

RESEARCH ARTICLE

Steller sea lions (*Eumetopias jubatus*) have greater blood volumes, higher diving metabolic rates and a longer aerobic dive limit when nutritionally stressed

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ABSTRACT

Marine mammal foraging behaviour inherently depends on diving ability. Declining populations of Steller sea lions may be facing nutritional stress that could affect their diving ability through changes in body composition or metabolism. Our objective was to determine whether nutritional stress (restricted food intake resulting in a 10% decrease in body mass) altered the calculated aerobic dive limit (cADL) of four captive sea lions diving in the open ocean, and how this related to changes in observed dive behaviour. We measured diving metabolic rate (DMR), blood O₂ stores, body composition and dive behaviour prior to and while under nutritional restriction. We found that nutritionally stressed sea lions increased the duration of their single long dives, and the proportion of time they spent at the surface during a cycle of four dives. Nutritionally stressed sea lions lost both lipid and lean mass, resulting in potentially lower muscle O₂ stores. However, total body O₂ stores increased due to rises in blood O₂ stores associated with having higher blood volumes. Nutritionally stressed sea lions also had higher mass-specific metabolic rates. The greater rise in O₂ stores relative to the increase in mass-specific DMR resulted in the sea lions having a longer cADL when nutritionally stressed. We conclude that there was no negative effect of nutritional stress on the diving ability of sea lions. However, nutritional stress did lower foraging efficiency and require more foraging time to meet energy requirements due to increases in diving metabolic rates and surface recovery times.

KEY WORDS: Steller sea lion, Blood volume, Nutritional stress, Diving metabolism, Oxygen store, Dive behaviour

INTRODUCTION

The foraging ability of marine mammals, such as Steller sea lions (*Eumetopias jubatus* Schreber 1776), is tied to their diving ability – particularly the duration and depths of dives that they can regularly perform in order to obtain food. Limitations in diving capacity will affect both the quantity and type of food that is accessible and hence the amount of nutrition they can obtain. Nutritional stress is hypothesized to have contributed to the decline of Steller sea lions in the wild (for a review, see Trites and Donnelly, 2003) through changes in the abundance, distribution or species composition of their prey (Benson and Trites, 2002; Trites et al., 2007).

Nutritional stress can lead to increased time spent foraging and diving to overcome this deficit. However, physiological and

anatomical changes resulting from nutritional stress may negatively affect the foraging ability of Steller sea lions. Resulting decreases in total body O₂ stores (TBO) or increases in diving metabolic rate (DMR) could affect overall diving capacity or increase the energetic cost of diving and, therefore, have an impact on subsequent energy intake (Rosen et al., 2007). For example, increases in resting metabolism (hypermetabolism) associated with an animal's 'hunger response' (Cornish and Mrosovsky, 1965; Collier, 1969) may increase DMR. Conversely, fasting-induced hypometabolism could reduce the rate of O₂ use (Guppy and Withers, 1999), thereby decreasing foraging costs.

Previous studies have shown varying metabolic responses to fasting and food restriction in pinnipeds while on land. However, little is known about these metabolic responses whilst diving. Among Steller sea lions and harbour seals (*Phoca vitulina* Linnaeus 1758), periods of fasting or restricted diets of low quality prey result in decreased resting metabolic rates, typical of a 'fasting' response to conserve energy (Markussen, 1995; Rosen and Trites, 1999; Rosen and Trites, 2002). However, restricted diets of high quality prey causes the metabolic rate of Steller sea lions to rise, which is indicative of a 'hunger' response that may be related to increased foraging effort (Rosen and Trites, 2002). The degree to which either response might be exhibited in an actively foraging animal is unknown. The only study to have examined changes in DMR in fasted sea lions found that DMR increased in winter and remained unchanged in summer following a 10-day fast (Svärd et al., 2009). It is unclear whether the same response would be seen in an animal nutritionally stressed over a longer time period, or how a change in DMR corresponds to changes in body composition or translates into changes in foraging behaviour.

Net energy gained during foraging depends on the energy consumed (amount and nutritional quality of prey) and the energy needed to dive, and is considered most energetically efficient if done aerobically (i.e. relying only on O₂ stores). This aerobic diving ability is generally expressed as the aerobic dive limit (ADL, or diving lactate threshold) (Butler, 2006). This can be calculated (cADL), using measures of O₂ stores and DMR, and used as a proxy for the measured ADL; this was originally defined by Kooyman et al. (Kooyman et al., 1980) as the dive duration after which post-dive blood lactate levels rise beyond resting levels due to anaerobic metabolism. Anaerobic metabolism is energetically expensive (Kooyman, 1989) and results in longer recovery times for a given dive duration – hence relying on anaerobic metabolism while diving could reduce net energy gained during a foraging bout depending on the quality, abundance and distribution of prey available. A potential increase in DMR in a nutritionally stressed animal would result in a decrease in the cADL, either limiting foraging time or increasing reliance on anaerobic metabolism, thereby decreasing foraging efficiency through increased metabolic overhead.

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List of symbols and abbreviations

BV	blood volume
DMR _{cycle}	diving metabolic rate, measured over the 'dive event'
DMR _{dive}	diving metabolic rate (excess O ₂ to the dive only)
LBM	lean body mass
M _b	body mass
[Mb]	myoglobin concentration
MRS	pre-dive (surface) metabolic rate
PV	plasma volume
TBO	total body oxygen stores
\dot{V}_{CO_2}	carbon dioxide production rate
\dot{V}_{O_2}	oxygen consumption rate

In addition to diving metabolic rate, the cADL also depends on TBO. TBO consists of the O₂ stored in the lungs, blood and muscle, and generally scales to body mass (M_b). Marine mammals have adaptations that result in higher O₂ stores through increased mass-specific blood volume, hematocrit (packed cell volume), hemoglobin concentration and myoglobin concentration ([Mb]). Studies with captive animals have shown that Steller sea lions experience changes in body composition due to a loss of lipid stores and lean body mass when on restricted or low-quality diets (for a review, see Rosen, 2009). Such nutrition-induced changes in body composition could potentially alter TBO through changes in muscle mass (due to loss of lean body mass) or blood volume (BV; possibly due to changes in body water content). TBO has been shown to change with age in several species of pinnipeds, as young animals develop O₂ stores and diving ability (Burns et al., 2005; Noren et al., 2005; Richmond et al., 2006; Fowler et al., 2007; Weise and Costa, 2007). However, aside from a few studies of seasonal changes in TBO (MacArthur, 1990; Villegas-Amtmann and Costa, 2010; Villegas-Amtmann et al., 2012), plasticity in O₂ stores in adult animals has received relatively little attention, and there are no studies examining how changes in nutritional status or body composition could alter TBO in adult individuals.

