg^{-1}$, sDMR, dive+surface interval) and duration of the surface interval for three Steller sea lions diving repeatedly to 10–50 m depths. Each dive was separated by a surface interval determined by the sea lion (spontaneous trials; open circles) or by the researcher (conditioned trials; filled squares). Surface intervals during conditioned trials were long enough for $\dot{V}_{O_2}$ to return to pre-dive levels between each dive. sDMR was estimated using a mass exponent of 1.0. The solid grey line indicates pre-diving resting metabolism (±1 s.d., *N*=191; grey dashed line) for all sea lions.

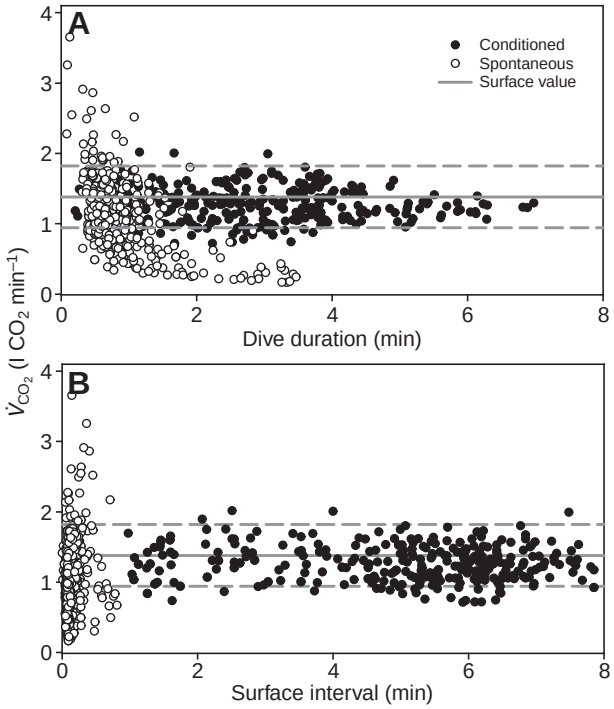

Fig. 4. Relationship between the rate of CO_2 production ($\dot{V}_{CO_2}$) and (A) duration of dives or (B) surface intervals between dives for three Steller sea lions diving repeatedly to 10–50 m depths. Each dive was separated by a surface interval determined by the sea lion (spontaneous trials; open circles) or by the researcher (conditioned trials; filled circles). Surface intervals during conditioned trials were long enough for $\dot{V}_{CO_2}$ to return to pre-dive levels between each dive. The solid grey line indicates the pre-diving $\dot{V}_{CO_2}$ (±1 s.d., *N*=191; grey dashed line) for all sea lions.

Rate of CO_2 production ($\dot{V}_{CO_2}$)

Average surface $\dot{V}_{CO_2}$ was 1.38±0.44 $l\,CO_2\,min^{-1}$ for all trials and did not differ between spontaneous (1.30±0.34 $l\,CO_2\,min^{-1}$) or conditioned (1.42±0.47 $l\,CO_2\,min^{-1}$) trials (P>0.1). Diving $\dot{V}_{CO_2}$ decreased exponentially with dive duration for spontaneous trials but it remained more or less constant for conditioned trials (Fig. 4A). For SI<1 min (spontaneous dives), diving $\dot{V}_{CO_2}$ was more variable compared with conditioned trials (Fig. 4B).

DISCUSSION

Scholander (Scholander, 1940) showed that the O_2 taken up during the recovery period after a forced submersion was less than expected based on the assumption that the metabolic rate during the dive and surface interval would be similar to the resting metabolic rate. Scholander (Scholander, 1940) concluded that this indicated diving induced hypometabolism, a probable adaptation in diving animals to extend aerobic dive duration by reducing oxygen consumption rates. Studies on voluntarily diving California sea lions (*Zalophus californianus*) (Hurley and Costa, 2001) and Steller sea lions (Hastie et al., 2007) confirmed that the rate of O_2 uptake during a dive event was lower than the resting rate at the surface. O_2 uptake rates during dive events in California sea lions decreased most noticeably for dives longer than 3 min (Hurley and Costa, 2001). Although initial studies with Steller sea lions did not indicate a difference in $\dot{V}_{O_2}$ with duration spent underwater (Hastie et al., 2007), maximum dive durations were only 3 min. However, later studies reported an exponential decrease in O_2 uptake with increasing dive duration (Fahlman et al., 2008b), and that a reduction in DMR below MR_S was most pronounced and consistent during dives longer than 3 min [see Fig. 2 in Fahlman et al. (Fahlman et al., 2008b)].

