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RESEARCH ARTICLE

Dive behaviour impacts the ability of heart rate to predict oxygen consumption in Steller sea lions (*Eumetopias jubatus*) foraging at depth

Beth L. Young[1,*], David A. S. Rosen[1], Allyson G. Hindle[1], Martin Haulena[2] and Andrew W. Trites[1]

[1]Marine Mammal Research Unit, University of British Columbia, Vancouver, BC, Canada, V6T 1Z4 and [2]Vancouver Aquarium, PO Box 3232, Vancouver, BC, Canada, V6B 3X8

*Author for correspondence (b.young@fisheries.ubc.ca)

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SUMMARY

The predictive relationship between heart rate (f_H) and oxygen consumption ($\dot{V}_{O_2}$) has been derived for several species of marine mammals swimming horizontally or diving in tanks to shallow depths. However, it is unclear how dive activity affects the f_H:$\dot{V}_{O_2}$ relationship and whether the existing equations apply to animals diving to deeper depths. We investigated these questions by simultaneously measuring the f_H and $\dot{V}_{O_2}$ of Steller sea lions (*Eumetopias jubatus*) under different activity states (surface resting or diving), types of dives (single dives or dive bouts), and depths (10 or 40 m). We examined the relationship over dives only and also over dive cycles (dive + surface interval). We found that f_H could only predict $\dot{V}_{O_2}$ over a complete single dive cycle or dive bout cycle (i.e. surface intervals had to be included). The predictive equation derived for sea lions resting on the surface did not differ from that for single dive cycles. However, the equation derived over dive bout cycles (multiple dives + surface intervals) differed from those for single dive cycles or surface resting, with similar f_H for multiple dive bout equations yielding higher predicted $\dot{V}_{O_2}$ than that for single dive bout cycles (or resting). The f_H:$\dot{V}_{O_2}$ relationships were not significantly affected by dive duration, dive depth, water temperature or cumulative food consumed under the conditions tested. Ultimately, our results demonstrate that f_H can be used to predict activity-specific metabolic rates of diving Steller sea lions, but only over complete dive cycles that include a post-dive surface recovery period.

Key words: Steller sea lion, diving, heart rate, metabolism.

INTRODUCTION

While there are numerous methods for measuring the energy expenditure of marine mammals in the field, not all are equal in reliability, precision or ease of use. The three most common current methods employ measures of doubly labelled water (DLW) turnover, body acceleration metrics and heart rate (f_H). The DLW method provides a mean estimate of field metabolic rate over a finite period that is not activity specific, with individual error estimates in pinnipeds ranging from –39 to +44% (Sparling et al., 2008). Body acceleration metrics, such as overall dynamic body acceleration (ODBA) and flipper stroking, are limited to predicting metabolic rate during active behaviours, and cannot account for changes in physiological state such as digestion (Bevan et al., 1997; Hays et al., 2004; Williams et al., 2004; Fahlman et al., 2008b; Green et al., 2009; Halsey et al., 2009). The f_H method estimates oxygen consumption rate ($\dot{V}_{O_2}$, an indirect measure of energy expenditure) from measured f_H (Fick, 1870). f_H can therefore provide activity-specific estimates of energy expenditure on a much finer time scale and for longer periods of time than the DLW method (Boyd et al., 2004; Butler et al., 2004; Ponganis, 2007), with comparable error estimates to ODBA under steady-state conditions (Green et al., 2009; Halsey et al., 2009).

The f_H method requires deriving species-specific predictive equations between f_H and $\dot{V}_{O_2}$ in a controlled environment before the technique can be used to predict $\dot{V}_{O_2}$ in the field (for a review, see Green, 2010). Previous studies in marine mammals and birds have made f_H–$\dot{V}_{O_2}$ comparisons while animals were submerged in a shallow swim mill (Woakes and Butler, 1983; Williams et al., 1991; Butler et al., 1992; Boyd et al., 1995; Ponganis et al., 1997; McPhee et al., 2003), walking on a treadmill (Froget et al., 2001; Green, 2001; Froget et al., 2002; Green et al., 2005), swimming horizontally in open water (Williams et al., 1993) or diving in a shallow tank (Bevan et al., 1992; Webb et al., 1998a). However, the artificial modes of locomotion and environments employed in these studies raise questions of applicability to animals that spend a considerable time diving to depth.

Despite the current use of f_H to predict $\dot{V}_{O_2}$ of diving marine mammals in the wild (i.e. Hindell and Lea, 1998; Boyd et al., 1999), it is not clear whether the f_H method is accurate for pinnipeds foraging at natural depths and for realistic dive durations. Accurate estimates of metabolic rate in the wild require f_H:$\dot{V}_{O_2}$ relationships that are derived under controlled conditions which encompass dive durations and dive depths that are representative of free-ranging animals. Previous studies that have investigated f_H and $\dot{V}_{O_2}$ in diving marine mammals have been limited by maximum tank depth (Webb et al., 1998a; Sparling and Fedak, 2004). Our study derived relationships between f_H and $\dot{V}_{O_2}$ in trained Steller sea lions freely diving in the open ocean to depths up to 40 m and for durations of 1–6 min, which reflect dive characteristics comparable to those of free-ranging animals (Merrick and Loughlin, 1997; Rehberg et al., 2009). Our specific objectives were therefore to (1) simultaneously measure and determine the relationship between f_H and $\dot{V}_{O_2}$ while Steller sea lions were foraging and diving to depths of up to 40 m and (2) determine whether f_H could be used to predict average metabolic rate (AMR) or diving metabolic rate (DMR) over either a single dive or a series of continuous dives (dive bout).

MATERIALS AND METHODS

Data collection

Our study was conducted from August to September, 2008, using three trained female Steller sea lions (*Eumetopias jubatus*, Schreber 1776) housed at the UBC Open Water Research Laboratory (Port Moody, British Columbia, Canada). Animals were housed in a specially designed floating pen that provided access to seawater and haulout space [for a full description, see Hastie et al. (Hastie et al., 2007)]. Two of the sea lions were 11 years old (body mass of F97SI, 218±4.4 kg; F97HA, 172±0.6 kg) and one was 8 years old (F00BO, 145±5.1 kg; total mass range 130–224 kg). All sea lions had been raised in captivity and had previously been trained to use all experimental apparatus. On non-trial days, animals were fed a diet of herring (*Clupea pallassi*) supplemented with vitamin tablets. Sea lions were fasted overnight, and then weighed on a platform scale prior to trials (±0.5 kg). All procedures and protocols were conducted in accordance with the guidelines of the Canadian Council on Animal Care (University of British Columbia no. A07-0413), and under permits from the Department of Fisheries and Oceans Canada (no. MML 2007-0001) and the Vancouver Aquarium. All animal work was conducted voluntarily under trainer control.