In addition to physiological changes, nutritional stress may also alter dive behaviour, whereby animals increase dive depth and durations, or alter O₂ management strategies to compensate for changes in abundance and distribution of prey. For example, animals may have to decrease dive durations to maintain efficient foraging within O₂ store limitations if TBO decreases, or if the cost of diving is elevated by a higher DMR. Conversely, animals may be more likely to dive closer to their physiological limits to maximize prey capture (including relying more heavily upon anaerobic metabolism) if prey abundance or distribution is limited, or in order to overcome past nutritional deficits.

Our objective was to determine whether nutritional stress alters the aerobic diving ability and behaviour of Steller sea lions. Four captive animals trained to dive in the open ocean were subjected to a period of restricted food intake to simulate a nutritional restriction in the wild. We measured dive behaviour and metabolic rates of animals actively diving in the open ocean prior to and while under nutritional stress – and estimated changes in TBO by measuring changes in body composition (to estimate loss of muscle mass) and blood O₂ stores. We then used TBO and DMR to calculate the ADL for our sea lions before and during nutritional stress, and compared this with observed changes in dive behaviour. The results of our study can be used to assess potential impacts of changes in diving ability due to changes in body composition and physiology on foraging costs, and infer whether nutritionally stressed sea lions in the wild have to expend more energy to dive and obtain food.

RESULTS**Oxygen stores and body composition**

Total M_b of the four Steller sea lions dropped by an average of 10.1% (9.1–10.9%) during the initial 3-week period of food restriction, while significant increases occurred in total plasma volume (PV), total BV and mass-specific PV and BV (Table 1). There was an increase in hematocrit, due to an increase in red blood

Table 1. Mean, minimum and maximum values for body composition, blood parameters, estimated O₂ stores, diving metabolic rate and calculated aerobic dive limit at normal body mass and while nutritionally stressed in Steller sea lions (N=4)

Parameter	Units	Normal			Stressed			Difference		P
		Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean	s.d.	
Mass	kg	194	157	225	174	140	201	-19.6	3.90	<0.001
Total body water	kg	116	93.0	138	109	85.9	131	-6.44	0.51	<0.001
Total body lipid	kg	28.1	24.0	35.2	18.4	14.3	23.5	-9.77	3.78	0.002
Lean body mass	kg	166	133	197	156	122	186	-9.86	0.68	<0.001
Body water	% body mass	59.8	58.6	61.3	62.7	61.4	65.5	2.94	1.00	0.001
Body lipid	% body mass	14.6	12.6	16.2	10.8	7.1	12.5	-3.83	1.31	0.001
Lean body mass	% body mass	85.5	83.8	87.4	89.4	87.5	92.9	3.83	1.31	0.001
Plasma volume	litres	10.9	8.7	12.8	12.5	10.3	15.8	1.68	1.07	0.016
Blood volume	litres	18.9	15.1	21.7	23.2	18.1	29.8	4.32	2.77	0.016
Mass-specific PV	ml kg ⁻¹	56.0	55.3	56.8	71.7	64.6	78.9	15.7	5.82	<0.001
Mass-specific BV	ml kg ⁻¹	97.5	96.0	101	132	122	149	34.8	13.0	<0.001
Hemoglobin concentration	g ml ⁻¹	152	141	160	161	152	166	9.00	11.3	0.065
MCH	pg	39.5	38.3	41.0	38.9	37.1	39.9	-0.60	0.84	0.148
MCV	fl	111	105	120	111	105	116	-0.58	4.89	0.787
Hematocrit	%	0.43	0.41	0.44	0.46	0.43	0.47	0.03	0.02	0.008
RBC count	×10 ¹² l ⁻¹	3.9	3.6	4.1	4.2	4.1	4.3	0.30	0.24	0.014
Blood O ₂ store	litres O ₂	2.74	2.26	3.13	3.66	2.65	4.87	0.92	0.68	0.027
TBO	litres O ₂	6.71	5.48	7.74	7.30	5.52	9.14	0.59	0.68	0.096
Mass-specific blood O ₂	ml O ₂ kg ⁻¹	14.2	13.0	15.7	20.7	18.9	24.3	6.51	3.08	<0.001
Mass-specific TBO	ml O ₂ kg ⁻¹	34.7	33.5	36.2	41.6	39.4	45.6	6.90	3.44	<0.001
Mass-specific DMR cycle (single)	ml O ₂ kg ⁻¹ min ⁻¹	11.5	10.8	11.9	12.7	12.1	13.3	1.23	0.73	0.004
Mass-specific DMR cycle (bout)	ml O ₂ kg ⁻¹ min ⁻¹	12.9	11.2	14.3	14.4	12.0	16.1	1.44	0.57	<0.001
cADL	min	3.0	3.0	3.1	3.3	3.0	3.4	0.25	0.16	0.008

Means and standard deviations of the difference in each parameter (final–initial value) are presented. *P*-values are from linear mixed effects models accounting for repeated measures between animals.

cell (RBC) count and no change in mean corpuscular volume (MCV), and a small but insignificant increase in hemoglobin concentration and decrease in mean corpuscular hemoglobin (MCH); hence hemoglobin production must have increased slightly following the increase in BV. Combined, absolute blood O₂ stores increased 32% (range 12–55%) from an average of 2.74±0.36 to 3.66±0.99 litres O₂. This translated into a 48% increase on a mass-specific basis, from 14.2±1.1 to 21.0±2.5 ml O₂ kg⁻¹. The mean values and range for each measured parameter before and during nutritional stress, as well as the mean, standard deviation and significance of the difference between pre- and post-restriction values are reported in Table 1.

We saw a significant decrease in total lean body mass (LBM), total body lipid and total body water (Table 1). Taking changes in M_b into account, there was a significant decrease in mass-specific body lipid and an increase in mass-specific LBM and mass-specific body water (Table 1). In absolute terms, LBM decreased by an average of 9.9±0.7 kg, which assumedly resulted in a decrease in muscle O₂ stores. We recognize that not all of the lost LBM may have been skeletal muscle, and that [Mb] could have increased during nutritional stress. However, we were unable to measure these parameters. To conservatively estimate TBO given these uncertainties, we assumed that all of the LBM lost was derived from skeletal muscle and that [Mb] remained constant. This means that – at most – muscle O₂ stores would have decreased on average from 2.37 litres O₂ (12.3 ml O₂ kg⁻¹) to 2.05 litres O₂ (11.8 ml O₂ kg⁻¹).

Lung O₂ stores were assumed to remain constant during nutritional stress and estimates were based on pre-nutritionally stressed mass. The increase in blood O₂ stores was much greater than the estimated loss of muscle O₂ stores, resulting in a slight increase in absolute TBO and a significant increase in mass-specific TBO (Table 1). Given that we assumed the maximum amount of muscle O₂ store loss and that [Mb] may also have been higher in nutritionally stressed animals, the increase in TBO was probably even greater than our calculation indicates.

