

Condition and mass impact oxygen stores and dive duration in adult female northern elephant seals

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SUMMARY

The range of foraging behaviors available to deep-diving, air-breathing marine vertebrates is constrained by their physiological capacity to breath-hold dive. We measured body oxygen stores (blood volume and muscle myoglobin) and diving behavior in adult female northern elephant seals, *Mirounga angustirostris*, to investigate age-related effects on diving performance. Blood volume averaged 74.4 ± 17.0 liters in female elephant seals or $20.2 \pm 2.0\%$ of body mass. Plasma volume averaged 32.2 ± 7.8 liters or $8.7 \pm 0.7\%$ of body mass. Absolute plasma volume and blood volume increased independently with mass and age. Hematocrit decreased weakly with mass but did not vary with age. Muscle myoglobin concentration, while higher than previously reported ($7.4 \pm 0.7\%$), did not vary with mass or age. Pregnancy status did not influence blood volume. Mean dive duration, a proxy for physiological demand, increased as a function of how long seals had been at sea, followed by mass and hematocrit. Strong effects of female body mass (range, 218–600 kg) on dive duration, which were independent of oxygen stores, suggest that larger females had lower diving metabolic rates. A tendency for dives to exceed calculated aerobic limits occurred more frequently later in the at-sea migration. Our data suggest that individual physiological state variables and condition interact to determine breath-hold ability and that both should be considered in life-history studies of foraging behavior.

Key words: age, dive duration, elephant seal, oxygen stores.

INTRODUCTION

The diving behavior of air-breathing marine vertebrates is ultimately constrained by their physiological capacity to breath-hold dive (Castellini et al., 1985; Costa et al., 1998). Increased oxygen stores result in greater diving capacity in a number of species (Burns et al., 2005; Dolar et al., 1999; Horning and Trillmich, 1997; Lenfant et al., 1970; Ridgway and Johnston, 1966; Trillmich et al., 2008). For the deepest divers, stored oxygen is held primarily in blood and muscle reservoirs (Costa et al., 2000; Kooyman, 2006; Thornton et al., 2001). Large blood volume, elevated hematocrit (Hct) and high concentrations of blood hemoglobin (Hb) and muscle myoglobin (Mb) comprise the large oxygen stores that enable marine mammals to prolong dives aerobically (Butler and Jones, 1997; Kooyman, 1989; Kooyman and Ponganis, 1998; Ponganis et al., 1999).

A change in the capacity to store oxygen as a function of growth or age has the potential to alter the foraging ability of deep-diving animals and ultimately constrain life-history patterns. Nevertheless, there are relatively few studies on wild populations of long-lived, known-age animals in which variation in oxygen storage capacity is known. To date, most research on foraging ability has focused on developmental processes for insights into how behavioral and physiological constraints differ between juveniles and adults (Burns et al., 2005; Fowler et al., 2007a; Fowler et al., 2007b; Fowler et al., 2006; Gunst et al., 2008; Richmond et al., 2006; Spence-Bailey et al., 2007; Weise and Costa, 2007; Zeno et al., 2008) or in relation to how dimorphism influences segregation of foraging strategies between males and

females (Breed et al., 2006; Kamilar and Pokempner, 2008; Le Boeuf et al., 2000; Weise and Costa, 2007).

Physiological changes that occur over long life spans are potentially as important as changes that occur during early development. The northern elephant seal (NES), *Mirounga angustirostris* (Gill 1866), is an ideal system in which to investigate these changes. Age at first breeding is 3–4 years, and females have produced offspring until 20 years old. For reproductive females, the demands for successful foraging are considerable, as they must accumulate sufficient energy reserves to support the fasting mother and her pup for a month over the lactation period on land. NES dive continuously to foraging depths between 300 and 1600 m (Le Boeuf et al., 2000; Le Boeuf et al., 1988). At sea for the vast majority of their lives, females range throughout the northeast Pacific and, like southern elephant seals (*Mirounga leonina*), follow preferred foraging routes (Bradshaw et al., 2004), returning to breed and molt for a month at a time in natal colonies (Le Boeuf et al., 2000). Females forage for 2–3 months before returning to land to molt their pelage. After the molt, females depart again to sea for 8 months while gestating a fetus, before returning to the colony to give birth.