Repeated dives and the effect of the O_2 debt on DMR

Fahlman et al. (Fahlman et al., 2008b) noticed that the first dive in a series of repeated spontaneous dives had the lowest DMR whereas the last dive had the highest DMR. Other studies have adjusted for this phenomenon by only analyzing dives that have post-dive recovery periods long enough for O_2 level to return to within 2% of the pre-dive resting rate (Williams et al., 2004). This approach assumes that each dive performed in a bout is independent. However, a series of repeated dives in a bout followed by a short surface interval may lead to continuing changes in O_2 stores and/or accumulation of CO_2 and its by-products (lactate, acid base balance disturbance). Therefore, the last dive before an extended surface interval may be affected by the previous dive sequence and estimates of DMR may be biased if only this last dive is analyzed.

We hypothesize that the much lower DMR of the first dive event reflects incomplete restoration of O_2 stores, and that the sea lions that undertake a series of continuous dives do so with an O_2 debt that is incurred during the first dive. We investigated this by comparing spontaneous diving trials to trials consisting of a series of dives where the sea lion stayed in the respirometry dome until the O_2 had returned to baseline levels between dives (conditioned dive). Results from these conditioned trials showed that the O_2 uptake rate during repeated dives was independent of the order of the dive.

The explanation for such a pattern in energy cost with repeated dives relies on a strategy of minimizing time spent on the surface and maximizing time at depth. Blood and tissues should be fully saturated with O_2 before the first dive in a dive bout, with levels of P_{O_2} decreasing during the first dive. The overall decline of P_{O_2} for a completely aerobic dive will depend on the metabolic rate of the tissues and the dive duration.

As the animal returns to the surface and begins breathing, O_2 will be taken up and CO_2 removed. Assuming no pulmonary diffusion limitations, uptake of O_2 into the blood is governed by:

$$dP_{v_{O_2}}dt^{-1} = (P_{L_{O_2}} - P_{v_{O_2}})\tau^{-1}, \quad (4)$$

where $P_{L_{O_2}}$ is the partial pressure of O_2 in the lung, $P_{v_{O_2}}$ is the partial pressure of O_2 in mixed venous blood and τ is a time constant that determines the time to equilibrium. The time constant is physiologically relevant and related to the solubility of the gas and the blood flow rate.

Sea lions have a modified respiratory system allowing most of the air in the lungs to be exchanged during a single breath (Denison and Kooyman, 1973). Therefore, dead space mixing can be assumed to be negligible and $P_{L_{O_2}}$ close to the ambient P_{O_2}. Assuming that lung blood flow is constant during a surface interval, O_2 uptake will depend on the difference between $P_{v_{O_2}}$ and $P_{L_{O_2}}$.

When the animal returns to the surface there is a large P_{O_2} gradient between lung and mixed venous blood, favouring diffusion of O_2 into the blood. The large P_{O_2} gradient and the shape of the O_2 dissociation curve allow large amounts of O_2 to be taken up with small changes in $P_{v_{O_2}}$. $P_{v_{O_2}}$ increases as O_2 is taken up by the tissues, thereby reducing the partial pressure gradient and the O_2 uptake rate. For this reason, complete restoration of the O_2 stores is not as profitable due to the temporal decrease in the O_2 uptake rate (Kramer, 1988).

Without complete restoration of the O_2 stores, the total O_2 uptake after the first dive is lower than the actual O_2 used during the dive event. During subsequent dives, the surface interval is adjusted to maximize the O_2 uptake rate and restore the O_2 that was used during the dive to avoid an accumulating O_2 debt. In other words, the animal will work to restore O_2 at the steep part of the O_2 dissociation curve where O_2 gain is maximized while at the same time minimizing the surface interval. Consequently, small changes in surface interval duration may result in large differences in the total amount of O_2 taken up during. This explains the large variability in estimated DMR for short dives which have a short surface interval (Fahlman et al., 2008b).