Measurement of f_H

f_H was measured using subcutaneous electrodes connected to a wireless transmitter, coupled to a datalogger. f_H monitoring equipment composition and placement are detailed elsewhere (Young, 2010; Young et al., 2010). Briefly, animals were outfitted with f_H monitoring equipment while anaesthetized (0–5% isoflurane in O_2), and all equipment was removed after each trial while the animals were under trainer control. The f_H monitoring system consisted of (1) a f_H datalogger (HTR, Wildlife Computers, Redmond, WA, USA) which recorded the R–R interval and (2) a f_H transmitter (HRX, Wildlife Computers) that had two ~80 cm leads. To reduce infection risk, we spliced 30 gauge, 99.9% pure silver Teflon-coated wire (Grass Technologies, Longueuil, QC, Canada) to the terminal ends of the electrode leads, and these ends were sterilized and inserted subcutaneously prior to each trial. The f_H transmitter and f_H datalogger were carried by the sea lions on a custom-fitted harness. The harness also held a time–depth recorder (TDR) that measured dive time, surface time and dive depth (sampling frequency 1 Hz, SU-05272, ReefNet Inc., Mississauga, ON, Canada). Animals were allowed to fully recover from anaesthesia in a dry area before the start of the trial. f_H was continuously measured immediately after both f_H electrodes were inserted.

Measurement of $\dot{V}_{\mathrm{O}_2}$

We measured $\dot{V}_{\mathrm{O}_2}$ using open-circuit gas respirometry as previously described for Steller sea lions (Hastie et al., 2007). Fractional oxygen and carbon dioxide concentrations within a desiccated (*via* $CaSO_4$) subsample of the excurrent airstream were measured using Sable System FC-1B and CA-1B analysers, coupled to a 500H mass flow generator and controller (Sable Systems Inc., Las Vegas, NV, USA).

Trials were conducted either at a dive site next to the holding pen or after the animals were transported to a nearby dive site in a 7 m research boat. A second boat towing a floating barge carried the respirometry equipment to the dive site. The floating barge had an opening in the middle containing a cage (152×152×250 cm) used to contain the animal at the surface before and after dives, and a floating transparent Plexiglas® respirometry dome. Air was drawn through the respirometry dome at a constant rate of 475 l min^{-1}. The excurrent airstream was continuously subsampled, and averaged every 0.5 s (Sable Data Acquisition System, Sable Systems Inc.). Fractional gas concentration readings were corrected for electronic drift against ambient air before and after each trial. Barometric pressure and excurrent air temperature were also recorded (Airguide Instruments, Chicago, IL, USA) to correct readings to standard temperature and pressure, dry (STPD). Water temperature ranged between 14.4 and 17.5°C (mean 15.8±1.0°C, *N*=15 trials).

Trial protocol

The sea lions were trained to remain stationary within the floating respirometry dome, and then dive between two feeding tubes positioned at the same set depth (10 or 40 m) ~6 m apart (mean animal length ~2 m). The feeding tubes were used to maintain swimming activity at depth. These trial depths reflect the lower and upper range of dive depths commonly noted for free-ranging

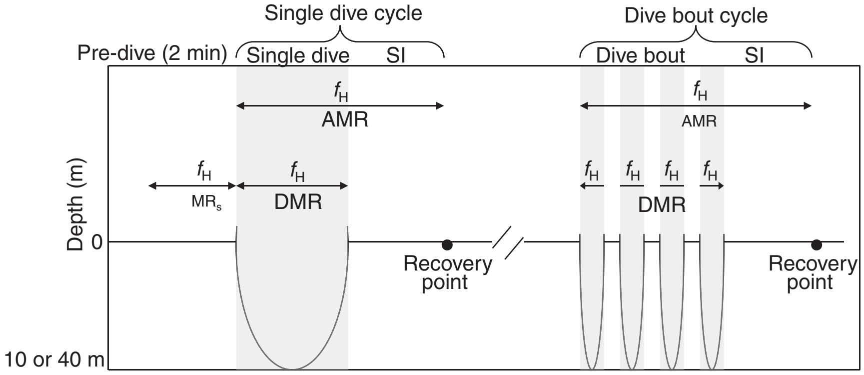

Fig. 1. Schematic diagram of a Steller sea lion dive trial showing dive depth (0–40 m) on the *y*-axis and time on the *x*-axis. The figure illustrates the varying intervals over which mean heart rate (f_H) and oxygen consumption rate ($\dot{V}_{\mathrm{O}_2}$) were used to calculate different predictive equations during dives (highlighted in grey) and surface intervals (depth 0 m). Surface metabolic rate or pre-dive $\dot{V}_{\mathrm{O}_2}$ (MR_s) was a mean calculated over 2 min prior to the initial dive. Average metabolic rate (AMR) for a single dive or dive bout used f_H and $\dot{V}_{\mathrm{O}_2}$ data from the start of the dive up until the post-dive recovery point (set at ±2% of MR_s), which were averaged across the total dive cycle duration (dive time plus surface interval, SI). Diving metabolic rate (DMR) over a single dive or dive bout included f_H data during the underwater portions only, and $\dot{V}_{\mathrm{O}_2}$ data only in excess of MR_s averaged across total dive duration.

females (Merrick and Loughlin, 1997; Rehberg et al., 2009). Each complete dive trial was repeated three times at each depth in random order for each animal (*N*=6 trials per animal), with only one trial per animal per day. Sea lions were not tested on sequential days. In the end it was necessary to discard one entire trial from each animal because of noise in the dataset (*N*=5 trials analysed per animal, *N*=15 bouts, *N*=25 single dives).

A trial consisted of (1) a 6–10 min resting period where sea lions were stationed in the respirometry dome that provided the baseline measurements of surface metabolic rate (MR_s), (2) a single dive with recovery period (see below), (3) a 4-series dive bout with recovery period at the end of the bout, and (4) a second single dive with recovery period, of longer dive duration than the first dive (40 m only, Fig. 1). Animals remained at the surface in the respirometry dome during post-dive recovery surface intervals (recovery periods), that ended when measured $\dot{V}_{O_2}$ returned to within 2% of MR_s (Fig. 1).