Our objective was to combine data on dive duration, a proxy for physiological demand, from known-age, free-ranging females with concurrent measurements of oxygen stores on the same individuals to test the hypothesis that age-related changes in the capacity to store oxygen will impact the duration of dives. This was accomplished over a 4-year period with 112 known-age females between 4 and 17 years old.

MATERIALS AND METHODS

Field site and study sample

We conducted this study at Año Nuevo State Reserve, San Mateo County, California, USA during semiannual female elephant seal haul-outs. For seven consecutive molt and breeding cycles between 2004 and 2008, we collected data on diving behavior in sampled animals. Individual seals were sampled 1–6 times over the study period. We collected 257 measurements of mass, plasma volume and Hct in 112 known-age individual females at instrument deployment and recovery, prior to departure and after arrival from sea. Muscle myoglobin concentration was measured from 128 samples in 59 individuals.

For each breeding and molt haul-out between 2004 and 2008, 19–25 females between 4 and 17 years old were sampled and instrumented. Age of females was determined from identification codes on plastic flipper tags (Jumbo roto-tags, Dalton Company, Oxon, UK) that were attached shortly after weaning. Instruments were deployed on breeding females at least 22 days post-partum. Females returning to breed were observed for 5 days post-partum to ensure that the mother–pup bond was established before they were immobilized, instruments were recovered and samples were taken. We are not aware of data for whether blood volume changes after parturition in marine mammals but it does not change in ewes or cows and it has been suggested that maternal blood volume is maintained throughout lactation (Metcalf and Parer, 1966; Reynolds, 1953). The effect of pregnancy on dive duration was examined in females that had instruments recovered after the post-molt foraging trip at the beginning of the breeding season. Elephant seals exhibit embryonic diapause, and implantation is thought to occur during the molt-fast to facilitate synchronous annual reproduction. Thus, effects of pregnancy on dive duration will only occur during the foraging trip following the molt. All females were observed throughout the breeding season to determine if they were pregnant. Females that did not give birth were observed for two weeks, identified as non-pregnant, and instruments were removed. None of these females subsequently gave birth. For females returning to molt, instruments were recovered as soon as possible after arrival on shore (usually 1–3 days after arrival). Deployments were made late in the fast, after females had molted completely and their hair had grown in. Time on shore before recovery or after deployment averaged 6 ± 5 days. Females were immobilized with an intramuscular injection of Telazol (1 mg kg^{-1} ; Aveco, Fort Dodge, IN, USA) following established protocols (Le Boeuf et al., 2000). When necessary, immobilization was maintained intravenously with ketamine hydrochloride (Briggs et al., 1975). Animals were weighed on a canvas sling attached to a hand winch and a tension dynamometer (MSI, Seattle, WA, USA; capacity $1000 \pm 1 \text{ kg}$) suspended from a lightweight aluminum or carbon fiber tripod.

Plasma volume, Hct and blood volume

Blood samples were collected from the extradural vein immediately following sedation and stored in glass, heparinized vacutainers on ice until arrival at the lab. Within 6 h of collection, samples were centrifuged at 3000 g for 15 min, and supernatant containing plasma or serum was frozen and stored at -20°C until analysis. To determine blood volume, each seal received an 8 ml intravenous injection ($0.13\text{--}0.40 \text{ mg kg}^{-1}$) of Evans Blue dye at a concentration of 10 mg ml^{-1} (El-Sayed et al., 1995). A sequence of three 10 ml blood samples was drawn at approximately 10-min intervals post-injection. Syringes were gravimetrically calibrated to determine precise injection volumes ($\pm 0.001 \text{ g}$).

Absorbance of Evans Blue dye in the plasma or serum supernatant was determined at 624 and 740 nm. Values at 740 nm, which do not absorb blue, were used to calculate the blank optical density at 624 nm and correct for hemolysis and precipitate (Foldager and Blomqvist, 1991; Weise and Costa, 2007). Values from serially collected Evans Blue samples were log-transformed, fit to a regression line, and the y -intercept was used to determine the instantaneous dilution volume (El-Sayed et al., 1995). Blood volume was calculated from the maximum measured Hct and plasma volume for each procedure. Hct was determined using standard microhematocrit centrifugation.

