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ORIGINAL PAPER

High diving metabolism results in a short aerobic dive limit for Steller sea lions (*Eumetopias jubatus*)

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Abstract The diving capacity of marine mammals is typically defined by the aerobic dive limit (ADL) which, in lieu of direct measurements, can be calculated (cADL) from total body oxygen stores (TBO) and diving metabolic rate (DMR). To estimate cADL, we measured blood oxygen stores, and combined this with diving oxygen consumption rates (VO_2) recorded from 4 trained Steller sea lions diving in the open ocean to depths of 10 or 40 m. We also examined the effect of diving exercise on O_2 stores by comparing blood O_2 stores of our diving animals to non-diving individuals at an aquarium. Mass-specific blood volume of the non-diving individuals was higher in the winter than in summer, but there was no overall difference in blood O_2 stores between the diving and non-diving groups. Estimated TBO (35.9 ml O_2 kg^{-1}) was slightly lower than previously reported for Steller sea lions and other Otariids. Calculated ADL was 3.0 min (based on an average DMR of 2.24 L O_2 min^{-1}) and was significantly shorter than the average 4.4 min dives our study animals performed when making single long dives—but was similar to the times recorded during diving bouts (a series of 4 dives followed by a recovery period on the surface), as well as the dive times of wild animals. Our study is the first to estimate cADL based on direct measures of VO_2 and blood oxygen stores for an Otariid and indicates they have a much shorter ADL than previously thought.

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Abbreviations

ADL	Aerobic dive limit
cADL	Calculated aerobic dive limit
TBO	Total body oxygen stores
VO_2	Oxygen consumption rate
DMR	Diving metabolic rate
MR_S	Pre-dive (surface) metabolic rate
RMR	Resting metabolic rate

Introduction

Marine mammals that exploit resources at depth rely on physiological adaptions to extend the time they can remain submerged to increase their foraging efficiency (Houston and Carbone 1992). Foraging ability for a marine mammal inherently depends on overall diving ability, with the most efficient foraging thought to be done aerobically. This aerobic diving capacity is typically defined by the aerobic dive limit (ADL or diving lactate threshold; Butler 2006). The ADL is measured as the maximum dive duration before there is an increase in post-dive blood lactate concentration beyond resting levels (Kooyman 1985). An increase in post-dive lactate indicates anaerobic metabolism is being used to conserve onboard oxygen stores for critical systems that can only function aerobically (Kooyman et al. 1980). It is thought that marine mammals should preferentially dive within their ADL (Kooyman 1989); the extra time needed to recover from anaerobic dives would result in a lower overall foraging efficiency, as the surface interval needed to recover increases disproportionately with longer dive times (Kooyman et al. 1980).

The ADL has only been measured in five species of marine mammals; two Cetaceans, two Phocid seals and one Otariid, the California sea lion (Ponganis et al. 1993, 1997a, b; Williams et al. 1993; Shaffer et al. 1997). Due to the challenges of measuring blood lactate in a freely diving animal, the calculated ADL (cADL) is commonly used to compare diving ability among species (Butler 2006). The cADL is a simplified estimate of aerobic diving capacity based on the amount of oxygen stored in the body and the rate at which this oxygen is depleted (Ponganis et al. 1993; Castellini et al. 1992; Kooyman et al. 1980). In general, marine mammals have higher total body oxygen stores (TBO) than their terrestrial counterparts (Lenfant et al. 1970; Kooyman and Ponganis 1998). In addition, marine mammals can extend their cADL by decreasing the rate at which they use oxygen stores, such as by suppressing metabolic rate (Butler and Jones 1997; Kooyman and Ponganis 1998). They also have a high tolerance to very low partial pressures of oxygen in the blood and can control the rate and pattern of oxygen store utilization (Ponganis et al. 2011; Meir et al. 2009).

TBO consists of the oxygen carrying capacity of the blood, muscle and lungs. Unlike Phocids (true seals), in Otariids (sea lions and fur seals) the lung can make a significant contribution to onboard oxygen stores, since they inhale before a dive. Blood oxygen stores are a function of blood volume, red blood cell concentration (measured as hematocrit) and hemoglobin concentration. Muscle oxygen stores are a function of myoglobin concentration, which has also been shown to be higher in marine than in terrestrial mammals (Castellini and Somero 1981; Kooyman and Ponganis 1998). Blood and muscle oxygen stores (and hence diving ability) have been shown to develop with age (Richmond et al. 2006; Weise and Costa 2007; Fowler et al. 2007), and generally correlate with the time of weaning and the beginning of independent foraging (Horning and Trillmich 1997; Burns et al. 2005). Activity level and exposure to diving may also affect body oxygen stores during development (Noren et al. 2001), but the influence of exercise and diving on TBO of adult marine mammals post-development is unknown.