Animals were fed small pieces of herring (20 g) during trials to facilitate cooperation. A tube system allowed fish delivery to set depths and into the respiratory dome without altering the integrity of the flow-through measurements. Sea lions were encouraged (*via* altered underwater fish delivery rates) to execute dives of 0.5–2 min duration; field data demonstrate that 45% of free-ranging dives are 0–1 min and 33% are 1–2 min for female Steller sea lions (Merrick and Loughlin, 1997). For the 40 m trials only, an additional, longer single dive was encouraged at the end of the trial by providing food underwater at higher rates, which enabled consideration of the influence of dive duration independent of depth on the f_H:$\dot{V}_{O_2}$ relationship.

The cumulative amount of food consumed from the time the animals were outfitted with the experimental gear until the end of each specific event (i.e. end of dive or end of dive + surface interval) ranged from 0.4 to 2.8 kg. The amount of food sea lions were fed during a dive trial was intentionally minimized as previous work has indicated that the consumption of a 4 or 6 kg meal changes the f_H:$\dot{V}_{O_2}$ relationship in Steller sea lions resting in water (Young et al., 2010). However, the previous study did not account for the potential effects of diving or feeding on smaller amounts of prey at depth on the f_H:$\dot{V}_{O_2}$ relationship. Therefore, in this study we explicitly tested the effect of cumulative food consumption on the f_H:$\dot{V}_{O_2}$ relationship.

Data analysis

Calculating metabolic rate

$\dot{V}_{O_2}$ was calculated from fractional O_2 and CO_2 concentrations [see eqn 3b in Withers (Withers, 1977)] using Datacan Data Analysis software (V 1.0.24; Sable Systems Inc.) and then exported into Microsoft Excel. For resting MR_s, $\dot{V}_{O_2}$ was measured for 6–10 min after $\dot{V}_{O_2}$ had reached a steady state and averaged over the 2 min preceding the first dive of a trial. We analysed underwater O_2 consumption in two ways to maximize the comparability of our data to previously published work. First, we calculated AMR across a dive cycle (dive + surface interval). Second, we calculated DMR over solely the underwater time. Both analyses were performed on both single dives and dive bouts. AMR over a single dive cycle or dive bout cycle was calculated by dividing the total integrated volume (l) of O_2 consumed during the dive and surface interval by the entire dive cycle or dive bout cycle duration. DMR was calculated as the integrated post-dive $\dot{V}_{O_2}$ above the MR_s baseline, divided by time underwater. For single dives, time underwater was the actual dive duration. For dive bouts, time underwater was the cumulative dive duration.

Unfortunately, there is not a consensus on whether $\dot{V}_{O_2}$ should be scaled with body mass and, if so, which exponent to use (Packard and Boardman, 1999; Brown and West, 2005; White and Seymour, 2005; Savage et al., 2007). Overall, model results with non-mass-corrected $\dot{V}_{O_2}$ ($\mathrm{l\ O_2\,min^{-1}}$) and mass-corrected metabolic $\dot{V}_{O_2}$ ($\mathrm{ml\ O_2\,min^{-1}\,kg^{-0.66}}$ or $\mathrm{ml\ O_2\,min^{-1}\,kg^{-0.75}}$) did not differ from each other (i.e. different equations, but the same factors and models were significant). In addition, mass could not be incorporated into the equations as a covariate because of either convergence errors or a lack of significance as a factor.

Ultimately, we chose the most parsimonious approach and decided to use $\dot{V}_{O_2}$ units that did not assume a scaling exponent $\dot{V}_{O_2}$ ($\mathrm{l\ O_2\,min^{-1}}$). Therefore, all equations presented should only be applied to wild Steller sea lions within our experimental mass range (130–224 kg). To facilitate comparison with previous studies that did scale metabolic rate with body mass [including Young et al. (Young et al., 2010)], all model equations and figures using $\dot{V}_{O_2}$ ($\mathrm{ml\ O_2\,min^{-1}\,kg^{-0.75}}$) are available elsewhere [see pp. 50–71 of Young (Young, 2010)].

f_H

f_H measurements stored in the f_H datalogger were downloaded and analysed with Microsoft Excel and R 2.9.2 (R Core Development Team, 2009). First, R–R intervals were converted to instantaneous f_H ($f_{H,inst}$ in beats $\mathrm{min^{-1}}$). A series of algorithms were applied to systematically remove any $f_{H,inst}$ data that were artifacts of muscle or wire movement [detailed in Young et al. (Young et al., 2010)]. Finally, $f_{H,inst}$ values were averaged across appropriate intervals to match the $\dot{V}_{O_2}$ integrations of the same time periods, yielding mean f_H (beats $\mathrm{min^{-1}}$; Fig. 1). Specifically, resting f_H was calculated over the 2 min directly preceding the first dive. Mean f_H was calculated over the entire dive and surface period, from the start of the dive (or first dive in a bout) until the end of the surface recovery interval. Diving f_H was calculated over either the underwater portion of a single dive or the cumulative underwater duration for dive bouts (Fig. 1).

It has previously been suggested that otariid f_H may not scale linearly with body mass (Castellini and Zenteno-Savin, 1997). Furthermore, preliminary analysis of our data showed that there were no significant linear relationships between body mass and mean f_H over a single dive cycle (ANOVA *F*-test, $F_{1,21}$=0.44, *P*=0.52 for slope) or over a dive bout cycle (ANOVA *F*-test, $F_{1,11}$=0.90, *P*=0.36 for slope). These results, combined with our goal of consistency between f_H and $\dot{V}_{O_2}$ units, contributed to our decision not to scale f_H to body mass.

Statistical analysis

Data from each animal within a trial and data from each animal across trial types were treated as a repeated measures dataset using linear mixed-effects (LME) models in R 2.12.1 [nlme library from Pinheiro and Bates (Pinheiro and Bates, 2000)]. LME models consider the correlation between repeated measurements within and among animals, while also characterizing individual animal variation relative to the mean of the population (Pinheiro and Bates, 2000). As animals were allowed to recover to pre-dive $\dot{V}_{O_2}$ levels between single dives and dive bouts (i.e. $\dot{V}_{O_2}$ returned to within 2% of MR_s), single dives and dive bouts within the same trial were considered to be statistically independent. 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 inference from the sample captive population to the free-ranging population) and fixed effects explored included: dive duration (1.0–1.99 min, 2.0–3.99 min, >4.0 min), dive depth (10 m, 40 m), activity (resting at the surface or diving) and cumulative amount of food consumed (0–1.0 kg or >1.0 kg, max. 2.8 kg).