Dive metabolism and behaviour

There was no change in absolute pre-dive (surface) metabolic rate (MR_s) or DMR_{cycle} (calculated over the ‘dive event’) for either single

or bout dives attributable to the nutritional stress event. However, due to the resultant decreased M_b , there was a significant increase in mass-specific MR_s and DMR_{cycle} for both single dives (Fig. 1, $P<0.001$) and dive bouts (cycle of four dives; Fig. 2, $P<0.001$) during the period the animals were nutritionally stressed. Mass-specific DMR_{cycle} for bout dives also depended on bout cycle duration ($P=0.005$) for both stressed and non-stressed states. The same relationship is found whether mass-specific metabolic rates are scaled to $M_b^{0.75}$ (as is shown in Figs 1 and 2 as a function of dive duration) or to M_b directly.

The duration of single dives significantly depended on nutritional state, increasing from an average of 4.6 to 5.2 min when nutritionally stressed (Fig. 3, $P=0.018$). Time to reach baseline MR post-dive (recovery in terms of \dot{V}_{O_2}) for single dives was dependent on dive duration (increased with longer dives) but was not significantly dependent on nutritional state. As a result, total dive cycle duration (dive and recovery) also increased in nutritionally stressed animals ($P=0.006$).

This effect of nutritional state on single dive duration confounded measures of DMR_{dive} (calculated by dividing all excess O₂ consumed in the post-dive recovery period above baseline levels by dive duration only), which decreased significantly with increasing dive duration in both stressed and non-stressed sea lions. In other words, independent of any changes due to nutritional stress, DMR_{dive} should be lower in nutritionally stressed sea lions partly as a function of the longer dives they undertook. Therefore, to examine whether DMR_{dive} changed independent of dive duration, we compared the relationship between DMR_{dive} and dive duration for stressed and non-stressed animals. The relationship between absolute measures of DMR_{dive} and duration did not change with nutritional state, but nutritional state did significantly affect mass-specific DMR_{dive} such that, for a given duration, mass-specific DMR_{dive} is higher when animals were nutritionally stressed (Fig. 4, $P<0.001$). Hence, as with MR_s and DMR_{cycle}, nutritional state had little effect on absolute values of DMR_{dive} but did result in increases in mass-specific values, seen when scaled to either $M_b^{0.75}$ (as shown in Fig. 4) or to M_b directly.

As with single dive cycle duration, the total duration of a bout dive cycle (four dive cycles and recovery) increased with nutritional stress ($P<0.001$); however, this was due to the

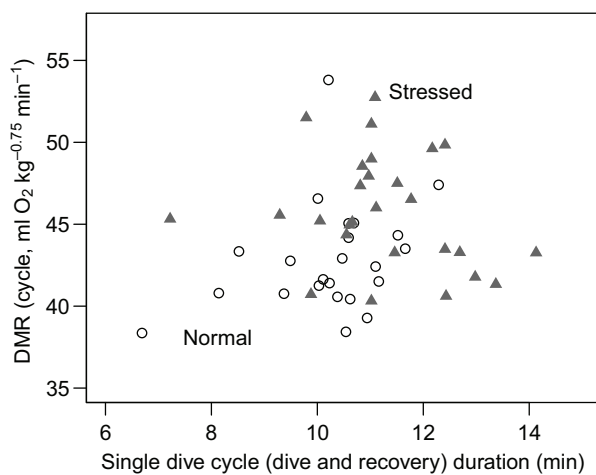


Fig. 1. Diving metabolic rate as a function of dive cycle (dive and post-dive recovery) duration for single long dives in four adult Steller sea lions. DMR is scaled to $M_b^{0.75}$ and calculated as DMR_{cycle} (excess O₂ averaged over the entire ‘dive event’). DMR_{cycle} was independent of dive duration, but higher when nutritionally stressed ($P<0.001$, triangles) than at normal mass (circles).

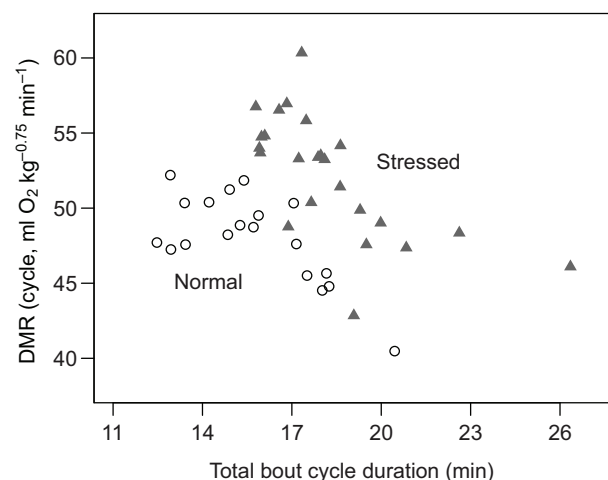


Fig. 2. Diving metabolic rate as a function of bout (4 dives with surface intervals and post-dive recovery) cycle duration in four adult Steller sea lions (DMR_{cycle} calculated over the dive ‘event’ and scaled to $M_b^{0.75}$). DMR_{cycle} was higher during nutritional stress (triangles, $P<0.001$) than at normal mass (circles) and depended on bout duration ($P=0.005$).

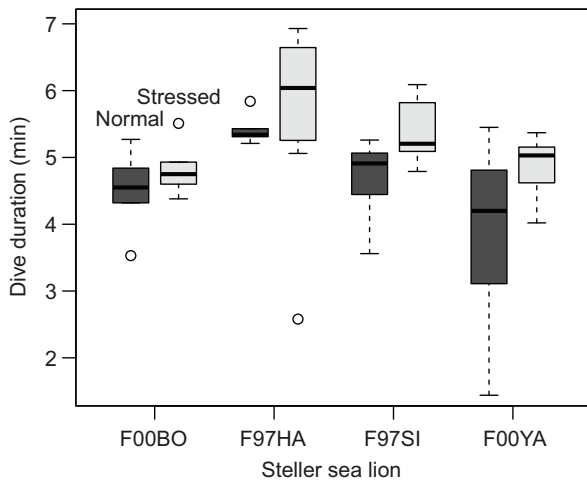


Fig. 3. Average duration of single long dives for each of four adult Steller sea lions under each nutritional state (5–6 single dives per animal per state). Duration increased when nutritionally stressed (light grey, $P=0.018$) compared with normal mass (dark grey). Open circles represent outliers.

consistent increase in post-bout recovery time (time to return to baseline MR in terms of \dot{V}_{O_2} , $P=0.002$; Fig. 5) rather than dive duration. Of the four animals, only two increased the duration of dives in a bout, whereas the other two decreased bout dive duration. Hence, in contrast to single dives, there was no overall increase in the dive duration in the bouts during nutritional stress (Fig. 6A; average duration of bout dives was 2.2 ± 1.2 min for unstressed sea lions and 2.5 ± 1.6 min when stressed).