In addition to knowing TBO, obtaining an accurate estimate of the cADL also depends on calculating the rate at which oxygen stores are used during diving. Much of the large disparity among cADLs within and between species can be attributed to the considerable variation in how diving metabolic rate (DMR) is measured; such as estimated from field metabolic rate (Costa and Gales 2000, 2003; Fowler et al. 2007) or calculated from allometric scaling (Richmond et al. 2006). The most accurate measure of metabolic rate is made directly from oxygen consumption rates (VO_2), but even this can vary depending on the method of calculation, the environment in which it is measured (restricted aquarium tanks vs. freely diving in open water), and on behavioral or physiological differences between trained and wild animals. This variation makes interspecific comparisons of cADL measurements difficult, as well as those among multiple studies on the same species.

Despite the difficulties of estimating ADL, the assumption that most dives are aerobic appears to be true for most species with an empirically measured ADL (Butler and Jones 1997; Kooyman et al. 1980; Costa et al. 2001). In contrast, many species of Otariids appear to be diving beyond their calculated aerobic limits (Costa et al. 2001). The discrepancy might be attributable to the fact that few studies have reported cADL based on direct measures of DMR (via VO_2) on actively diving animals, and none have simultaneously measured TBO.

The only measured ADL for an Otariid is 2.3 min for a juvenile California sea lion and is significantly lower than previously estimated cADLs of up to 15 min for Steller sea lions (Ponganis et al. 1997a, b; Richmond et al. 2006). This estimated cADL is also high compared to the typical dive behavior of Steller sea lions in the wild. Studies on adult and juvenile Steller sea lions report dives being normally short, shallow and frequent, with most dives <4 min and the longest recorded dive being ~ 12 min (Merrick et al. 1994; Loughlin et al. 1998; Merrick and Loughlin 1997; Pitcher et al. 2005).

Our objective was to calculate the ADL of Steller sea lions by measuring blood oxygen stores (to calculate TBO) and DMR of a group of adult females trained to dive regularly in the open ocean. We compared the cADL to their actual dive durations, as well as to those of Steller sea lions recorded in the wild. We also investigated the effect of previous exercise on blood O_2 stores and compared this between seasons in non-diving animals. Our study is the first to calculate the ADL for an Otariid based on concurrent measurements of blood O_2 stores and direct measurements of DMR via VO_2, and results in one of the most accurate estimates of cADL for a marine mammal.

Methods

Study design/data collection

We used eight adult, female Steller sea lions that were collected from breeding rookeries as pups and raised in captivity at the Vancouver Aquarium (British Columbia, Canada). All animals were previously trained to use experimental equipment and performed all trials voluntarily under trainer control. Four of the sea lions (between 12 and 15 years old) were housed at the Open Water Research Laboratory (Port Moody, BC), and have been actively diving in the open ocean for research purposes since 2005 or 2007. Animals were fed a diet of herring (*Clupea pallassi*) and market squid (*Doryteuthis opalescens*) supplemented with vitamins. All experiments were conducted under UBC Animal Care Permit #A07-0413.

The four sea lions at the Open Water facility (OW) each underwent several trials to determine DMR from May–July 2011. Trials were conducted concurrent with a study on the effect of altered inspired gas concentrations on dive behavior and recovery time, but there was no significant affect of inspired gas on DMR. Briefly, TBO was calculated based on direct measures of blood oxygen stores and estimates from previous studies for muscle and lung oxygen stores (detailed below). This was done concurrently with the DMR trials (July 2011) for the sea lions at the OW facility and on four sea lions (9 years of age) at the Vancouver Aquarium (February and May 2012) for comparison of diving and non-diving individuals, and seasonal differences within the non-diving group. Calculated ADL was estimated by dividing TBO by DMR. These values were then compared to the voluntary dive behavior of the sea lions.

Diving metabolic rate

Diving metabolic rate was determined from four Steller sea lions diving voluntarily over a series of dive trials. Dive trials were conducted at a depth of either 10 or 40 m (to match behavior typical of wild sea lions). The experimental setup consisted of a floating platform with a square opening in the middle containing a submerged cage (1.52 m × 1.52 m × 2.5 m) and a floating transparent Plexiglas respirometry dome (100 L). Metabolic rate was measured in the dome using flow through respirometry. Air was drawn through the dome at 350 L min^{-1}. Air was sub-sampled and scrubbed of water vapor, then fractional concentrations of oxygen and carbon dioxide were measured using Sable System FC-1B and CA-1B analyzers, coupled to a 500H mass flow generator and controller (Sable Systems Inc., Las Vegas, NV, USA). Gas concentrations in the excurrent air stream were recorded every 0.5 s (Sable Data Acquisition system, Sable Systems Inc.). Barometric pressure, relative humidity, and air temperature were also recorded.