Myoglobin measurements

A small scalpel incision was made through the epidermis prior to insertion of a 6-mm trocar to obtain a single biopsy of blubber and muscle, approximately 500 mg and 50 mg, respectively, from each female during anesthesia. The biopsy sites were cleaned with Betadine[®] before and after the procedure. Biopsies were taken from the longissimus dorsii (LD), which is the primary locomotor swimming muscle in phocids. Muscle samples were transferred to 2 cm^3 O-ring sealed cryovials, further sealed with wraps of Parafilm[®] to prevent desiccation, and stored on ice. After arrival at the lab, muscle samples were frozen at -80°C until analysis. Myoglobin content ($\text{g Mb } 100 \text{ g}^{-1}$ wet muscle) was determined following Reynafarje's method (Reynafarje, 1963) using blanks for a control and California sea lion (*Zalophus californianus*) muscle to compare with published values (Weise and Costa, 2007).

Oxygen stores

Total oxygen stores were calculated using a combination of measured and estimated values. Blood oxygen stores were based on measured Hct and assumed Hb content. Mean corpuscular hemoglobin concentration (MCHC) was assumed to be $0.4408 \text{ g Hb } 100 \text{ ml}^{-1}$ of blood per 1% Hct ($N=97$; D.E.C. and D.P.C., unpublished data). This estimate results in values near 25 g dl^{-1} , which is typical of prior determinations in the literature (Zuckerman, 2004). Calculations of oxygen stores were made with the following assumptions from the literature (Kooyman, 1989): (1) initial blood oxygen from arterial blood was 95% saturated and depleted to 20% over the course of a dive; (2) venous blood was two-thirds of the total blood volume, 5 vol.% less than arterial blood, and was depleted to zero oxygen content; and (3) oxygen-binding capacity of hemoglobin and myoglobin was $1.34 \text{ ml O}_2 \text{ g}^{-1}$. Muscle oxygen stores were calculated using the average (7.5%) from our myoglobin subsample on the larger dataset. Mb concentration in the LD was assumed to represent musculature throughout the body, resulting in a likely overestimate of whole-body average Mb concentration. Muscle mass was estimated as 28% of total body mass and was assumed to be uniformly distributed throughout the body (Kooyman et al., 1999). Diving lung volume was assumed to be 27.3 ml kg^{-1} , and oxygen concentration in the lung was estimated as 15% (Ponganis et al., 1993).

Calculation of cADL

In order to determine how well oxygen stores predict observed dive durations, we calculated the aerobic dive limit (cADL) by dividing the available oxygen stores by metabolic rate (Kooyman, 1989). The aerobic dive limit (ADL), also known as the diving lactate threshold (DLT), reflects the point during a dive where there is a greater reliance on anaerobic metabolism such that lactate begins to accumulate in the blood (Butler, 2006). Given that metabolic rates are not fixed, we used a mean diving metabolic

Table 1. Summary statistics for blood oxygen stores by female age and at handling periods corresponding to instrument deployment and recovery

Age	N	Mass (kg)	Hct (%)	Plasma volume (l)	% Body mass	Blood volume (l)	% Body mass
4	42	312±61	59±4	25.5±4.8	8.1±0.5	61.6±11.6	20.2±1.9
5	36	363±80	57±4	30.2±6.9	8.3±0.5	70.1±15.3	19.4±2.1
6	43	355±73	55±4	31.0±7.1	8.7±0.9	69.8±15.4	19.7±1.8
7	37	375±71	57±5	33.1±6.7	8.8±0.5	76.6±13.5	20.4±1.7
8	28	370±76	57±4	32.6±8.4	8.7±0.7	75.8±15.1	20.4±1.6
9	24	405±81	55±4	37.0±7.9	9.1±0.5	83.0±17.9	20.4±1.7
10	22	377±62	59±4	33.5±5.5	8.9±0.6	81.9±14.4	21.8±2.3
11	14	417±14	57±3	37.7±9.3	9.0±0.5	87.7±21.3	20.9±1.8
12	2	468±0	58±5	44.3±0.8	9.5±0.2	106.2±12.3	22.7±2.6
13	2	456±23	57±4	41.3±4.8	9.1±0.6	94.9±3.3	20.8±0.3
14	4	425±32	57±4	40.1±3.1	9.5±0.7	93.7±9.2	22.0±1.1
15	2	431±44	55±2	38.4±3.6	8.9±0.1	84.7±11.7	19.6±0.7
17	1	335	53	28.0	8.4	59.6	17.8
Deployment	140	323±58	57±4	28.0±5.9	8.6±0.7	66.2±13.7	20.4±1.9
Recovery	117	421±65	56±4	36.8±7.0	8.7±0.6	84.1±15.3	20.0±1.9