There was also an overall increase in surface interval duration (from 21 ± 14 to 25 ± 12 s; $P=0.031$) during bouts when nutritionally stressed. As a result, all nutritionally stressed sea lions spent a greater proportion of time at the surface breathing in the metabolic dome for a given bout of diving (four dives, with three inter-dive surface intervals; $P=0.001$, Fig. 6B), in addition to the longer post-bout recovery. Spending more time at the surface relative to dive duration makes sense if the sea lions were using O_2 stores and

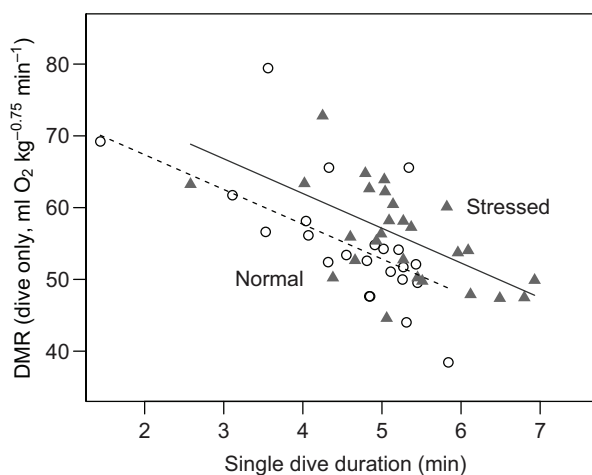


Fig. 4. Diving metabolic rate as a function of dive duration for single long dives in four adult Steller sea lions ($R^2=0.49$). DMR is scaled to $M_b^{0.75}$ and calculated as DMR_{dive} (excess O_2 consumed in the post-dive recovery period divided by dive duration only). DMR_{dive} was higher during nutritional stress ($P=0.017$, triangles, continuous line) than at normal mass (circles, interrupted line) and depended on dive duration ($P<0.001$).

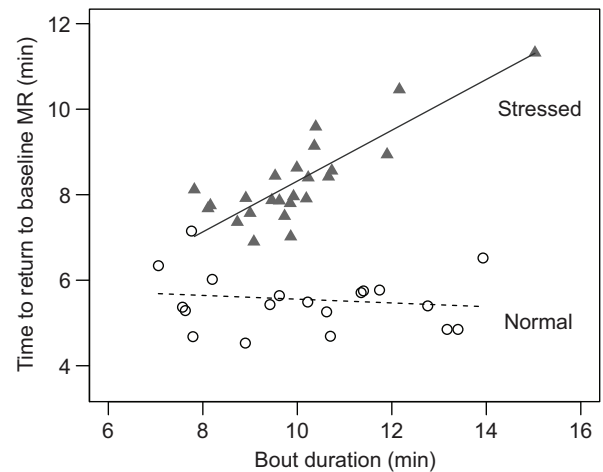


Fig. 5. Time to reach baseline metabolic rate (recovery of \dot{V}_{O_2} to baseline) following a cycle of bout dives as a function of bout duration in four adult Steller sea lions under each nutritional state, normal (circles) and stressed (triangles). Bout duration includes four dives to 40 m and three surface intervals, which comprised a higher proportion of the total bout duration in nutritionally stressed individuals. Significantly greater recovery time when stressed ($P<0.001$) suggests that they ended their bout with a greater depletion of O_2 stores, while the dependence on bout duration ($P<0.001$ for stressed dives only) suggests that they were not recovering O_2 stores between bout dives as shown by the constant recovery time following normal diving bouts.

producing CO_2 at a higher rate – which is consistent with the higher mass-specific DMR_{cycle} for bout dives seen when they were nutritionally stressed (shown in Fig. 2 as a function of bout duration). When nutritionally stressed, recovery following a bout of diving also depended significantly on bout duration (Fig. 5, $P<0.001$), indicating that nutritionally stressed animals were depleting O_2 stores to a greater level.

We calculated foraging efficiency as the amount of fish caught (energy ingested in kilojoules) versus energy expenditure (converting millilitres of O_2 to kilojoules) using mean measures of DMR_{cycle} and dive behaviour for each dive type and nutritional state (Table 2). The amount of prey available (fish delivery rate at depth) was kept constant between trials, and we assumed the energy needed to digest prey (digestive efficiency) did not vary with nutritional state. For single dives, the nutritionally stressed animals consumed more fish per minute of a dive cycle (dive and recovery duration) and had slightly higher foraging efficiency (~7% higher), despite having higher mass-specific DMRs. However, for bout dives, the amount of fish consumed per minute of dive cycle by the sea lions was lower and their mass-specific DMR was higher, which combined to reduce their foraging efficiency (~20% lower).

DISCUSSION

Diving capacity is largely influenced by the aerobic dive limit and is dependent on limited O_2 stores and the rate at which these are consumed (Kooyman et al., 1980). Nutritional stress can significantly affect body composition and metabolic rate (for a review, see Rosen, 2009) – and probably affects the aerobic dive limit as a result. Although developmental changes in both O_2 stores and metabolic rate have been demonstrated in pinnipeds, few studies have examined potential variation within individual adult animals. Specifically, it is unknown how changes in body condition or physiology due to nutritional stress affect the cADL or subsequent dive behaviour and ability to forage.