Animals were fasted overnight prior to trials and weighed each morning. They were fed less than 0.5 kg during transport to the dive site to minimize any effect of heat increment of feeding on metabolic rate (Rosen and Trites 1997). During trials, the animals wore a harness with a VHF transmitter and time depth recorder (ReefNet, Inc., Mississauga, ON, Canada). Pre-dive metabolic rate was measured at the start of each trial while animals rested inside the metabolic dome, as the last 3 min of 5–10 min period, when VO_2 was constant. Animals then dove voluntarily to the bottom of two feeding tubes that delivered ~20 g pieces of herring at depth (10 or 40 m) every 5 s for the duration of the dive.

The animals were asked to do either a single long dive or a 4-dive bout cycle, during which the animals chose both their dive and inter-dive surface interval durations. Following the single dive or dive bout, the sea lions remained at the surface in the respirometry dome for a post-dive 'recovery' measurement, defined as the time it took for the rate of oxygen consumption (VO_2) to return to within 5 % of pre- or post-dive baseline values, whichever was lower. The four diving animals each completed 5 or 6 dives of each type (single and bout) and at two different depths (10 or 40 m). To facilitate comparisons to other studies, DMR was calculated two different ways: by dividing the total oxygen consumed in excess of post-dive baseline in the recovery period over the dive duration only (DMR_{dive}; as per Hastie et al. 2007; Hurley and Costa 2001) and as the average VO_2 over the dive and recovery period (DMR_{cycle}; as per Fahlman et al. 2008; Kooyman et al. 1980). For bout dives, DMR_{cycle} was the average VO_2 for the entire cycle of dives and recovery period.

Metabolic data was analyzed using LabAnalyst X (Warthog systems, Mark Chappell, University of California). Data was corrected for electronic drift by baselining gas concentrations to ambient air at the beginning and end of the trial. The entire gas analysis system was periodically calibrated with gases at known concentrations. Rates of oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were calculated using equations 11.7 and 11.8 in Lighton (2008).

Resting metabolic rate (RMR) of non-diving animals

RMR of non-diving Steller sea lions was measured while animals were resting in water in a small tank at the Vancouver Aquarium using the same metabolic equipment as described above for diving animals. They were fasted overnight prior to measurements of VO_2 and all trials were completed within their assumed thermo-neutral zone. RMR was calculated as the lowest 20 min average of a 40–45 min trial period (when VO_2 had reached a steady state) and was measured once for each animal in each season.

Total body oxygen stores

Estimates of oxygen stores in the lung, blood and muscle were combined to determine TBO. We assumed full use of the lung O_2 stores during the dive. Lung O_2 stores were estimated based on actual measures of body mass (M_b), and assumed diving lung volume of 55 ml kg^{-1} (Lenfant et al. 1970) and 15 % O_2 content (Kooyman et al. 1971) such that:

$$\text{Lung } O_2\,(\text{mL}) = M_\text{b}(\text{kg}) \times 55\,\text{mL kg}^{-1} \times 0.15$$

Muscle O_2 stores were calculated assuming a muscle mass of 37 % of total body mass (Richmond et al. 2006), and a myoglobin concentration [Mb] for adult Steller sea lions of 28.7 mg g^{-1} and 20.0 mg g^{-1} of wet weight muscle, for swimming muscle and non-swimming muscle, respectively (Kanatous et al. 1999). Muscle was assumed to be 52 % swimming and 48 % non-swimming as measured on 1 month old pups (Richmond et al. 2006). Muscle O_2 stores were calculated assuming an oxygen binding capacity of 1.34 ml O_2 g^{-1} Mb (Kooyman and Sinnett 1982) using the equation:

$$\text{Muscle } O_2\,(\text{mL}) = 0.37 \times M_\text{b}(\text{kg}) \times 1.34\,\text{mL g}^{-1}\text{Mb} \times \%\text{muscle type} \times [\text{Mb}]$$

Blood oxygen stores were measured directly from each sea lion. Blood samples were taken from the caudal gluteal vein shortly after animals were anesthetized using isofluorane gas (maximum 5 % isofluorane) under veterinary control. Blood samples were taken as early in the anesthetic procedure as possible, as soon as animals were induced (10–15 min). Samples were analyzed by a commercial laboratory (Idexx Laboratories, Delta, BC) for hematocrit and hemoglobin values.

Plasma volume was measured using Evans blue dilution procedure (Gibson and Evans 1937). After taking background samples, Evans blue dye (0.5 mg kg^{-1} dose, Sigma-Aldrich; E2129) was injected into a vein in the rear flipper through an intravenous catheter. Serial samples were taken at approximately 8, 16, 24, and 32 min after injection (exact time noted per sample). Plasma was analyzed using a simplified technique whereby the relationship between the optical densities at two different wavelengths (624 and 740 nm) can be used to correct for the presence of plasma in dye samples (Nielsen and Nielsen 1962; Foldager and Blomqvist 1991; El-Sayed et al. 1995). A linear regression of the absorption over time was used to determine the concentration of dye at the time of injection from the *y*-intercept, which was then used to calculate instantaneous dilution volume using standard curves created from stock solutions.