For each analysis, the best model in terms of fixed effect factors was determined using an ANOVA. Execution on a single model generated a conditional *F*-test to determine the significance of model slope, intercept and fixed effects. As all intercepts proved to be highly significant ($P\leq0.001$), the *F*-values reported here are for model slopes only. 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 (i.e. whether addition of fixed effect significantly improved null model). All models presented only had one fixed effect applied at a time; therefore, for all LRT tests d.f.=1. Within LME analysis, both models must be linear (i.e. significant slope and intercept) as determined with an *F*-test before being compared with an LRT test. Therefore, unless noted otherwise, all models compared *via* LRT tests were linear and statistics reported are for LRT tests. All reported data are presented as means ± s.e.m., and statistical significance was set at α=0.05.

Confidence intervals (95% CI) for final predictive equations were calculated using standard bootstrapping methods with 1000 replicates per model (Venables et al., 1999; R Core Development Team, 2009; Whitlock and Schluter, 2009). The ordered 24th and 976th bootstrapped replicates were then plotted to represent the 95% CI for a model.

The error associated with the predictive equations is not constant and increases with the distance from the mean f_H value, but many studies evaluating other techniques to estimate energy expenditure report a single, average error (e.g. Boyd et al., 1995). To facilitate comparison among studies, we estimated model error using both standard error of the estimate (s.e.e.) as well as ‘average percent error’. The s.e.e. for $\dot{V}_{O_2}$ was calculated using the minimum f_H for each model (Zar, 1999) [see eqn 10 in Green (Green, 2001)]. It is important to note that the s.e.e. values are maximum estimates because the error at the tails of each model is greater than the error at the mean f_H value (see Appendix Table A1 for s.e.e. mean and maximum f_H values). All s.e.e. equations included the error associated with variation between sea lions in slope and intercept during model optimization. We also derived the average percent error associated with each model, which was a single representative percent of the error based on the standard residuals. We calculated the average percent error by dividing the mean absolute fixed residual by the median predicted $\dot{V}_{O_2}$ value. We then multiplied this by 100 to calculate a percent error. Standard error of the mean (s.e.m.) for the slope and intercept is also presented in Table 2.

RESULTS

Dive characteristics

Dive duration for all single dives (*N*=25 dives) recorded from the TDR ranged from 1.0 to 6.3 min, and cumulative dive time for bouts ranged from 2.6 to 8.0 min (*N*=13 bouts, Table 1). Maximum dive depth ranged from 11 to 14 m (10 m trials) or from 41 to 58 m (40 m trials) as animals occasionally swam below the feeding stations temporarily before feeding. Our dive characteristics were comparable to those observed in free-ranging female Steller sea lions (Merrick and Loughlin, 1997; Rehberg et al., 2009).

Relationship between f_H and resting metabolic rate

There was a significant relationship between f_H and $\dot{V}_{O_2}$ for animals resting at the surface in open water (MR$_s$). This relationship does not significantly differ from the f_H:$\dot{V}_{O_2}$ relationship derived for four other Steller sea lions resting in a swim mill (~16 h postprandial) (Young et al., 2010). Further, combining data from these two sets of trials creates a stronger composite predictive equation. This composite equation that described all animals resting in water (fasted in swim mill + MR$_s$ in open water) was designated resting metabolic rate (RMR), and was used to make all comparisons to the diving f_H:$\dot{V}_{O_2}$ relationships. Mean RMR ranged from 0.81 to 2.30 l O$_2$ min^{-1} and the corresponding f_H ranged from 57 to 108 beats min^{-1}.

Relationship between f_H and DMR

Overall, f_H and DMR data were normally distributed, had normally distributed errors, and had homogeneous variance across single dives and dive bouts. Diving f_H during a single dive ranged from 27 to 68 beats min^{-1}, and DMR ranged from 1.21 to 3.21 l O$_2$ min^{-1}. Over dive bouts, diving f_H ranged from 34 to 75 beats min^{-1} (subsurface time only), and DMR ranged from 1.40 to 3.00 l O$_2$ min^{-1}. Bradycardia was notable for both dive types (on average, dive f_H was reduced from resting by 64% in single dives and 44% in bouts). However, no significant differences in DMR (2.06±0.56 l O$_2$ min^{-1} for single dives, 2.22±0.47 l O$_2$ min^{-1} for bouts) relative to RMR (1.41±0.35 l O$_2$ min^{-1}) were noted.

None of the predictive f_H:$\dot{V}_{O_2}$ relationships for DMR were significantly linear under any analysis conditions (either without fixed effects or including appropriate fixed effects such as depth, dive duration, cumulative food consumption and dive type, or after log transformation, Fig. 2). For example, diving f_H:$\dot{V}_{O_2}$ relationships were not linear for dive bout data alone ($F_{1,11}$=0.89, *P*=0.365), when combined with data from animals resting in water ($F_{1,77}$=0, *P*=1.00) or when combined with single dive data ($F_{1,32}$=1.18, *P*=0.283). Given that no predictive equations for DMR were linear, further comparisons (such as DMR *vs* AMR or RMR) were not undertaken.

Table 1. Summary of dives conducted by three Steller sea lions

	Duration (min)				Maximum dive depth (m)			
Data	Mean	s.d.	Min.	Max.	Mean	s.d.	Min.	Max.
Single dive (*N*=25)	2.6	1.5	1.0	6.3	35	16.7	11	58
Single SI	3.0	0.9	1.4	4.6				
Dive bout (*N*=13)	5.6	1.7	2.6	8.0	31	18.4	11	57
Dive bout cumulative SI	3.3	1.4	0.5	5.3				
All dives	3.6	2.2	0.9	8.0	32	17.0	11	58
All SI	4.3	1.2	2.7	6.5				

Dive and surface interval (SI) durations are presented for single dives, for dive bouts and for dive types combined. Dive bout data are cumulative from the first dive until the last SI (see Fig. 1). Associated maximum dive depths are also listed. Sample size of useable single dives and dive bouts is also indicated.