During spontaneous diving bouts, the O_2 uptake during the surface interval depends on the previous history of dives and makes it difficult to accurately estimate the energetic cost of foraging in Steller sea lions on a dive-by-dive basis. Attempting to estimate the costs on a dive-by-dive basis would artificially decrease the mean and increase the variability of the DMR estimate for short dives with short surface intervals (Kooyman et al., 1973). Including the end-bout recovery period makes it possible to estimate the overall metabolic cost of an entire bout, but without an end-bout recovery period, a portion of the O_2 debt will not be accounted for and the average metabolic rate for the bout will appear lower than the actual cost. However, only analyzing dives with a long surface interval may overestimate the DMR. As a consequence, respirometry studies on continuously diving animals need to consider the entire dive bout as a functional unit for the purpose of estimating DMR.

Does CO_2 removal determine the length of the surface interval?

Our respirometry system did not allow measurement of breath-to-breath gas exchange unlike that used by Boutilier et al. (Boutilier et al., 2001). However, our instantaneous RER values were consistently <0.7 after the sea lion surfaced and then increased to >1.0 as the surface interval progressed (Fig. 1). This agrees with the RERs reported for the harbour porpoise (*Phocoena phocoena*) and grey seal (*Halichoerus grypus*) (Boutilier et al., 2001) suggesting that mostly O_2 is exchanged during the first couple of breaths followed by rapid exchange of CO_2 towards the end of the surface interval.

Although our data indicate that re-adjustment of the O_2 stores is not the principal variable that determines the length of the surface interval, O_2 stores are nevertheless not fully readjusted at the end of SIs<50 s (Boutilier et al., 2001). Instead, sea lions dive with a small O_2 debt that is not paid off until the end of the bout, similar to Weddell seals (Kooyman et al., 1973). The O_2 debt takes >50 s to fully restore and most of the surface intervals in a dive bout during spontaneous trials were shorter than this. Despite this, the O_2 stores are sufficiently restored within a few breaths to allow the sea lion to dive without significantly altering the aerobic dive duration. Following this, O_2 is slowly taken up while CO_2 is still removed and the surface interval ends when sufficient CO_2 has been removed, so that consecutive dives do not lead to a significant accumulation of CO_2. Sea lions therefore strive to optimize uptake of O_2 while also trying to remove sufficient CO_2 to be in a dynamic equilibrium that does not result in a continuous reduction in O_2 stores or accumulation of CO_2. This agrees with the output of optimal foraging models when applied to diving birds (Halsey and Butler, 2006).

During a few spontaneous dive trials, the sea lions dived after extremely short surface intervals (<10 s) for the entire diving bout. For such a dive pattern, O_2 may determine the length of the surface interval, leading to accumulation of CO_2. Such dive behaviour may be beneficial if a sea lion encounters a particularly dense prey patch, but would inevitably lead to elevated tissue and blood P_{CO_2}. However, elevated tissue and blood P_{CO_2} will ultimately force an animal to end a dive bout.

DMR and MR_S *versus* BMR; evidence for hypometabolism?

Blood and tissues are saturated with O_2 before each conditioned dive. The O_2 uptake during the recovery phase after a dive is therefore a direct reflection of the resting O_2 uptake rate during the surface interval and reloading of the tissue and blood O_2 that was used during the dive. This represents the true O_2 uptake rate during the dive event and is therefore a better reflection of the actual energetic cost of the dive. In addition, the cost is independent of previous dives and only depends on activity during that particular dive (Fahlman et al., 2008b). The results from the conditioned trials are particularly interesting as they suggest that diving metabolic rate is not much different from the pre-dive sMR_S, although sDMR decreases below sMR_S during long dives (Fig. 2).