Blood volume (BV) was calculated from hematocrit (Hct) and plasma volume (PV) using the equation:

$$\text{BV}(L) = \text{PV}(L) \times \frac{100}{(100 - \text{Hct})}$$

Blood O_2 stores were calculated using BV and hemoglobin concentration [Hb], assuming an oxygen binding capacity of hemoglobin of 1.34 ml O_2 g^{-1} Hb (Kooyman and Sinnett 1982). Blood was assumed to be 1/3 arterial, that it was 95 % saturated at the beginning of the dive and reduced to 20 % at the end of the dive, and 2/3 venous, assumed to be 5 vol % less than initial arterial saturation (Richmond et al. 2006; Ponganis et al. 1993). Hence:

$$\text{Arterial } O_2(\text{mL}) = 0.33 \times \text{BV}\,(\text{mL}) \times (0.95 - 0.20) \times (1.34\,\text{mL g}^{-1}\,\text{Hb}) \times [\text{Hb}](\text{g mL}^{-1})$$

$$\text{Venous } O_2(\text{mL}) = 0.67 \times \text{BV}(\text{mL}) \times (\text{arterial } O_2 \text{ content} - 5\text{vol}\%)$$

Statistical analysis

All data was analyzed using R software (R Development Core Team, 2011). Data from each animal were treated as repeated measures by including animal ID as a random effect, using linear mixed-effects models (lme) from the nlme package (Pinheiro et al. 2011). Models were run using the maximum likelihood method. Fixed factors for blood and TBO parameters included group (diving or non-diving) and season, and for dive data included dive duration (2–7 min), depth (10 m or 40 m) and type of dive (single or bout). If multiple fixed factors were significant, nested models (with or without a fixed effect) were compared using a log likelihood ratio test to determine the best overall model to fit the data (Pinheiro and Bates 2000). A repeated measures ANOVA on a single model was performed to determine if the slope and intercept were significant. For significant categorical factors, post hoc tests (using the Bonferroni method) were performed to compare the means between multiple groups. Values are reported as means (±SD) and significance was set at $\alpha = 0.05$.

Results

Blood oxygen stores of diving and non-diving sea lions

There were no significant differences in blood oxygen stores between the diving and non-diving groups of animals or between the seasons within the non-diving group (Table 1). Mass-specific blood oxygen stores averaged 15.5 (±2.6) ml O_2 kg^{-1}. Therefore, estimated TBO averaged 35.9 (±2.6) ml O_2 kg^{-1} (with lung and muscle O_2 stores comprising 8.25 and 12.2 ml O_2 kg^{-1}, respectively). All blood parameters measured (BV, PV, Hct, and [Hb]; Table 1) were slightly higher in winter (non-diving group), but only mass-specific blood volume showed a significant difference between seasons [Fig. 1; Season vs. BV (ml kg^{-1}); $p < 0.001$ for winter vs. both spring and summer] resulting in slightly higher blood O_2 stores as well ($p = 0.051$). Blood volume averaged 109 ml kg^{-1} in winter (non-diving) and 99.6 ml kg^{-1} and 97.5 ml kg^{-1} in the spring (non-diving) and summer (diving) groups (overall range 94–115 ml kg^{-1}). Plasma volume ranged from 51 to 63 ml kg^{-1}. We did not compare TBO between groups as the lung and muscle components were estimated based on mass scalars.

Metabolic rate

RMR in non-diving animals was 1.29 L O_2 min^{-1} (for animals averaging 172 kg), which was lower than pre-diving metabolism in diving animals (1.78 L O_2 min^{-1} for animals averaging 193 kg). However, metabolism was measured in different environments for the two groups; the non-diving animals spent up to 40 min in a pool compared with the diving animals that rested in a small open ocean pen prior to their first dive. As the latter was measured just prior to their dive it was likely higher than true resting values, and is defined as a "pre-dive" metabolic rate.

Diving metabolic rate was higher than pre-dive metabolism when calculated based on both the "dive event" (dive + recovery, DMR_{cycle}) and when all excess oxygen consumed was attributed to the dive only (DMR_{dive}). Mean DMR_{cycle} for single dives ($n = 43$ single dives, both depths included, grand mean calculated from the average for each individual) was 2.24 L O_2 min^{-1} and for a bout of four consecutive dives ($n = 40$ bout dives, both depths included, grand mean calculated from the average for each individual) was 2.44 L O_2 min^{-1} (Table 2). Mean DMR_{dive} for single dives was 2.88 L O_2 min^{-1} but also depended significantly on dive duration (Fig. 2; $p < 0.001$). A "minimum" DMR_{dive} was estimated as 2.68 L O_2 min^{-1} for dives >4.5 min, as DMR_{dive} for these dives no longer depended on dive duration (Fig. 2).