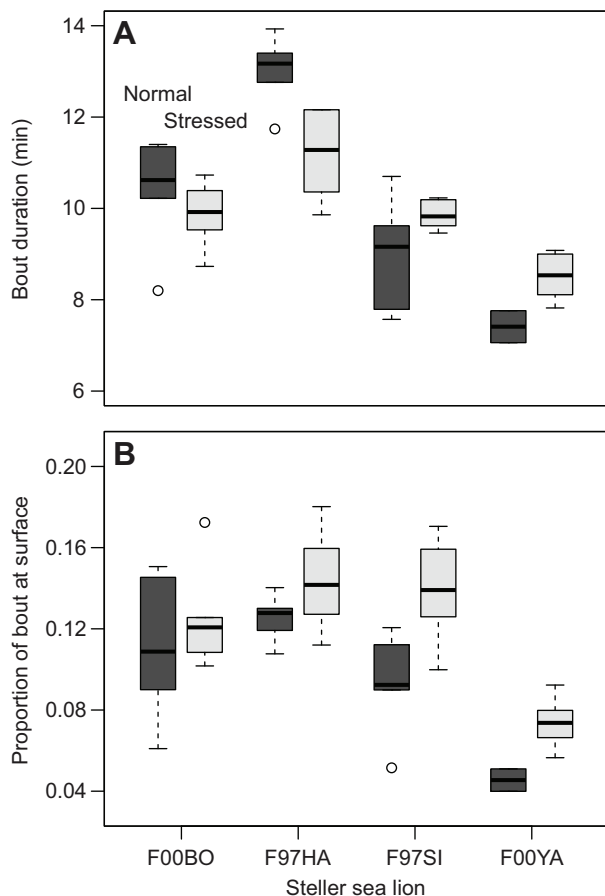


Fig. 6. Bout dive behaviour for each of four adult Steller sea lions under each nutritional state (normal; dark grey and stressed; light grey; 4–6 bouts per animal per state). (A) Total bout duration (four dives and three surface intervals, not including final recovery period). (B) Proportion of the total bout duration spent at the surface. When nutritionally stressed animals either increased surface interval duration resulting in an increase in bout duration (F97SI and F00YA), or decreased dive duration and maintained surface duration (F00BO and F97HA). This resulted in a greater proportion of a 4-dive bout cycle (total surface interval duration/total bout duration) being spent at the surface when nutritionally stressed ($P=0.001$), indicating that they required longer surface intervals to recover O_2 stores. Open circles represent outliers.

Given the hypometabolic response generally seen in fasting pinnipeds on land (Markussen, 1995; Rosen and Trites, 2002), it is possible that nutritionally stressed sea lions could extend their aerobic dive limit by lowering their metabolism while diving. Steller sea lions tend to have higher DMRs relative to their mass than phocid seals (e.g. Weddell seals) (Castellini et al., 1992), and may have a greater capacity to lower their metabolism whilst diving. However, it seems more likely that diving metabolism should already be lowered to minimal levels due to adaptations to conserve O_2 stores and extend the cADL (such as the dive response) that are employed whilst diving under normal physiological conditions. Such adaptations should preclude any further reduction in diving metabolism when stressed. Hence we expected the DMR of the sea lions in our study to fall due to a loss of body mass (but remain unchanged on a mass-specific basis), since these individuals were actively foraging and unlikely to be able to lower their metabolism as is seen in land-bound animals. However, counter to our expectations, we observed an increase in mass-specific metabolic rates and no reduction in either absolute DMR or absolute MR_s .

It is generally accepted that marine mammals subjected to nutritional stress will experience differential losses of lipid and lean mass because of their tendency to conserve LBM and use (largely metabolically inert) lipid from the blubber layer as their primary energy source while fasting (Oritsland, 1990), although this generalization is largely formulated from studies of phocid seals that have greater overall lipid reserves. Hence mass-specific DMR may increase simply due to the relative increase in the proportion of LBM. In our case, 48.4% (range 38.2–57.6%) of the mass loss of our study animals could be attributed to the lipid layer. However, when metabolism was scaled to LBM we still saw an increase in mass-specific MR_s and DMR, indicating that other factors besides the proportional increase in lean mass must have contributed to the overall increase in metabolic rate. An increase in resting metabolism was previously observed in Steller sea lions while consuming restricted quantities of high quality prey (Rosen and Trites, 2002), and DMR_{cycle} was also observed to increase following 9–10 day fasts (Svärd et al., 2009).

The increase in mass-specific MR we observed may reflect a ‘hunger’ response, as suggested for (non-diving) sea lions by Rosen and Trites (Rosen and Trites, 2002), which is congruent with the increased foraging effort seen in our nutritionally stressed animals when performing single long dives. Alternately, although mass was kept constant, it is also possible that body composition (% LBM) of the sea lions continued to change over the subsequent 3-week period during which dive trials were conducted and MR was measured. However, there was no observed change in MR over the course of the nutritionally stressed trials. There could also have been an added thermoregulatory cost due to loss of insulative lipid stores, as we saw increases in both mass-specific pre-dive MR_s and DMR, although the relatively warm summer water temperatures make this less likely.

It is reasonable to inquire whether the increased mass-specific DMR seen in the nutritionally stressed sea lions was a product of increased digestive costs. While diving and digestion are often considered incompatible physiological processes under normal foraging conditions (Sparling et al., 2007; Rosen, 2009), Svärd et al. (Svärd et al., 2009) suggested that the increased metabolism seen during diving in Steller sea lions following fasting might be due to the simultaneous costs of diving and the heat increment of feeding. Although such a strategy would decrease the efficiency of foraging, they argued that the immediate need to gain energy and replace depleted lipid stores from ingested food imposed by their previous mass loss overrides this consideration. Although a previous study found that MR did not increase until 60 min after a 2 or 4 kg meal in resting animals (Rosen and Trites, 1997), and the course of our dive trials typically lasted only about 40 min, the onset of digestion may be earlier in nutritionally stressed animals when diving. However, this possibility seems unlikely since the resting rates measured at the end of the dive trial were not elevated compared with the pre-dive rates, as should have been seen if digestion occurred towards the end of our dive trials. Hence increased digestion is unlikely to explain the higher mass-specific DMR seen in the nutritionally stressed sea lions.

Differences in activity levels may also explain the higher mass-specific DMRs we observed in nutritionally stressed animals. Although the trials were designed to elicit the same activity levels (i.e. same dive depth, two tubes delivering food to create the same prey patch) they could have differed if animals were more motivated to search for and catch prey while nutritionally stressed.

It is difficult to tease apart the effects of scaling and changes in M_b on metabolism – to know whether the observed increases in

Table 2. Foraging efficiency per dive type and nutritional state (based on an average 194 kg sea lion at normal mass, and 174 kg while stressed)

Dive type	Nutritional state	Mean single or bout dive duration	Mean recovery time	Total dive cycle/bout duration	Bottom time (% dive cycle)	Fish consumed (g min ⁻¹ of dive cycle)	Energy expended (kJ min ⁻¹)	Energy ingested (kJ min ⁻¹)	Efficiency (kJ ingested/expended)
Single	Normal	4.59	5.64	10.23	36.8%	88.2	45.2	681	15.1
Single	Stressed	5.20	6.03	11.23	38.9%	93.4	44.8	721	16.1
Bout	Normal	9.10	5.51	15.71	36.8%	88.3	51.0	681	13.4
Bout	Stressed	8.77	8.30	18.30	29.8%	71.5	51.1	552	10.8

Mean duration is for a single long dive or a bout of four dives (total duration of four dives). Total dive cycle duration includes mean recovery time and (for bouts) total surface interval duration. Mean bottom time accounts for an average of 0.83 min for animals to transit to a depth of 40 m and back. Fish consumed is based on total bottom time per cycle multiplied by ~0.02 kg pieces of fish delivered via feeding tubes every 5 s to depth and reported per minute of a total dive cycle. Energy expended is based on DMR_{cycle} (assuming 20.35 kJ ml⁻¹ O₂). Foraging efficiency is the total energy ingested (assuming an energy content of herring of 7.72 kJ g⁻¹) divided by the total energy consumed (dive depth and fish delivery rates were kept constant).