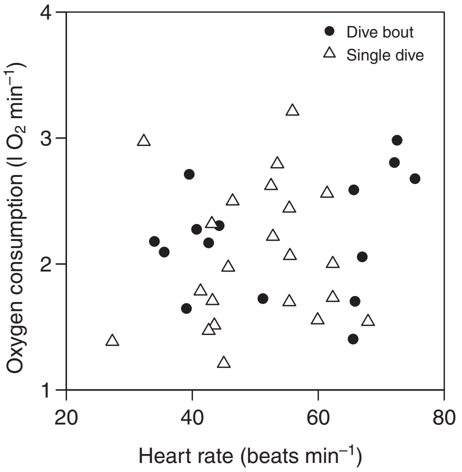

Fig. 2. The relationships between diving f_{H} and DMR for Steller sea lions over a dive bout or over a single dive were not significantly linear.

Relationship between f_{H} and AMR

There was a significant relationship between f_{H} and AMR, but this was not significantly affected by dive duration, dive trial depth, maximum dive depth or water temperature across single dive or dive bout cycles (dive + surface recovery interval). Cumulative meal size did not change the f_{H}:AMR$_{\mathrm{single}}$ relationship over dive cycles (LRT=1.14, *P*=0.89). Animals consumed less food during the first single dive cycle (mean 0.36±0.19 kg, 0–0.62 kg, *N*=17) compared with the last single dive cycle (mean 1.38±0.38 kg; 0.88–2.08 kg, *N*=8). Despite this, adding dive cycle order as a fixed factor (i.e. first or last dive bout) did not significantly change the f_{H}:AMR$_{\mathrm{single}}$ relationship (LRT=6.16, *P*=0.19). We therefore combined data from all single dive cycles for both trial depths for analysis.

Over a single dive cycle, average f_{H} ranged from 46 to 103 beats min^{-1} (mean 75±18.1 beats min^{-1}), and AMR$_{\mathrm{single}}$ ranged from 1.23 to 2.44 l O$_2$ min^{-1} (mean 1.82±0.35 l O$_2$ min^{-1}). The predictive equations describing the f_{H}:$\dot{V}_{\mathrm{O_2}}$ relationships over single dive cycles (Table 2: Eqn 2) and while animals were resting at the water surface (Table 2: Eqn 1) were statistically similar (LRT=10.73, *P*=0.030). Therefore, a single equation could be used to describe both data sets (Table 2: Eqn 3, Fig. 3A).

Over a dive bout cycle, average f_{H} ranged from 46 to 97 beats min^{-1} (mean 71±18.3 beats min^{-1}) and AMR$_{\mathrm{bout}}$ ranged from 1.59 to 2.70 l O$_2$ min^{-1} (mean 2.03±0.33 l O$_2$ min^{-1}). During dive bout cycles, sea lions were fed a maximum of 1.8 kg herring to facilitate cooperation (cumulative mean 1.0±0.33 kg). However, feeding did not affect the significantly linear f_{H}:$\dot{V}_{\mathrm{O_2}}$ relationship over a dive bout cycle (LRT=7.93, *P*=0.09; Table 2: Eqn 4). Combining data sets to test different factors yields slightly different predictive equations with mixed-effects models because of variability in degrees of freedom and replicate datasets from the same individuals across more than one trial type (i.e. Eqns 4, 5a and 6a in Table 2 for dive bouts are similar but not exactly the same). The f_{H}:$\dot{V}_{\mathrm{O_2}}$ relationship over a dive bout cycle differed from that for both RMR (LRT=34.66, *P*<0.0001; Fig. 3B; Table 2: Eqns 5a and 5b) and a single dive cycle (LRT=23.75, *P*=0.0001, Fig. 3C; Table 2: Eqns 6a and 6b). One aspect of these differences was that predicted AMR was often greater for bout cycles than for either single dive cycles or RMR at a given f_{H} (Fig. 3). Model convergence errors prevented direct comparison of the f_{H}:$\dot{V}_{\mathrm{O_2}}$ relationship between dive bout cycles (Table 2: Eqn 4) and the equation for pooled RMR and AMR$_{\mathrm{single}}$ data (Table 2: Eqn 3, Fig. 3A). However, given that dive bout equations were distinct from both single dive and RMR equations independently (Fig. 3A,B), we suggest that logically dive bouts are also different from the composite RMR + AMR$_{\mathrm{single}}$ line (Table 2: Eqn 3, Fig. 3A).

DISCUSSION

The predictive relationship between f_{H} and $\dot{V}_{\mathrm{O_2}}$ has been explored in several marine mammal species submerged in a shallow swim mill (Woakes and Butler, 1983; Williams et al., 1991; Butler et al., 1992; Boyd et al., 1995; Ponganis et al., 1997; McPhee et al., 2003), swimming horizontally in open water (Williams et al., 1993) or diving in a shallow tank (Webb et al., 1998b; Sparling and Fedak, 2004). The implicit or underlying assumption in applying these equations to animals in the wild is that these relationships remain constant independent of the animals' specific aquatic behaviour. This is an ambitious assumption given the range of cardiovascular and metabolic adjustments known to be employed by diving pinnipeds.

Table 2. Equations for selected linear models relating heart rate (f_{H}) to oxygen consumption ($\dot{V}_{\mathrm{O_2}}$) for Steller sea lions that were resting or diving

Eqn	Model description	*a*	Slope s.e.m.	*P*-value	*b*	Intercept s.e.m.	*P*-value	Food (kg)	N_1 animals	N_2 data points	s.e.e. at min. f_{H}
1	RMR resting in water	0.009	0.003	0.0026	0.713	0.231	<0.0001	<0.17	7	71	4%
2	AMR single dive cycle	0.010	0.004	0.0262	1.104	0.394	<0.0001	<2.08	3	25	22%
3	AMR single dive cycle + RMR	0.009	0.003	0.0002	0.708	0.212	<0.0001	<2.08	7	96	12%
4	AMR dive bout cycle	0.012	0.002	0.0001	1.104	0.114	<0.0001	<1.82	3	13	4%
5a	AMR dive bout cycle + RMR (factor=bout)	0.013	0.003	0.0268	1.058	0.316	<0.0001	<1.82	3	13	5%
5b	AMR dive bout cycle + RMR (factor=RMR)	0.013	0.003	0.0268	0.438	0.223	<0.0001	<0.17	7	71	8%
6a	AMR dive bout cycle + single dive cycle (factor=bout)	0.011	0.003	0.0001	1.229	0.213	<0.0001	<1.82	3	13	15%
6b	AMR dive bout cycle + single dive cycle (factor=single)	0.011	0.003	0.0001	0.830	0.352	<0.0001	<2.08	3	25	19%

Equations were of the form $\dot{V}_{\mathrm{O_2}}=a\times f_{\mathrm{H}}+b$, where *a* and *b* are the slope and intercept values, respectively.