Aerobic dive limit of Steller sea lions

Aerobic dive limit based on DMR_{cycle} for a single dive was 3.0 min and based on DMR_{dive} (for dives >4.5 min) was 2.5 min. Both of these estimates were shorter than the average single dive duration of the captive animals for our study, which was 4.4 (±0.5) min (Fig. 3). However, they were slightly longer than the average dive time for repeated dives in a bout, which was 2.0 (±1.3) min, with an average surface interval of about 18 (±14) s between these dives.

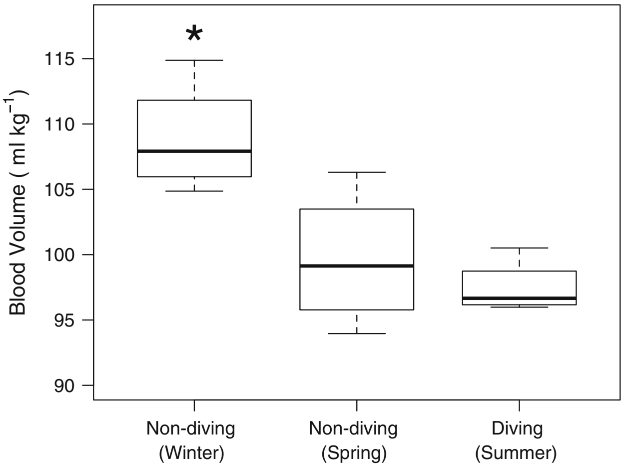

Fig. 1 Blood volume (ml kg^{-1}) of diving and non-diving Steller sea lions as measured by Evans blue dilution. Blood volume during winter (non-diving, $n = 4$) was significantly higher (*Asterisks*) than in the spring (non-diving, $n = 4$) or summer (diving, $n = 4$)

Table 1 Blood parameters including hematocrit (Hct), plasma volume (PV), blood volume (BV), hemoglobin concentration [Hb], blood O_2 stores, total body oxygen stores (TBO), and mass (day of blood O_2 store measurement) of diving and non-diving captive Steller sea lions

Animal	Group	Season	Mass (kg)	Hct	PV (ml kg^{-1})	BV (ml kg^{-1})	[Hb] (g/l)	Blood O_2 (ml kg^{-1})	TBO (ml kg^{-1})
F97BO	Diving	Summer	157	0.42	55.7	96.0	155	14.4	34.8
F00HA	Diving	Summer	175	0.44	56.3	100.5	160	15.7	36.1
F00YA	Diving	Summer	218	0.43	55.3	97.0	141	13.0	33.4
F97SI	Diving	Summer	225	0.41	56.8	96.3	150	13.9	34.3
Average			194	0.43	56.0	97.5	152	14.2	34.6
F03AS	Non-diving	Spring	171	0.40	56.4	94.0	137	12.1	32.5
F03RO	Non-diving	Spring	171	0.49	51.3	100.7	170	16.9	37.3
F03WI	Non-diving	Spring	178	0.50	53.1	106.3	166	17.3	37.8
F03IZ	Non-diving	Spring	180	0.40	58.6	97.6	137	12.6	33.0
Average			175	0.45	54.9	99.6	153	14.7	35.1
F03AS	Non-diving	Winter	174	0.49	55.5	108.8	175	18.9	39.3
F03RO	Non-diving	Winter	164	0.48	59.7	114.9	169	19.1	39.6
F03WI	Non-diving	Winter	176	0.49	54.6	107.1	173	18.4	38.8
F03IZ	Non-diving	Winter	169	0.40	62.9	104.9	137	13.5	33.9
Average			171	0.47	58.2	108.9[a]	164	17.5	37.9

[a] Mass-specific blood volume is significantly higher in winter than in spring or summer

Table 2 Metabolic rates and aerobic dive limit of Steller sea lions including pre-dive (surface) metabolic rate (MR_S), diving metabolic rate as a function of: the single dive cycle (DMR_{cycle}), dive time only (DMR_{dive}) and a dive bout cycle (DMR_{bout}). Mass (average for the trial period), calculated aerobic dive limit (cADL) based on DMR_{cycle} and on the minimum estimate of DMR_{dive}, mean dive duration of single long dives and mean dive duration to cADL ratio are also listed

Animal	Mass (kg)	MR_S (L O_2 min^{-1})	DMR_{dive} (L O_2 min^{-1})	DMR_{cycle} (L O_2 min^{-1})	DMR_{bout} (L O_2 min^{-1})	TBO (ml O_2 kg^{-1})	cADL (DMR_{cycle})	cADL (minimum DMR_{dive})	Mean dive duration (min)	Dive duration: cADL ratio
F97BO	158	1.42	2.54	1.88	2.17	34.8	2.9	2.5	3.8	1.3
F00HA	170	1.72	2.60	2.11	2.21	36.1	2.9	2.6	5.0	1.7
F00YA	216	1.77	3.05	2.34	2.59	33.4	3.1	2.4	4.2	1.3
F97SI	226	2.20	3.33	2.62	2.80	34.3	3.0	2.5	4.5	1.5
Average	193	1.78	2.88	2.24	2.44	34.6	3.0	2.5	4.4	1.5