Values are sample sizes (N), means and standard deviations (s.d.).

rate of two times the Kleiber rate [$0.11 \times M^{0.75}$, where M is body mass (Kleiber, 1961)], based on measured metabolic rates of freely diving Weddell seals (*Leptonychotes weddellii*), to calculate the cADL (Castellini et al., 1992). This approach has been validated in a number of other studies where the ADL (DLT) was empirically measured and compared to the cADL (Kooyman et al., 1980; Ponganis et al., 1997; Ponganis et al., 2009; Shaffer et al., 1997; Williams et al., 1993). For comparison, we also estimated the maximum O_2 depletion rate during the dive by assuming that total available body O_2 stores were completely used over the dive duration.

Instrumentation and analysis

Females were equipped with a time-depth recorder (MK9, MK10-V or MK10-AF; Wildlife Computers, Redmond, WA, USA). Dive recorders were attached to the pelage on the dorsal midline, posterior to the shoulders, using waterproof epoxy (Loctite Quickset™, Henkel Corp., Dusseldorf, Germany). Prior to deployment, instruments were encased in electronic splicing tape and mounted on black nylon seine mesh (Memphis Net and Twine, Memphis, TN, USA) with a combination of nylon and stainless steel cable ties. When seals returned to shore, instruments were removed by cutting the cable ties and splicing tape from the mesh base plate, which was left to fall off during the annual molt. Time-depth recorders (TDRs) sampled every 8 s for the long, post-molt migrations and every 4 s for shorter, post-breeding migrations.

Diving data were summarized in Matlab using the IKNOS-DIVE program from the IKNOS toolbox (Y. Tremblay, unpublished data). Dive data were zero-offset corrected, and dive durations were calculated as the amount of time below the surface for dives below 15 m. Effects of female traits on oxygen stores and the effects of female traits and oxygen stores on dive duration were assessed using linear mixed-effects models with individual seal as a random-effects subject term (Proc Mixed, SAS 9.1.3, SAS Institute, Inc., Cary, NC, USA). Covariance structure for the model fitting was evaluated using calculated Akaike information criteria (cAIC), and the model was fit using restricted maximum likelihood (REML). Secondary interactions between fixed effects were assessed and removed from the model if not significant. Model residuals were assessed for approximate normality. Multicollinearity was assessed for continuous

independent variables using the variance inflation factor (VIF) from Proc Reg in SAS. VIFs for all model independent variables were <1.5 for the models presented.

Measurements of oxygen stores can only be obtained on shore either at the beginning or end of a foraging trip. Nevertheless, dive duration changes over a foraging trip (Zeno et al., 2008) as well as when the animals move across the continental shelf (Le Boeuf and Crocker, 1996). For this reason, we compared dive duration and oxygen stores for their first week of pelagic diving after seals moved off the continental shelf and their final week of diving prior to coming onto the continental shelf at the end of the foraging trip. In the model, this parsed dataset is referred to as 'deployment/recovery status' to reflect dive durations that correspond to measurements made with deployment or recovery of TDRs.