We did not detect any changes in MR_S between dives during conditioned trials and there were no differences in pre-dive MR_S or the MR_S during the recovery phase following a bout as would have been expected if the sea lions had begun to digest their food (heat increment of feeding). Others have measured a significant increase in the metabolic rate of pinnipeds while resting on land several hours after their last dive. In particular, Sparling et al. (Sparling et al., 2007) noted that digestion was inhibited in grey seals throughout a foraging bout and that MR_S did not increase until long after the dive bout had ended. Given that it takes more than 30 min for MR_S to increase in Steller sea lions after a meal (Rosen and Trites, 1997) and that the resting period that ended the dive bouts of the sea lions in our study was a maximum of 10 min, the increase we recorded in DMR associated with the last dive of each bout cannot be attributed to digestion. Instead, our data are consistent with the finding of Sparling et al. (Sparling et al., 2007) that diving animals defer digestion while foraging.

The minimum measured MR_S values were $0.901 O_2 min^{-1}$ for a 219.5 kg sea lion, $0.711 O_2 min^{-1}$ for a 166.2 kg animal and

0.711O_2 min^{-1} for a sea lion weighing 129.9 kg. These values were 55% to 85% higher than those predicted from Kleiber's equation for basal metabolic rate (Kleiber, 1961). Average MR_S values, however, were as much as 230% higher than those predicted by Kleiber (Kleiber, 1961). However, the pre-dive MR_S values in water were lower for both sea lions F97SI and F00BO than their previously MR_S values measured in air in previous experiments (1.921O_2 min^{-1} and 1.361O_2 min^{-1}, respectively) (Hastie et al., 2007). The in-water MR_S values were similar to those reported for grey seals (Sparling and Fedak, 2004), but slightly lower than the results reported for California sea lions (Hurley and Costa, 2001).

Some of the differences between MR_S values for grey seals, California sea lions and Steller sea lions may be due to differences in thermoregulatory costs. Seasonal air and water temperatures in our study of Steller sea lions (Vancouver, Canada) and that of grey seals (St Andrews, Scotland) were similar. However, the MR_S for California sea lions was measured at higher water temperatures (15–20°C) (Hurley and Costa, 2001) compared with the water temperatures in our study (4.3–18.9°C). The relationship between MR_S and water temperature was negative (Eqn 1) with MR_S falling by 24% when resting in water at 18.9°C compared with 4.3°C. Despite a shorter surface resting period and a water temperature as low as 4.3°C, sMR_S for female Steller sea lions was lower (8.4 mlO_2 min^{-1} kg^{-1}) than that measured in California sea lions. Thus, our data suggest that the sMR_S in female Steller sea lions at water temperatures ranging from 4°C to 19°C is lower than for California sea lions.

Kooyman (Kooyman, 1989) defined hypometabolism as a metabolic rate that is lower than that measured under standard conditions of resting in a post-absorptive state. Average DMR of the Steller sea lions decreased by ~8% when dive duration was extended from 1 min to 6 min (Fig. 3; Eqn 3). Overall, 32% (N=160) of all conditioned dives were below MR_S and 24% (N=118) were below minimum MR_S (Table 1; MR_{Smin}) for each sea lion. Results from the conditioned dives showed that the energetic cost of a dive event was only 8% higher than the average MR_S. Thus, DMR did not differ substantially from MR_S (Fig. 3).

Our results agree with those of others indicating that DMR is close to MR_S (Hurley and Costa, 2001; Sparling and Fedak, 2004). However, the relative decrease in DMR compared with MR_S is much less in our study than values reported for grey seals and California sea lions. This could highlight methodological differences in the respective experiments. In our study, the animals were unrestrained, while the grey seals were active but restrained by the size of the pool, and the California sea lions were inactive while submerged (see below).

The 8% decrease in DMR observed during longer dives in our study could be due to reduced cardiac work by a reduction in heart rate, i.e. the dive response. In Weddell seals, the metabolic cost of the heart was estimated to be ~12% of the total energy consumption (Davis and Kanatous, 1999). Assuming that cardiac work is directly related to heart rate means that lowered heart rates would reduce overall metabolic rate by 6% based on a 50% reduction in heart rate reported during a 6 min dive in California sea lions (Ponganis et al., 1997). Another possibility is that activity is lower during longer dives, thereby decreasing overall DMR (Fahlman et al., 2008b). This may facilitate a reduction in DMR and allow the sea lions to dive for longer without exceeding the calculated aerobic dive limit (cADL). Consequently, future studies should measure underwater activity concurrently with DMR to resolve whether the decrease in DMR during longer dives is a true hypometabolic state or whether it is caused by a behavioural adjustment in activity.