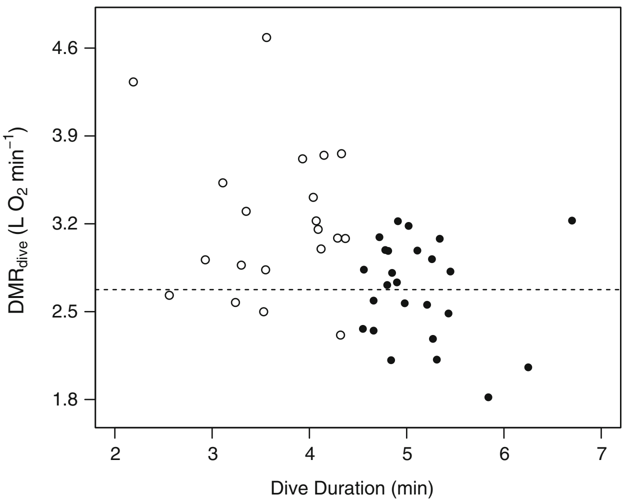

Fig. 2 Diving metabolic rate as a function of dive duration. DMR is calculated versus dive time only (DMR_{dive}). DMR_{dive} decreases significantly as dive duration increases. The *dashed line* represents the average DMR_{dive} for dive durations >4.5 min (*filled circles*) used to calculate ADL

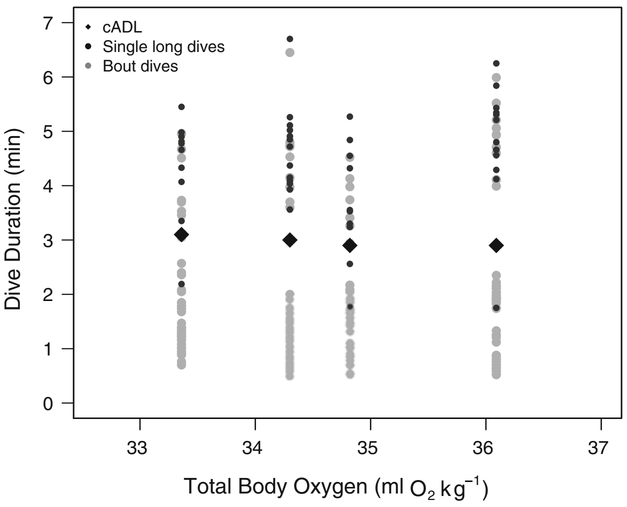

Fig. 3 Calculated aerobic dive limit (cADL) and dive durations as a function of total body oxygen stores (ml O_2 kg^{-1}) for each of the four diving animals. Single long dives (*dark circles*) are typically longer than the cADL (*diamonds*, based on DMR_{cycle}). Bout dive durations (*grey circles*) for each animal are also shown

Discussion

Oxygen stores and consumption

The average TBO of adult female Steller sea lions in our study was 35.9 ml O_2 kg^{-1}, which was slightly lower than estimates from previous studies of 38.8 and 40.4 ml O_2 kg^{-1} conducted on younger sea lions (Richmond et al. 2006; Lenfant et al. 1970). The effect of exercise and diving activity on TBO in adult animals is unknown. We predicted actively diving animals may have higher hemoglobin concentrations or higher blood volumes that would result in higher blood oxygen stores. However, blood O_2 stores did not differ significantly between the sea lions that were on an active diving regime and those that were not. Although development of oxygen stores in Otariids occurs over the first few years of development (Fowler et al. 2007; Richmond et al. 2006; Weise and Costa 2007), it may be relatively static in later years. Both groups of sea lions had been initially raised in the same captive environment that did not allow them to dive beyond 3 m during the developmental period. Thus, the group that was later moved to the open water site did not have the opportunity to develop a strong diving ability during this formative period, resulting in the lack of differences between groups that was not overcome by later differences in exercise regimes. Conversely, blood O_2 stores may not be significantly affected by early diving experience, and therefore our values are representative of animals in the wild. Surprisingly, we found that blood volume was higher in non-diving animals. However, these differences in blood volume did not translate into significantly greater blood oxygen stores and were only higher in the winter season. Thus, we suspect the differences reflected seasonal changes in physiology rather than previous diving activity.

Differences in TBO due to activity or exercise could be manifested as changes in myoglobin concentration or muscle mass, which we were unable to measure. This has not been studied in Pinnipeds, but myoglobin concentrations have been shown to be higher in dive-trained tufted ducks and exercising bar-headed geese than inactive controls (Stephenson et al. 1989; Saunders and Fedde 1991). Based on this, it is conceivable that our diving sea lions may also have had elevated myoglobin concentration (that would have resulted in higher TBO) due to their years of diving experience, even though they were not diving at a young age.