RESULTS

For females, age 4–17 years, mass at handling ranged from 218 to 600 kg, averaging 367 ± 79 kg. Mean blood oxygen store measurements across age classes are shown in Table 1. Blood volume averaged 74.4 ± 17.0 liters in female elephant seals or $20.2 \pm 2.0\%$ of body mass. Plasma volume averaged 32.2 ± 7.8 liters or $8.7 \pm 0.7\%$ of body mass. Across this size range, scaling of blood volume was isometric with body mass ($M=0.97 \pm 0.03$, $r^2=0.80$, $P<0.01$; Fig. 1). Hct averaged $56 \pm 4\%$ and Mb averaged 7.5 ± 0.7 g%. We obtained 116 complete diving records from 174 individuals. Mean dive durations for the week closest to deployment measures were 20.01 ± 2.88 min and were 27.33 ± 3.43 min for the week closest to recovery measures.

Effects of age, pregnancy status and mass on blood and muscle oxygen stores

Body mass had the strongest positive effect on plasma volume ($F_{1,144}=1827.31$, $P<0.001$). There was also a significant positive effect of age on plasma volume ($F_{1,143}=30.59$, $P<0.001$; Fig. 2). Hematocrit did not vary as a function of age ($P>0.05$) but it weakly decreased with body mass ($F_{1,144}=17.33$, $P<0.001$).

Blood volume, which is derived from Hct and plasma volume, increased strongly as a function of body mass ($F_{1,143}=892.22$, $P<0.001$; Fig. 1). Blood volume also increased significantly with age ($F_{1,143}=24.40$, $P<0.01$; Fig. 2). Seventeen percent of the variation in blood volume was attributed to significant differences between

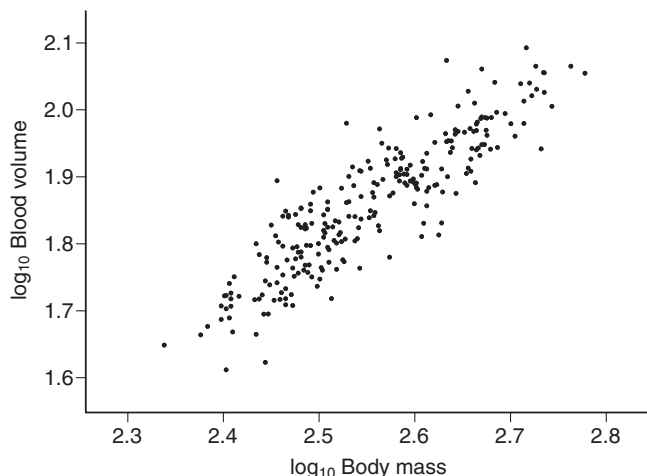


Fig. 1. Isometric increase of blood volume with body mass in female northern elephant seals. Blood volume synthesizes variation in measured plasma volume and hematocrit. The slope parameter estimate was 0.97 ± 0.03 . Due to the repeated measures of some of the data, statistics were presented using a linear mixed-effects model.

individuals (Wald test, $Z=1.76$, $P=0.04$). Myoglobin concentration did not vary as a function of age or mass ($P>0.05$; Table 2).

Pregnancy status did not influence any of the variables related to O_2 stores, including Hct, plasma volume, blood volume or Mb ($P>0.05$), and was removed from the models.

Effects of traits on dive duration

The initial linear mixed-effects model contained pregnancy status, deployment/recovery status, mass-specific plasma volume, Hct, mass, age and Mb as fixed effects and therefore the sample size was restricted to the subset of seals for which Mb samples had been collected. Mb did not significantly affect dive duration ($F_{1,76}=0.14$, $P=0.75$), so Mb effects were excluded from future models and the larger data set was used. Similarly, pregnancy status had no significant impact on dive durations ($P>0.05$) and was therefore

Table 2. Summary statistics for muscle oxygen stores by female age and at handling periods corresponding to instrument deployment and recovery

Age	Mass (kg)	N	Mb (g per 100 g muscle)	Muscle O_2 stores (ml kg^{-1} muscle)*
4	32	16	7.7±1.1	103.4
5	363	6	7.6±1.4	102.4
6	355	7	6.5±3.3	87.4
7	375	3	7.0±1.2	93.3
8	370	8	7.4±1.5	99.0
9	405	7	7.9±1.3	106.1
10	377	5	6.3±1.4	84.8
11	417	5	7.7±1.7	102.9
12	468	—	—	—
13	456	1	8.5	113.5
14	425	1	8.2	109.7
15	431	—	—	—
17	335	—	—	—
Deployment	323	37	7.8±1.3	104.5
Recovery	421	22	7.1±1.4	95.1

Values are sample size (N), means and standard deviations (s.d.).