mass-specific MR_S and DMR_{cycle} were an artifact of decreases in M_b , or whether they described true physiological changes. The longer surface intervals (but not longer dive times) that occurred between the consecutive dives in a bout by our animals suggests that the increase in mass-specific metabolic rate was an actual added cost to diving, as the animals spent more time at the surface refilling O₂ stores and removing accumulated CO₂ relative to dive durations (Fig. 5). Additionally, although total bout duration was not significantly greater, the first dive performed by the animals in a bout was longer when nutritionally stressed (increasing from 4 to 5 min). This increase in initial dive times also could have contributed to the greater surface interval durations we observed during bout dives when animals were nutritionally stressed. Finally, the way in which DMR_{cycle} was calculated – by dividing O₂ consumed over the dive and surface durations – may also have masked actual increases in DMR. Specifically, if surface duration was longer, the calculated O₂ consumption would have been spread over a longer period and therefore would have been artificially lowered. However, calculating DMR by dividing only over dive duration (excluding surface intervals, DMR_{dive}) results in an increase in absolute DMR_{dive} (although not significant, $P=0.064$) in nutritionally stressed animals. Hence although neither measure of absolute DMR was significantly affected by nutritional status (probably due to individual animal variation), the slight increase in dive duration as well as absolute DMR_{dive} in nutritionally stressed animals may explain the longer surface durations relative to dive durations.

Animals can dive past their physiological (aerobic) limits and rely more on anaerobic metabolism, which could also explain the significantly longer recovery times of our study animals following a diving bout of equal length when nutritionally stressed. The longer recovery times following a series of dives occurred despite longer surface intervals between those dives that, all other things being equal, should have decreased the recovery time needed following the bout. In fact, the time to return to baseline MR increased with the duration of the bout of diving. This indicates that the nutritionally stressed sea lions were not recovering their O₂ stores between dives, and were thus depleting their O₂ stores to a lower level (and were probably accumulating greater amounts of CO₂).

The longer recovery times of the nutritionally stressed sea lions contrasts sharply with the constant recovery times that followed the diving bouts (independent of duration) when the sea lions were not nutritionally stressed (Fig. 5). This suggests that the surface interval duration may be more dependent on CO₂ elimination than on refilling of O₂ stores, as it typically takes longer for CO₂ to be fully removed from the body than for O₂ stores to be refilled (Boutillier et

al., 2001; Gerlinsky, 2013). The time to return to baseline \dot{V}_{CO_2} was also slightly higher in nutritionally stressed animals (Gerlinsky, 2013), although the effect of nutritional stress on CO₂ recovery was not nearly as great as the increase in O₂ recovery time.

Although a higher mass-specific DMR increased the cost of diving, it did not shorten the cADL of Steller sea lions as they had significantly higher blood O₂ stores as well. We had expected nutritionally stressed sea lions to have lower TBO simply because of mass loss (and related muscle loss). Loss of LBM has an impact on muscle O₂ stores due to a loss of skeletal muscle mass as protein stores are catabolized. Unfortunately, we were unable to directly measure muscle mass. However, we assumed that substantial loss of muscle O₂ stores must have occurred given the significantly lower LBM of our sea lions after the episode of nutritional stress. Although it is unclear how much of the LBM lost during periods of food restriction or fasting comes from skeletal muscle, it is probably a significant portion (see Cherel et al., 1994). By our calculation, the amount of LBM lost over the initial 21-day period of food restriction represented ~14±2.9% of initial muscle mass, assuming the ‘worst-case scenario’ (i.e. that 100% of LBM lost was muscle). The only study to have directly measured muscle mass (by computed tomography) during a mass loss event found that grey seal pups (*Halichoerus grypus* Fabricius 1791) lost ~20% of their muscle mass during a 31-day post-weaning fast (Nordøy and Blix, 1985).

In addition to the amount of muscle mass an animal has, resultant muscle O₂ stores will also depend on [Mb], which could increase as skeletal muscle protein is utilized. Several studies have shown increases in muscle [Mb] concurrent with mass loss due to hibernation or fasting (Galster and Morrison, 1976; MacArthur, 1990; Noren et al., 2005). This potential increase in muscle [Mb] implies that loss of muscle O₂ stores may be significantly less than estimated based on changes in muscle mass alone, and that mass-specific muscle O₂ stores may actually be higher in nutritionally stressed animals.

Changes in body composition also affected blood O₂ stores through changes in BV. BV scales to M_b between species and individuals, but the effects of M_b changes within an individual are less clear. Significant increases in the BV of sea lions in our study were accompanied by increases in body water as a percentage of M_b , although total body water decreased on an absolute basis. This may indicate that the sea lions were retaining more water during the food restriction as lipid and protein stores were catabolized, which could have contributed to the increase in PV despite the loss of mass. Higher protein concentration in the blood (caused by catabolism of protein stores) may also have an osmotic influence, causing retention of water. Increases in urea and lower blood urea nitrogen (BUN):creatinine ratios generally indicate dehydration in

nutritionally stressed animals (Dierauf and Gulland, 2001). However, we saw opposite trends in these parameters, which may be indicative of a high level of hydration that contributed to the increase in PV. Decreases in urea (as opposed to the expected increase) were also seen in previous studies of Steller sea lions on restricted herring diets (Rosen et al., 2004). The changes in BV we observed may also have been transient in nature (i.e. more a consequence of losing mass, than being at a lower mass or altered body composition).

Blood O₂ stores were also measured in a group of sea lions (non-diving; i.e. in an aquarium) before and after an episode of nutritional stress during another study where the sea lions were consuming different types of prey (Gerlinsky, 2013). Overall, the effect of nutritional stress on PV was less pronounced in these animals, but the data also indicate that the effect could depend on prey quality. Of the four animals measured, one sea lion fed the same restricted diet (herring) as the diving sea lions in this study had higher PVs, whereas one individual fed a low-fat diet (Atka mackerel) had significantly lower PV. In general, it seems that animals on a restricted diet of higher quality prey show differing trends in body composition and utilization of lean and lipid mass stores than those on lower quality diets (Rosen and Trites, 2001; Jeanniard du Dot et al., 2008). Hence the effect of nutritional stress on PV may also depend on diet quality. However, it is important to note that these sea lions were also measured in a different season (winter) and were not actively diving – either of which could also explain the differences we observed.

Increased blood O₂ stores have also been seen in grey seal pups during the post-weaning fast, although this was due to higher hemoglobin concentrations as opposed to higher BV (Noren et al., 2005) and was attributed only to developmental changes (as opposed to a reaction to mass loss). We saw a small, but insignificant increase in hemoglobin concentration in our study (paralleled with a small decrease in MCH), indicating that there was a small increase in hemoglobin production, but not enough to match the increase in red blood cells. However, all of these changes were fairly small and probably not biologically significant as they were within the range normally seen in this group of animals (D.A.S.R., unpublished data).