In general, Otariids have much lower mass-specific TBO than has been found in Phocid seals (Lenfant et al. 1970; Burns et al. 2005, 2007; Kooyman et al. 1983; Ponganis et al. 1993; Noren et al. 2005). This is also reflected by shorter than expected dive times relative to their body size (Schreer and Kovacs 1997). Among Otariids, Steller sea lions also have a lower mass-specific TBO than most other sea lions and fur seals. This can be attributable to differences in either blood volume or muscle myoglobin concentration. For example, while blood volume of Steller sea lions in our study was similar to northern fur seals, young Steller sea lions and California sea lions (Lenfant et al. 1970; Ponganis et al. 1997b), adult female California sea lions have higher myoglobin concentration resulting in greater TBO (Weise and Costa 2007). Conversely, while the muscle myoglobin concentration in Steller sea lions is similar to that of New Zealand and Australian sea lions, these species have a much higher blood volume (i.e., 152 ml O_2 kg^{-1} in New Zealand sea lions and 178 ml O_2 kg^{-1} in Australian sea lions, vs. 97.5 ml O_2 kg^{-1} in our Steller sea lions) also resulting in greater TBO (Costa et al. 1998; Fowler et al. 2007). Clearly, a better understanding of the selective pressures and ecological consequences of differences in TBO among different Otariids would benefit from a more comprehensive study of actual blood and muscle oxygen store measurements across species.

In our study, diving VO_2 was higher than “pre-dive” metabolism (1.78 L O_2 min^{-1}; 9.44 ml O_2 kg^{-1} min^{-1}), whether measured over the entire dive cycle (DMR_{cycle}, 2.24 L O_2 min^{-1}; 11.6 ml O_2 kg^{-1} min^{-1}) or when all excess oxygen was attributed to the dive only (DMR_{dive}, 2.88 L O_2 min^{-1}; 15.0 ml O_2 kg^{-1} min^{-1}). A previous study on the same group of animals found slightly lower metabolic rates, but a similar pattern—DMR_{cycle} was 9.9 ml O_2 kg^{-1} min^{-1} and was slightly higher than resting rates of 8.2 ml O_2 kg^{-1} min^{-1} (Fahlman et al. 2008). These results are in contrast to an earlier study that found Steller sea lions had a relatively low DMR_{dive} which decreased to ~6 ml O_2 kg^{-1} min^{-1} from pre or post-dive resting rates (Hastie et al. 2007). However, the study by Hastie et al. (2007) only used one feeding tube at depth, so it is likely the animals were much less active while diving than in the previously mentioned studies that employed two feeding tubes to encourage an active dive, representative of foraging behavior. The apparent decrease in metabolism during diving may also have been because pre-dive MRs (~11 ml O_2 kg^{-1} min^{-1}) were slightly higher and recovery periods were assumed to be shorter, resulting in relatively less consumed oxygen being attributed to the dive itself. This highlights the importance of ensuring complete metabolic recovery when estimating the true costs of diving. A similar reduction in metabolism during diving from “pre-dive” metabolic rate (10.23 down to ~6 ml O_2 kg^{-1} min^{-1}) was seen in California sea lions (Hurley and Costa 2001). However, this study reported the “submersion” metabolic rate on animals in an aquarium, not from animals that were actively diving. The only other oxygen consumption rates that have been measured on freely diving animals are those of Weddell seals. Kooyman et al. (1980) originally found their DMR to be about twice as high as resting values, whereas Castellini et al. (1992) found that diving VO_2 was similar to metabolism at rest except for long dives in which there was a significant decrease in DMR that was also correlated with dive duration.

Aerobic dive limit

The cADL based on DMR_{cycle} for a single dive was 3.0 min, and was 2.5 min based on the estimate of DMR_{dive} for long dives. These values are shorter than the 3.7–15 min (Richmond et al. 2006) and 3.7–12.2 min (Hastie et al. 2007) that have been previously estimated for Steller sea lions. The 12.2 min estimate of cADL was based on a DMR that was much lower than we found in our study [and was also lower than the DMR estimated by Fahlman et al. (2008)]. The 3.7 min estimate by Richmond et al. (2006) was based on an estimated DMR that was very close to our value (but calculated to account for the cost of transport while diving; Rosen and Trites 2002)—but was longer due to a higher TBO estimate.