*Oxygen stores were calculated assuming $1.34 \text{ ml } O_2 \text{ g}^{-1} \text{ Mb}$.

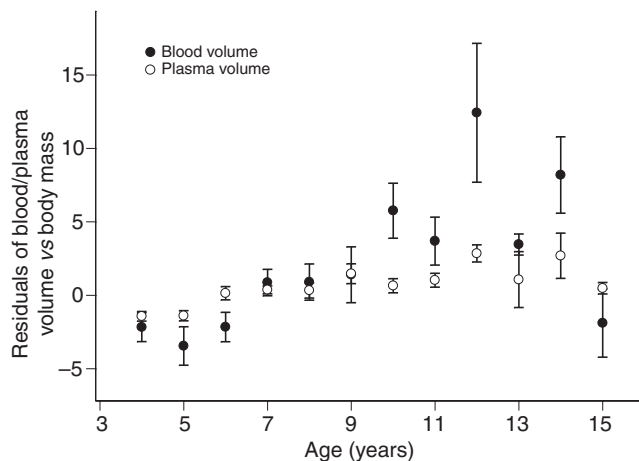


Fig. 2. Changes in the residual variation of mass vs blood volume (filled circles) and plasma volume (open circles) with age in adult female northern elephant seals. Increased variance of error bars (\pm s.e.m.) for blood volume is due to the plasticity of hematocrit.

removed from the model. By contrast, deployment/recovery status had a highly significant impact on dive durations, with substantially longer dives occurring towards the end of trips ($F_{1,98}=55.21$, $P<0.001$). Dive duration increased secondarily as a function of increasing body mass ($F_{1,98}=18.54$, $P<0.001$; Fig. 3), followed by Hct ($F_{1,98}=3.43$, $P<0.01$). Differences in female age, mass-specific plasma volume or differences between individuals as the random subject term in the model did not influence dive duration ($P>0.05$).

Total oxygen stores and cADL

Maximum dive durations ranged from 50 to 77 min, twice cADL values (range, 28–35 min), for all ages (Table 3). Assuming complete use of available oxygen stores over a dive, calculated total oxygen stores ranged from 27.1 to 47.01 O_2 , and maximal depletion rates ranged from 3.6 to 4.5 $\text{ml } O_2 \text{ min}^{-1} \text{ kg}^{-1}$. Expressed as multiples of predicted standard metabolic rate from Kleiber's equation, maximal oxygen consumption was $1.8 \times$ Kleiber after departure to sea and $1.4 \times$ Kleiber just prior to arrival. Dive duration increased with expansion of total available oxygen stores (Fig. 4). Considering the entire trip, we report the longest published dive for this species (103 min). This dive was flanked by a sequence of dives that double cADLs. Corresponding surface intervals averaged 2 min (Fig. 5).

DISCUSSION

Maternal age was not an important determinant of dive duration in one of the deepest and longest diving iteroparous breeders, the NES. Instead deployment/recovery status, body mass, and Hct were the only variables that significantly influenced how long seals could dive. This combination suggests that complex interactions between conditioning and physiological traits determine diving capacity. Mass-specific plasma volumes were relatively invariant, leaving variation in mass-specific blood oxygen stores to be driven mostly by Hct. This variation exerted a significant, but weak, influence on dive duration. Myoglobin concentration, and thus muscle oxygen stores, did not vary with mass, age or diving behavior (Table 2).