The decrease in DMR with dive duration in our study was not as large as was seen in California sea lions (Hurley and Costa, 2001). One possible reason for this difference between studies is that the sea lions we used swam freely and would have expended more energy while swimming. We have previously shown that activity is a good indicator of DMR (Fahlman et al., 2008b). Therefore, any metabolic suppression induced by greater dive duration may have been partly offset by the activity level during the dive, potentially explaining why DMR did not decrease with dive duration as much as in inactive California sea lions (Hurley and Costa, 2001).

Depth, and thereby the overall distance that the sea lion had to swim to the prey patches we created, did not affect foraging costs. We previously showed that metabolic savings by passive gliding during descent were similar to the additional cost of active swimming during ascent (Fahlman et al., 2008b), which explains why the metabolic cost of different dives to at least 50 m did not differ. During shallow dives, diving lung volume will significantly affect buoyancy and species that principally dive to shallow depths may adjust the inhaled air volume to adjust buoyancy (Fahlman et al., 2008a). During deeper dives, lung compression will reduce the effect of inhaled air volume on buoyancy (Bostrom et al., 2008), explaining why our results differ from those of deeper diving species (Williams et al., 2004; Williams et al., 1999). In fact, a previous study showed that New Zealand sea lions (*Phocarctos hookeri*) that dived to deeper depths spent more time gliding and had a lower field metabolic rate than individuals that made shallow dives (Costa and Gales, 2000).

Conclusions

In summary, the exchange of O_2 and CO_2 during a surface interval after breath-hold diving is a dynamic process. The first dive in a series of repeated spontaneous dives has the lowest O_2 uptake rate, whereas the last dive has the highest. This is because of adjustments of O_2 stores. In addition, variability in estimated DMR decreases with dive duration and surface interval duration during spontaneous dives, suggesting that estimated DMR from a dive with a short surface interval is unreliable. It is therefore difficult to accurately estimate the metabolic cost of an individual dive using exchange of O_2 when the surface interval is short between repeated spontaneous dives. Rather, O_2 exchange can be used to estimate metabolic cost of the entire diving bout. For dives where the surface interval is more than 50 s, O_2 uptake rate is a suitable estimate of the energetic cost of the dive event in adult female Steller sea lions. The RER increases throughout the surface interval, reflecting an initial rapid exchange of O_2 followed by mobilization of tissue and blood CO_2 that shows a delayed removal. The duration of the surface interval is determined by the dual need to restore sufficient O_2 and remove CO_2 so that neither becomes limiting. The delayed removal of CO_2 means that the surface duration is in most cases determined by the need to remove CO_2. When accounting for the O_2 debt, DMR appears to be almost the same as MR_S in Steller sea lions. DMR decreases with increasing dive duration, but it is not clear if this is secondary to a reduction in activity or reflecting a true hypometabolic state.

LIST OF ABBREVIATIONS

D	depth
DD	dive duration
DMR	metabolic rate of a dive event (dive+surface interval)
DMR_{Cond}	metabolic rate of conditioned dive
DMR_{Spon}	metabolic rate of spontaneous dive
M_b	body mass
MR_S	surface metabolic rate
MR_{Smin}	minimum MR_S

P_{CO_2}	partial pressure of CO_2
P_{LO_2}	partial pressure of O_2 in the lung
P_{O_2}	partial pressure of O_2
P_{VO_2}	partial pressure of O_2 in mixed venous blood
RER	respiratory exchange ratio ($\dot{V}_{CO_2}/\dot{V}_{O_2}$)
sDMR	mass-specific DMR
sMR_S	mass-specific MRs
SI	surface interval
T_{depth}	water temperature at depth
T_s	surface water temperature
$\dot{V}_{CO_2}$	rate of carbon dioxide expiration
$\dot{V}_{O_2}$	rate of oxygen uptake

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