The substantial increase in blood O₂ stores of the sea lions experiencing nutritional stress more than compensated for the potential loss of muscle O₂ stores. This resulted in an increase in both absolute and mass-specific TBO, in spite of the lower mass of the nutritionally stressed animals. Despite a higher mass-specific DMR, this increase in O₂ available for use during the dive increased the cADL (from 3.0 to 3.3 min) and was consistent with the significant increase in the average duration of the single long dives (from 4.6 to 5.2 min).

The single long dives performed by the animals in our study were significantly longer than their cADL, under both stressed and unstressed nutritional states. As we did not directly measure ADL, it is plausible the cADL is too short. However, the cADL does coincide with the behaviour of Steller sea lions in the wild that typically undertake dives ~2–2.4 min long (Merrick and Loughlin, 1997; Loughlin et al., 1998; Gerlinsky et al., 2013). Additionally, the average duration of the four dives our sea lions performed in a cycle of dives (bout), was much shorter (2.5 min when stressed, and 2.3 min when unstressed) than the single long dives, and closer to the cADL estimate. As our study sought to determine whether nutritional stress limited dive behaviour or ability, our dive trials encouraged the sea lions to dive as long as possible (i.e. food delivery was at a high rate to encourage longer dives, and animals

were fed minimally when not participating in trials). We therefore believe that the animals were diving beyond their aerobic limits for their single long dives, especially when nutritionally stressed. It is conceivable that captive animals may be naïve to the consequences of diving beyond their aerobic limits or less concerned with developing an O₂ debt (Gerlinsky et al., 2013). As transit time was constant, the increase in dive duration for these single dives increased their effective foraging (bottom) time by 17%, thereby allowing them to increase their intake of energy for a single dive cycle. However, we saw evidence that this strategy was not feasible when performing several consecutive dives (there was no overall significant increase in bout dive duration).

The drawback to the sea lions undertaking longer dives (with a higher DMR) when nutritionally stressed is that they require longer post-bout recovery (Fig. 5) and longer surface intervals between consecutive dives. This extra inter-dive time is a consequence of having a higher DMR when nutritionally stressed, which would deplete onboard O₂ stores faster and cause CO₂ to accumulate faster. As a consequence, the sea lions that were nutritionally stressed decreased their foraging efficiency by having to spend more time and energy gaining food – and more time recovering from their dives (possibly because they were diving anaerobically). This was particularly evident during bout dives, when they spent a greater proportion of their bout at the surface (Fig. 6B) and decreased their foraging efficiency (energy ingested/energy expended) by 20% (Table 2).

It is interesting to note that the sea lions in our study seemed to use two distinct foraging strategies to compensate for the increased DMR. Whilst two animals increased both dive and surface durations, the other two animals chose to decrease dive duration, such that they did not have to increase surface duration between dives in a bout (Fig. 6A). This resulted in no overall change in the total duration of these bouts of several dives due to nutritional stress (as opposed to the increase in the duration of single long dives).

Conclusions

In summary, our study has shown that changes in the nutritional status of adult sea lions can lead to significant variation in body O₂ stores. Nutritional stress did not directly limit the diving ability of our sea lions (but actually enhanced it) because it increased their O₂ stores. However, nutritional stress did increase the energetic cost of a bout of diving – and required the sea lions to spend more time recovering on the surface relative to the durations of their dives. Nutritional stress may also have resulted in the sea lions relying more on energetically expensive anaerobic metabolism. Thus nutritionally stressed sea lions experienced an overall decrease in foraging efficiency during a bout of diving and required more time than an unstressed animal to obtain comparable amounts of energy. Such a decrease in foraging efficiency is further compounded by the need for nutritionally stressed individuals to increase food intake to recover lost body mass.

The extent to which animals may be able to significantly increase foraging time in the wild may be limited. An increase in foraging time means less time for other aspects of life history, and could negatively affect survival by exposing adults to greater risk of predation and increasing fasting times for pups. Combining increased cost of diving with changing prey distributions could significantly affect foraging costs, net energy intake and subsequent pup condition and juvenile survival. Animals faced with unexpected episodes of nutritional stress would have to increase their foraging times to maintain energy intake and acquire the extra energy needed to replace lost mass and fat stores.

MATERIALS AND METHODS

Study design and data collection

We measured changes in the blood O₂ stores, body composition, metabolism and dive behaviour before and during a period of nutritional stress of four adult, female Steller sea lions in May–August 2011. All sea lions were collected from breeding rookeries as pups and raised in captivity at the Vancouver Aquarium (British Columbia, Canada). The animals (between 12 and 15 years old) were housed at the Open Water Research Station (Port Moody, BC, Canada), and have been actively diving in the open ocean for research purposes for 3–8 years (since 2003: F00SI, F00BO; 2005: F00HA; or 2008: F00YA). All animals were previously trained to use the experimental equipment and performed all trials voluntarily under trainer control. All experiments were conducted under UBC Animal Care Permit no. A07-0413.

Animals were fed a diet of herring (*Clupea pallasii*) and market squid (*Doryteuthis opalescens*) supplemented with vitamins. Animals were nutritionally stressed by restricting daily food intake, such that the animals lost ~10% (9.1–10.9%) of their initial M_b over a 3 week period. We then continued to restrict their daily intake to maintain them at ~10% below their initial mass (to a maximum of 15% as per Animal Care regulations) for an additional 3 weeks, while the nutritionally stressed dive trials were conducted. Blood O₂ stores and body composition were measured at the beginning and end of the initial 3 week period of food restriction. Trials took place concurrently with a study to assess how inspired gas concentrations affect post-dive recovery of O₂ stores and elimination of CO₂. This included exposure to hypercapnia (to 3% inspired CO₂) and hypoxia (to 19% inspired O₂) in some trials, but at levels too low to significantly affect DMR or dive behaviour. Altered inspired gas trials were statistically compared with ambient air trials before being included in analysis (Gerlinsky, 2013). Inspired gas concentrations were altered prior to the animal entering the metabolic dome and remained constant throughout the trial, such that we could use altered baseline O₂ and CO₂ concentrations to calculate DMR.