Model slopes and intercepts (±s.e.m.) are presented along with associated *P*-values (ANOVA, *F*-test), maximum cumulative food provided for any trial within a given dataset, number of animals N_1 and number of data points N_2 included in each model. Standard error of the estimated $\dot{V}_{\mathrm{O_2}}$ (s.e.e.) at the minimum f_{H} was calculated with eqn 10 from Green et al. (Green, 2001) (see Appendix Table A1 for details). Model description abbreviations (AMR, RMR) are defined in the list of abbreviations. Equations predicting $\dot{V}_{\mathrm{O_2}}$ are for resting metabolic rate (RMR) or average metabolic rate (AMR) over a dive cycle or dive bout (dive + surface interval). Equations were derived using mixed-effects linear models with a repeated measures framework. Models were considered linear if both the slope and intercept were significant (ANOVA, *F*-test).

Eqns 1–4 are the most appropriate models for field application. Eqns 1 and 2 should be used when appropriate behaviour (i.e. resting at surface *vs* single dives) can be identified. However, as these two equations were not significantly different, Eqn 3 can be used if such behaviour cannot be differentiated. Eqn 4 should always be used for dive bouts. Eqns 5 and 6 provide statistical support for selecting Eqns 1–4 (as illustrated in Fig. 3).

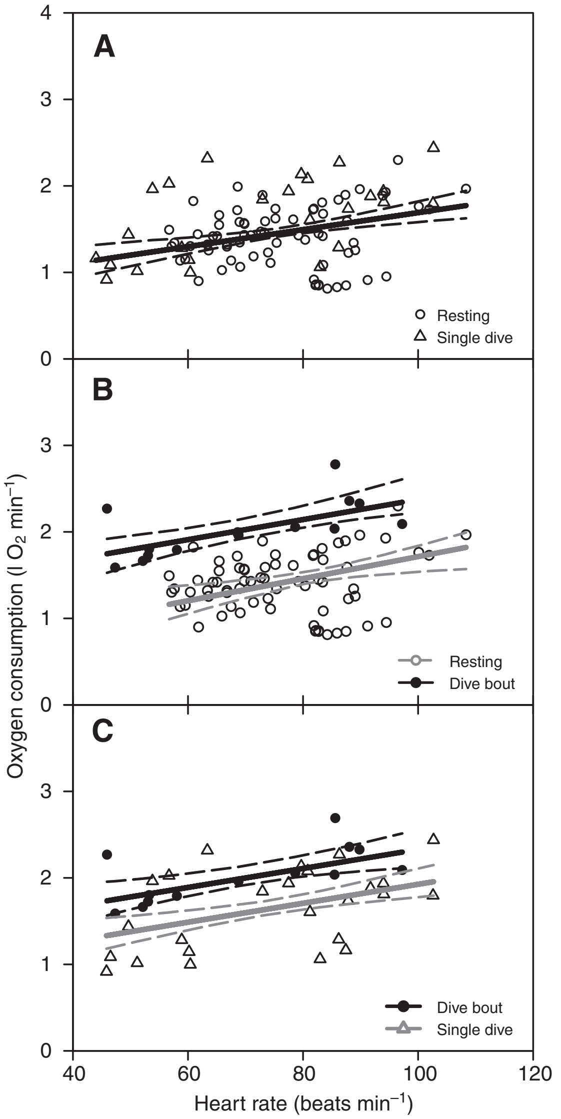

Fig. 3. The relationship between f_{H} and $\dot{V}_{\mathrm{O_2}}$ when Steller sea lions were resting (RMR, open circles) did not differ from that for predicting AMR over a single dive cycle (open triangles, A), but did differ from the relationship for predicting AMR over a dive bout cycle (filled circles, B). The relationship over a dive bout cycle (filled circles) differed from the relationship over a single dive cycle (open triangles, C). Equations were derived using mixed-effects linear models with a repeated measures framework.

Our results demonstrate that f_{H} can be used reliably to predict $\dot{V}_{\mathrm{O_2}}$ in diving Steller sea lions, but only when averaged over single dive cycles or dive bout cycles where animals are allowed to recover fully from any potential O$_2$ debt incurred during diving. Further, while the predictive equation for single dive cycles did not differ from that for animals resting in water, the relationship for dive bout cycles was significantly different from that for both single dives and resting. In contrast to previous studies that have simply pooled the data for several animals together, our conclusions are strengthened by using mixed-effects models within a repeated measures framework, with each animal treated as a random sample from the population. The statistical strength of our predictive models – despite our sample size – strongly indicates that detectable differences in the f_{H}:$\dot{V}_{\mathrm{O_2}}$ relationships represent a true biological change rather than a statistical artifact.

The results of our study also imply that predictive f_{H}:$\dot{V}_{\mathrm{O_2}}$ relationships must be derived from controlled studies that incorporate representative dive characteristics of free-ranging animals if f_{H} is to accurately estimate the metabolic rates of diving endotherms in the field. While many previous studies investigating the f_{H}:$\dot{V}_{\mathrm{O_2}}$ relationship in diving marine mammals have been limited by tank depth (Webb et al., 1998a; Sparling and Fedak, 2004), our open ocean site allowed sea lions to dive freely with dive characteristics (depths and dive durations) similar to those of sea lions in the wild (Merrick and Loughlin, 1997). The duration of such dives is probably shorter than the estimated aerobic dive limit of adult female Steller sea lions [7.5 min (Richmond et al., 2006); mean 7.1–7.6 min (Hastie et al., 2007)]. It is also important to collect calibration data from foraging behaviour approximating field conditions, given that locomotion can potentially increase the total energetic costs of diving (Williams et al., 2004; Hastie et al., 2007). Our use of two separated feeding tubes created active feeding and underwater exercise, which appeared to elevate DMR (cf. Hastie et al., 2007) to levels that were comparable to pre-dive values.