Interestingly, our estimate of cADL coincides with the only study to compare the cADL and measured “lactate” ADL in an Otariid. In this case, a field metabolic rate corresponding to 4.8 × basal metabolic rate (BMR; Kleiber 1975) was used to calculate an ADL of 1.8–2.0 min for juvenile California sea lions, which underestimated their measured ADL of 2.3 min (Ponganis et al. 1997b). For California sea lions, a DMR of ~4 × BMR would be needed to accurately predict the measured ADL. Coincidentally, the measured DMR over a dive cycle (DMR_{cycle}) in our study was 4 × BMR, suggesting that we determined an accurate estimate of the ADL in Steller sea lions. However, further direct measures of ADL in Otariids are needed to provide support for this value, as it contrasts with studies comparing the measured and calculated ADL in Phocids and Cetaceans that suggest assuming a DMR of 2 × BMR best approximates the ADL (Williams et al. 1999; Noren et al. 2012).

The cADL of 2.5–3.0 min for Steller sea lions is also much shorter than the average duration of their single dives of 4.4 min. Although this may imply the cADL is underestimated, Steller sea lions in the wild rarely dive this long. Dives of adult female Steller sea lions tend to be brief (averaging 1.9–2.4 min), shallow (averaging 21–53 m), and frequent (Merrick et al. 1994; Loughlin et al. 1998). The longest recorded dives for Steller sea lions have been for juveniles at >12 min but such long dives are extremely rare (0.04 % of 692,000 recorded dives) and their mean maximum durations were ~6 min (Pitcher et al. 2005). In fact, 92 % of dives in winter and 98 % of dives in summer made by adult females were <4 min (Merrick and Loughlin 1997). This suggests that anaerobic processes allowed the longer, single dives we recorded to extend beyond the ADL. Although the animals in our study were diving freely in the open ocean to depths typical of a wild animal, there were also significant behavioral differences in their "motivation" to dive. Our captive animals only did one dive trial per day and had several days to rest and recover. Hence, they may not have been as concerned about developing a long term "anaerobic debt" or may simply have been more naive about the consequences of diving beyond their ADL.

In contrast to the single long dives, dive durations of our sea lions when performing consecutive dives in a bout became progressively shorter and were, on average, ~2.2 min each, slightly less than their cADL, and more similar to the behavior of wild animals. Our sea lions also spent short (average 18 s) surface intervals between their dives, which may have been insufficient to fully replenish their oxygen stores—indicating a delayed payoff of oxygen debt. If there is no immediate need to fully balance oxygen stores or process accumulated lactate, single dives may be "uncoupled" from aerobic limits (Horning 2012). Hence a consecutive bout of diving (such as what animals in the wild perform) may be more constrained by aerobic limits than a single dive (Horning 2012) and the average duration of dives in a bout may be more similar to the ADL.

The cADL of Steller sea lions is low compared to other marine mammals, and is much lower than expected given they are the largest species of Otariid. A review by Schreer and Kovacs (1997) defining allometric relationships for maximum dive duration and depth with body mass between all groups of diving mammals suggested that the maximum dive duration of a 200 kg Steller sea lion should be 12.9 min. However, maximum dive durations of Otariids do not correlate with body mass—unlike for Phocids (Schreer and Kovacs 1997). Thus, this high ADL predicted from body allometry seems unreasonable, particularly in light of our direct measurements of metabolism and blood O_2 stores.

Some Otariids have a greater tendency to dive beyond their cADL than others. This apparent reliance on anaerobic metabolism is likely more related to different foraging strategies and dive behavior than on physiological limits (Costa et al. 2001, 2004). For example, benthic hunters are more likely to dive beyond their cADL than those that forage pelagically. It is thought this maximizes bottom time by decreasing transit time, especially when foraging for a more predictable prey source (Costa et al. 2004). Although using anaerobic metabolism may result in inefficient foraging in terms of submergence to surface times, other foraging strategies, or other O_2 management strategies may maximize prey capture by relying on some amount of anaerobic metabolism.

ADL has yet to be directly measured in Steller sea lions. However, we have shown that Steller sea lions have a much shorter cADL than previously thought and that blood oxygen stores are not related to the level of physical activity or fitness. Given their relatively low body oxygen stores and higher diving metabolism, it would be interesting to determine whether the measured ADL of Steller sea lions is also lower than expected. Our findings agree with the only study that compared measured and calculated ADL, and is the first to estimate cADL based on direct measures of VO_2 for an Otariid. We believe that the relatively short 2.5–3 min dive limit accurately reflects the cADL for Steller sea lions despite the consistently longer single dives performed by the animals in our study. In fact, most marine mammals are capable of diving well beyond their aerobic limits if needed; the maximum recorded dive duration of a Weddell seal is 82 min, which is 4 times their ADL of 20 min (Ponganis et al. 1993; Kooyman et al. 1980; Castellini et al. 1992), and the ADL of a juvenile California sea lion at 2.3 min, is much shorter than their longest recorded dive of 9.9 min (Feldkamp et al. 1989; Ponganis et al. 1997b). While limited evidence shows that wild Steller sea lions typically dive within the limits of our cADL, our study on captive animals shows that Steller sea lions can voluntarily dive much longer. However, extending dives beyond 3 min likely requires Steller sea lions to use anaerobic metabolism.

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