Diving metabolic rate

DMR was determined for four Steller sea lions diving voluntarily over a series of dive trials. The experimental dive set-up and measurement of diving metabolism are described by Gerlinsky et al. (Gerlinsky et al., 2013). Briefly, the animals were trained to dive between a 100 l metabolic dome floating at the surface and feeding tubes that delivered fish pieces at depth. The rates of O₂ consumption (\dot{V}_{O_2}) and carbon dioxide production (\dot{V}_{CO_2}) were measured using flow through respirometry. Specifically, a dried subsample of excurrent air was analyzed for O₂ and CO₂ concentrations using Sable System FC-1B and CA-1B analyzers, respectively, coupled to a 500H mass flow generator and controller (Sable Systems Inc., Las Vegas, NV, USA) pulling air at 350 l min⁻¹. Metabolic data were analyzed using LabAnalyst X (Warthog Systems, University of California, CA, USA) and equations from Lighton (Lighton, 2008):

$$\dot{V}_{O_2} = FR_e[(F_iO_2 - F_eO_2) - F_iO_2(F_eCO_2 - F_iCO_2)]/(1 - F_iO_2), \quad (1)$$

$$\dot{V}_{CO_2} = FR_e(F_eCO_2 - F_iCO_2). \quad (2)$$

Animals were fasted overnight prior to trials and weighed each morning. They were fed <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). MR_s was measured at the start of each trial while animals rested inside the metabolic dome, and was calculated from the last 3 min of a 5–10 min measurement period, when \dot{V}_{O_2} was constant.

The animals undertook a single long dive, which was then followed by a 4-dive bout cycle. Following completion of either the single dive or dive bout, the sea lions remained at the surface in the respirometry dome until they were physiologically recovered (\dot{V}_{O_2} and \dot{V}_{CO_2} returned to pre-dive levels, ~8–12 min). During the dives, animals received ~20 g pieces of herring every 5 s from two feeding tubes set at a depth of 40 m. The feeding rate was constant for both dive types and both nutritional states. Single dives were only included if >1.5 min, and dive bouts continued regardless of individual dive duration as long as the animal continued to surface inside the metabolic dome. Animals were previously trained for this experimental protocol (to complete a single dive, then a 4-dive bout separated by a full

recovery) so may have had some prior knowledge as to what dive type was expected of them. They chose both their dive and (in the case of dive bouts) inter-dive surface interval durations.

To facilitate comparing our results with those from other studies, we calculated DMR in two ways: first, by dividing the total O₂ consumed in excess of post-dive baseline in the recovery period over only the dive duration (DMR_{dive}) and, second, as the average \dot{V}_{O_2} over the entire dive and following recovery period (DMR_{cycle}). In the case of bout dives, DMR_{cycle} was calculated as the average \dot{V}_{O_2} for the entire cycle of dives (including inter-dive surface periods) and subsequent recovery period.

Blood oxygen stores

Blood O₂ stores were measured for each of the four diving sea lions at the Open Water Research Station as per Gerlinsky et al. (Gerlinsky et al., 2013). Briefly, blood samples were taken from the caudal gluteal vein shortly after animals were anaesthetized using isoflurane gas. Blood O₂ stores were calculated from measures of BV and hemoglobin concentration (see Ponganis et al., 1993; Richmond et al., 2006), where:

$$BV = PV \times \frac{100}{(100 - Hct)}. \quad (3)$$

BV was calculated from hematocrit (Hct) and PV, which was determined using Evans Blue dilution procedure (Gibson and Evans, 1937). Hct, hemoglobin concentration (using the Sysmex sodium lauryl sulfate method), RBC count, MCH and MCV values were determined by a commercial laboratory using a Sysmex automated hematology analyzer (Idexx Laboratories, Delta, BC, Canada).

Blood O₂ stores were calculated using BV and hemoglobin concentration, assuming an O₂ binding capacity of hemoglobin of 1.34 ml O₂ g⁻¹ Hb (Kooyman and Sinnett, 1982). Blood was assumed to be one-third arterial, that it was 95% saturated at the beginning of the dive and reduced to 20% at the end of the dive, and two-thirds venous, assumed to be 5 vol% less than initial arterial saturation (Ponganis et al., 1993; Richmond et al., 2006).

Body composition

Body composition was determined by deuterium dilution (Reilly and Fedak, 1990). After obtaining a background blood sample, animals were injected intramuscularly with a measured dose of deuterium oxide (D₂O, 0.10–0.15 mg kg⁻¹ dose). A second blood sample was taken after a 2 h equilibrium period. Samples were centrifuged at 1397 g for 5 min and serum was stored at -70°C until analysis. Serum and dose samples were analyzed by Metabolic Solutions Inc. (Nashua, NS, USA) for isotope concentrations to determine total body water (TBW, in kg). TBW was used to calculate total body lipid (TBL, in kg) using the equation from Arnould et al. (Arnould et al., 1996) derived for Antarctic fur seals (*Arctocephalus gazella* Peters 1875):

$$TBL = 0.927M_b - 1.309TBW + 0.265. \quad (4)$$

The remaining mass of the sea lions was assumed to represent LBM and was calculated by subtracting TBL from M_b (Arnould et al., 1996).

Aerobic dive limit

We calculated an ADL for our Steller sea lions before and during the time they were nutritionally stressed, taking into account the changes in TBO and DMR. Lung O₂ stores (based on a diving lung volume of 55 ml kg⁻¹ and 15% O₂ content) and muscle O₂ stores were calculated as per previous studies (Richmond et al., 2006; Gerlinsky et al., 2013) and we assumed no change in lung O₂ stores. We were unable to measure [Mb], and there is no published evidence to suggest that it changes when animals are nutritionally stressed. Our calculations of ADL therefore assumed that [Mb] was constant and independent of nutritional state. We assumed that muscle mass was initially 37% of M_b and, for the calculation of TBO and ADL, that all LBM lost was muscle mass (i.e. maximum muscle mass loss). Lung, muscle and measured blood O₂ stores were then combined to estimate TBO, which was divided by DMR_{cycle} to estimate cADL.

Statistical analysis

All data were 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 from the nlme package (Pinheiro et al., 2011). Models were run using the maximum likelihood method. 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). Conditional R^2 statistics (describing the proportion of variance explained by both the fixed and random effects) were calculated using the package lmmfit, version 1.0 for R (Maj, 2011). Values are reported as means \pm s.d. and significance was set at $\alpha=0.05$.

For measures of blood parameters, body composition, MR_s , DMR_{cycle} and dive behaviour (dive and surface interval duration), linear mixed models were used to determine if there was a difference between pre- and post-treatment values. A repeated measures ANOVA on a single model was performed to determine if there was a linear relationship between DMR and dive duration (tested as a fixed covariate), with nutritional state tested as a fixed factor to determine if this altered the relationship. Metabolic rates were tested as absolute values, scaled to $M_b^{0.75}$ and scaled to M_b directly.

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Competing interests

The authors declare no competing financial interests.

Author contributions

C.D.G. carried out all data collection and analysis and was lead author on the manuscript. C.D.G., A.W.T. and D.A.S.R. were all involved in the conception and design of the project as well as writing and editing the manuscript.

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