Deployment/recovery status

Visual inspection of diving records for all females showed a steady increase in dive duration after about a month of diving, without

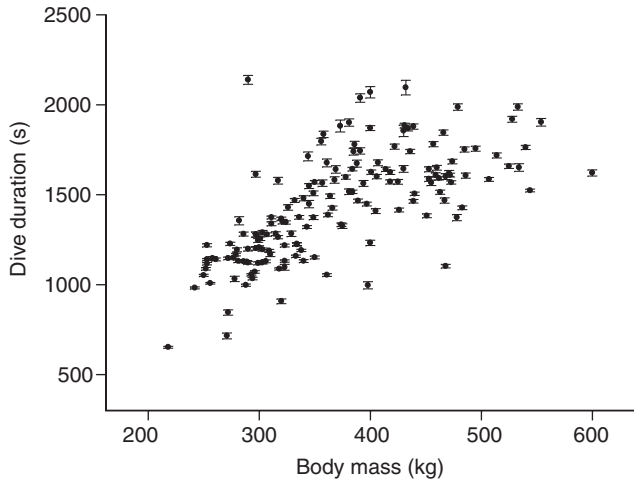


Fig. 3. Changes in mean dive duration with body mass \pm s.e.m. The values were calculated for the first and last week of diving off the continental shelf.

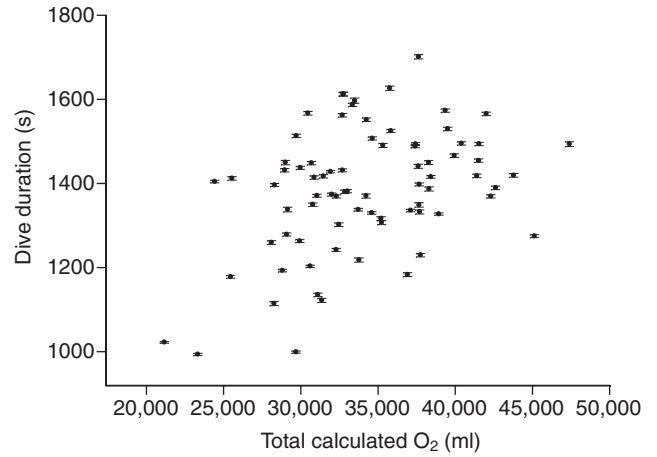


Fig. 4. Changes in dive duration (\pm s.e.m.) with estimated total oxygen stores. Oxygen stores were estimated using measured blood volumes and Mb concentrations from both deployment and recovery measurements. A regression line was not fit due to lack of independence among measurements.

a similar increase in post-dive surface intervals. When seals are ashore, fasting while engaging in energetically costly processes of breeding and molting likely results in loss of diving condition and reduced oxygen storage capacity. Approximately $29 \pm 20\%$ of dives exceeded cADL during the week of diving towards the end of foraging trips, while only $1 \pm 2\%$ of dives exceeded cADLs for the corresponding week at the beginning. After lean body reserves have been replenished towards the end of a foraging trip, proportions of adipose tissue increase while blood volume remains a constant proportion of body mass (Fig. 1). Such changes in body composition may result in a lower mass-specific metabolism (Rea and Costa, 1992) for an equivalent proportion of stored oxygen, therefore contributing to a greater ADL.

Increased dive durations across trips could potentially be influenced by increases in lean mass-specific blood volume as seals increase blubber content. However, the changes in blubber composition over the short, post-breeding trip are small, with females

recovering significant lean tissue mass that was lost during the lactation fast. Analyses of lean mass-specific O_2 stores against dive durations do not support this argument (D.E.C. and D.P.C., unpublished data). Our data do not suggest that changes in blood oxygen stores could underlie these differences (Table 1). The decline in estimated maximum rate of O_2 uptake (\dot{V}_{O_2}) over the foraging migration suggests that metabolic adjustments may bring about some of these changes.

Pregnancy

Previous investigations have attributed increases in diving ability over the course of a foraging trip to increases in blood volume associated with late-term pregnancy (Le Boeuf, 1994). In the present study, our model indicates that pregnancy is not a significant factor and that dive duration is primarily influenced by how long seals remain at sea. The lack of a pregnancy effect is surprising, as it is well known that human blood volume

Table 3. Measured dive durations, calculated aerobic dive limit (cADL), and estimated maximal rates of oxygen consumption (\dot{V}_{O_2}) in known-age female northern elephant seals