Relationship between f_{H} and DMR

We determined that f_{H} could be used to predict AMR over a dive cycle, for either single dives or dive bouts, but could not predict DMR. The lack of predictive power for DMR could be a function of the temporal disconnect between measurements of $\dot{V}_{\mathrm{O_2}}$ (measured as post-dive excess O$_2$ consumption above resting levels) and f_{H} (measured directly during submergence) (Fig. 1). This comparison assumes that the post-dive increase in $\dot{V}_{\mathrm{O_2}}$ is independent of any post-dive tachycardia that can facilitate increased gas exchange upon surfacing (see below). By contrast, the f_{H}:AMR relationship was formulated from data with a greater temporal overlap (Fig. 1). Specifically, the f_{H} component encompassed the dive itself and any surface recovery periods, and thus incorporated both diving bradycardia and post-dive tachycardia.

The lack of a linear relationship between f_{H} and DMR supports the concept that the dive cycle is a discrete and fundamental unit for diving that cannot be partitioned physiologically into underwater and surface elements (Fedak et al., 1988; Butler and Jones, 1997). Several similar studies on diving vertebrates concluded that f_{H} and $\dot{V}_{\mathrm{O_2}}$ should be measured over complete dive cycles because their relationships over dives alone were inconsistent (Fedak, 1986; Fedak et al., 1988; Bevan et al., 1992; Butler, 1993). Dives and subsequent surface intervals are physiologically linked because gas exchange and removal of metabolic byproducts (i.e. CO$_2$) only occur at the surface (Kramer, 1988). Furthermore, repetitive dives in many species are associated with a progressive O$_2$ debt, which is not repaid until the bout is completed (Kooyman et al., 1973; Ponganis et al., 1993; Fahlman et al., 2008a). It is therefore not surprising that mean diving f_{H} was not useful in predicting DMR for Steller sea lions.

Relationship between f_{H} and AMR

Our study found significant relationships from which AMR can be predicted using f_{H} over periods of rest, single dive or multiple dive bouts. The relationship was similar when the animals were resting at the surface or performing single dive bouts. The similar relationship between single dive cycles and surface resting suggests that the O$_2$ debt incurred over a single dive cycle was not sufficient to alter the relationship.

However, a unique equation was needed to describe the relationship during multiple dive bouts (Fig. 3). While the range and pattern of f_{H} and $\dot{V}_{\mathrm{O_2}}$ across single dive cycles and dive bout cycles overlapped, dive bouts yielded slightly higher $\dot{V}_{\mathrm{O_2}}$ for a given f_{H}

because of a greater intercept value for the dive bouts (Fig. 3C). More detailed analyses of the dive bradycardia data collected here further demonstrated that mean diving f_H was similar across single dives and dive bouts (Hindle et al., 2010), as has been demonstrated in California sea lions (*Zalophus californianus*, dive durations 1–3 min) (Ponganis et al., 1997).

Although single dives and dive bout cycles are subject to similar physiological and environmental effects, dive bouts incur the cumulative effects of multiple dives, most notably a progressive O_2 debt (Kooyman et al., 1973; Ponganis et al., 1993; Fahlman et al., 2008a). Unfortunately, the relationship between f_H and $\dot{V}_{O_2}$ is not constant across levels of oxygen debt. This is partly because rates of reoxygenation are affected by the P_{O_2} gradient (Kramer, 1988), such that oxygen is taken up faster into the bloodstream with greater levels of depletion. Therefore, a greater oxygen debt (as from dive bouts) will result in a greater effective $\dot{V}_{O_2}$ upon resurfacing without similar changes in f_H.

Whereas single dive cycles probably incorporate minimal exercise (and limited O_2 debt), dive bouts often encompass longer cumulative dive time, and more frequent changes in depth and pressure relative to single dives. Although dive depth and dive duration were not significant factors (when averaged over a complete dive cycle or dive bout cycle), it is possible that instantaneous changes in these parameters during the dive bout influenced the overall f_H:$\dot{V}_{O_2}$ relationship. Ultimately, the oxygen debt appears sufficiently pronounced in multiple dive bouts to result in a f_H:$\dot{V}_{O_2}$ relationship that is distinguishable from both AMR during surface resting and metabolic rate over a single dive cycle.

Errors associated with using the f_H technique

The model error for our 'best' models over single dive cycles and periods of rest was 17% average percent error (12% s.e.e., Table 2, Appendix Table A1), and the model error over all of the dive bout cycles was 9% average percent error (4% s.e.e., Table 2, Appendix Table A1). The model error for our recommended predictive equations was greater than the error recorded for equations predicting $\dot{V}_{O_2}$ from f_H of submerged swimming California sea lions [mean ~3%, range –28 to +23% (Boyd et al., 1995)], and slightly greater than the estimates derived from ODBA on Steller sea lions [range –4 to +7% (Fahlman et al., 2008b)]. Both of our types of error estimates were also greater than those of the DLW method for Antarctic fur seals [mean ~3%, range –11 to +20% (Costa and Trillmich, 1988)], but considerably less than error estimates for California sea lions using the same technique [mean ~36%, range –10 to +86% (Boyd et al., 1995)]. It is relevant to note that caution should be used when comparing error estimates from different studies as calculation methods vary widely among studies.

Our percent error estimates assume that the appropriate predictive equation will be applied to f_H data collected in the field. Dataloggers can be used to measure f_H, dive depth and dive duration and to determine when an animal is resting at the surface of the water, executing a single dive, or executing multiple dives in series (Ponganis, 2007). These data are essential for applying the most appropriate f_H:$\dot{V}_{O_2}$ equation from Table 2 to estimate metabolic rate in the field.

Given the absence of statistical differences between predictive equations for animals resting in water or completing a single dive cycle, we recommend using the composite equation encompassing all trials for animals resting at the surface or when performing single dives (Table 2: Eqn 3). Given that statistical differences exist between the resting and dive bout equations, it follows that the impact of using the 'wrong' equation to predict AMR of dive bouts should be considered. We therefore calculated the percent error for using the 'wrong' equation by comparing the predicted AMRs for a mean resting f_H of 100 beats min^{-1} among appropriate equations in Table 2. Incorrectly using the predictive equation for animals resting in water (Table 2: Eqn 1) to estimate $\dot{V}_{O_2}$ of a free-ranging Steller sea lion performing multiple dives in a series (Table 2: Eqn 4) would overestimate $\dot{V}_{O_2}$ by ~25% (i.e. 0.90 *vs* 1.20 l O_2 min^{-1}).

Given the large potential error associated with using an inappropriate equation, and knowing that it is reasonably easy to determine dive behaviour *via* small TDRs in situations were f_H is already being telemetered, Eqn 3 in Table 2 should be used when animals are resting at the surface or executing single dives, and Eqn 4 should be used when animals are executing a series of multiple dives (Table 2). The errors incurred by applying the wrong equation will only alter calculation of energy expenditure during the time when that specific activity is occurring. Therefore, the cumulative error for estimates of daily energy expenditure will depend directly on the activity budget. That is, if energy expenditure is miscalculated for an infrequent activity, the resulting error on daily expenditure calculations will be small. For example, of the 48% of the time female Steller sea lions spend foraging at sea, only ~22% is spent diving (Rehberg et al., 2009).

Logistically, it is not always possible to distinguish single recovery dive cycles from dive bout cycles in free-ranging animals (i.e. Hindell and Lea, 1998; Boyd et al., 1999). Wild female Steller sea lions dive quite frequently and dive behaviour may differ between the declining western Alaska sea lion population (mean of 17.5 dives h^{-1} for approximately 5 h day^{-1} (Merrick and Loughlin, 1997) or the slowly increasing eastern Alaska population (mean of 13.5 dives h^{-1} for approximately 3 h day^{-1} (Rehberg et al., 2009). Consequently, dive bout cycles are more likely to accompany successful foraging compared with single dive cycles; single isolated

Table A1. Standard error of the estimate (s.e.e.) for mixed-effects linear models

		At minimum f_H			At mean f_H			At maximum f_H		
Eqn	Model description	$f_{H,min}$	s.e.e.	s.e.e. (%)	$f_{H,mean}$	s.e.e.	s.e.e. (%)	$f_{H,max}$	s.e.e.	s.e.e. (%)
1	RMR resting in water	57	0.047	4%	77	0.046	3%	108	0.050	3%
2	AMR single dive cycle	46	0.341	22%	74	0.339	19%	103	0.341	16%
3	AMR single dive cycle + RMR	44	0.134	12%	76	0.133	9%	108	0.134	8%
4	AMR dive bout cycle	46	0.073	4%	70	0.070	5%	97	0.074	3%
5a	AMR dive bout cycle + RMR (factor=bout)	46	0.085	5%	75	0.087	4%	108	0.094	4%
5b	AMR dive bout cycle + RMR (factor=RMR)	46	0.085	8%	75	0.087	6%	108	0.094	5%
6a	AMR dive bout cycle + single dive cycle (factor=bout)	46	0.254	15%	73	0.253	13%	103	0.254	8%
6b	AMR dive bout cycle + single dive cycle (factor=single)	46	0.254	19%	73	0.253	16%	103	0.254	13%

RMR, resting metabolic rate; AMR, average metabolic rate; f_H, heart rate.

dives with a full surface recovery of O_2 stores (AMR_{single}) are less likely to occur than dive bouts when sea lions are exploiting a prey patch. We therefore recommend using the predictive equation for AMR over a dive bout cycle (AMR_{bout}, Table 2: Eqn 4) for any ambiguous diving behaviour when using f_H to predict field metabolic rate of diving Steller sea lions.

Prudent application of the appropriate predictive equations will facilitate obtaining reasonable estimates of activity-specific energy expenditure in wild Steller sea lions. This information can then be used to quantify the energetic cost of observed or predicted changes in sea lion behaviour related to changes in their biotic or abiotic environment.

APPENDIX

The s.e.e. made using a mixed-effects linear model (Table A1) can be estimated using the following formula [eqn. 10 in Green (Green, 2001); p. 339 in Zar (Zar, 1999)]. The formula includes additional error terms incorporating the error associated with random intercepts and random slopes [p. 202 in Venables et al. (Venables et al., 1999); p. 110 in Zuur et al. (Zuur et al., 2009)]:

$$\text{s.e.e.}_{\dot{V}_{O_2,i}} = \sqrt{\left(d_1 + d_2 + d_3\right)^2 \left(\frac{1}{N_1}\right) + e^2 \left[\frac{1}{N_2} + \frac{(f_{H,i} - f_{H,mean})^2}{\sum f_H^2}\right]} \,. \quad \text{(A1)}$$

In the equation $(d_1+d_2+d_3)^2$ is the variance associated with random effects between animals (intercept, slope and fixed factor if appropriate), N_1 is the number of sea lions in each model, e^2 is the residual error (scatter around regression line), N_2 is the number of data points included in each model, $f_{H,i}$ is the heart rate value for which $\dot{V}_{O_2,i}$ is predicted, $f_{H,mean}$ is the mean heart rate for the entire model and Σf_H^2 is the sum of all squared f_H values used in the model. It is important to note that model error is not a single number but, rather, a range that is smallest at the mean f_H value, and increases towards both tails. We therefore calculated s.e.e. at the minimum, mean and maximum f_H values for each model to clearly represent the range of error for each model. Standard error of the estimate was also expressed as a percentage of the estimated $\dot{V}_{O_2}$ (example below with minimum f_H) such that:

$$\text{s.e.e.\%}_{\text{at min } f_H} = \left(\frac{\text{s.e.e.}_{\text{at min } f_H}}{\text{Estimated } V_{O_2} \text{at } f_{H,\min}}\right) \times 100 \,. \quad \text{(A2)}$$

LIST OF SYMBOLS AND ABBREVIATIONS

AMR	average metabolic rate ($\dot{V}_{O_2}$) averaged over the dive cycle (dive + T_{MR_s})
DLW	doubly labelled water
DMR	diving metabolic rate ($\dot{V}_{O_2}$) averaged over dive
f_H	heart rate (beats min^{-1})
$f_{H,inst}$	instantaneous heart rate (beats min^{-1})
MR_s	$\dot{V}_{O_2}$ resting at the surface in open water (pre-dive)
ODBA	overall dynamic body acceleration
RMR	resting metabolic rate (MR_s + resting swim mill trials) (Young et al., 2010)
SI	post-dive surface interval (min)
T_{MR_s}	SI until returned to ±2% of pre-dive $\dot{V}_{O_2}$ level (MR_s)
TDR	time–depth recorder
$\dot{V}_{O_2}$	oxygen consumption rate (l O_2 min^{-1})

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