Age	Mass (kg)	N	Diving duration (min)	N	TB O_2 (ml O_2)	cADL (min)	\dot{V}_{O_2} (ml O_2 min $^{-1}$ kg $^{-1}$)
4	312	33	21.7 \pm 5.0	42	28,723 \pm 5514	29 \pm 3	4.5 \pm 1.2
5	363	30	23.8 \pm 4.7	36	32,234 \pm 7076	29 \pm 3	3.9 \pm 1.2
6	355	19	23.0 \pm 7.0	40	31,756 \pm 6405	29 \pm 3	3.8 \pm 0.9
7	375	17	24.0 \pm 4.3	35	34,276 \pm 6200	31 \pm 3	4.0 \pm 1.0
8	370	17	23.1 \pm 5.4	28	34,062 \pm 6174	31 \pm 3	4.2 \pm 1.1
9	405	19	25.1 \pm 5.7	24	36,760 \pm 7927	31 \pm 4	3.9 \pm 1.1
10	377	19	23.3 \pm 3.8	19	36,685 \pm 6932	33 \pm 4	4.4 \pm 1.0
11	417	12	25.7 \pm 5.8	14	39,361 \pm 9337	32 \pm 3	3.8 \pm 0.8
12	468	1	26.7	2	47,026 \pm 6800	35 \pm 5	4.2 \pm 0.0
13	456	2	25.9 \pm 1.2	2	42,312 \pm 31	33 \pm 1	3.6 \pm 0.4
14	425	2	25.3 \pm 1.5	4	41,346 \pm 5289	33 \pm 3	4.0 \pm 0.1
15	431	2	23.3 \pm 4.4	2	37,755 \pm 5647	30 \pm 2	3.8 \pm 0.5
17	335	1	18.4	1	27,072	28	4.1
Deployment	323	89	20.0 \pm 3.0	135	29,998 \pm 5955	30 \pm 3	4.8 \pm 0.9
Recovery	421	85	27.0 \pm 3.0	114	37,986 \pm 6530	31 \pm 3	3.4 \pm 0.5

TB O_2 represents available O_2 stores calculated as described in the Materials and methods. Max \dot{V}_{O_2} was estimated assuming complete use of available stores over the dive duration. Deployment and recovery are estimates for the week of diving at the beginning and end of foraging trips, respectively. Values are sample size (N), means and standard deviations (s.d.).

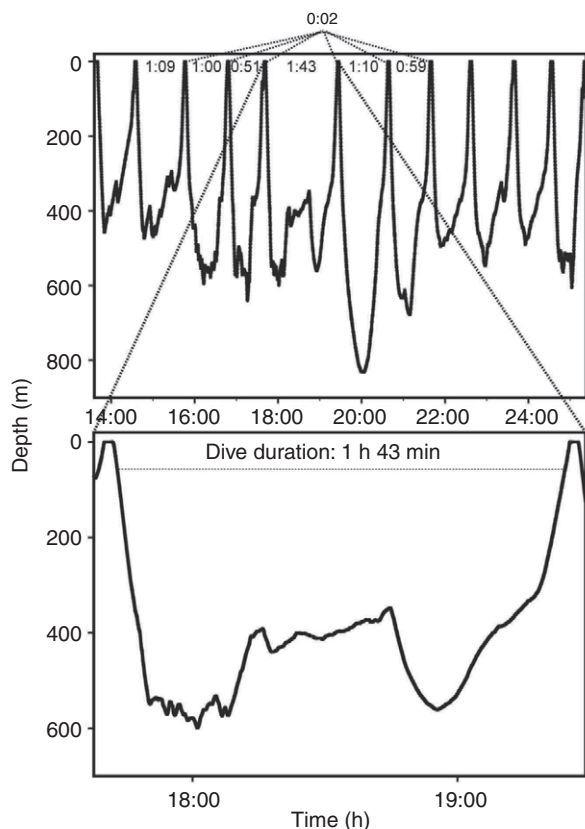


Fig. 5. The longest recorded dive for a northern elephant seal. Despite its length, this dive was flanked by a series of dives that also greatly exceeded calculated limits. Corresponding surface intervals were short and invariant.

increases during pregnancy (Prichard, 1965; Ueland, 1976). However, our data reveal a pattern of increasing dive durations across a trip, evident in post-breeding foraging trips before implantation occurs and post-molt trips from non-pregnant females. This pattern has also been observed in juvenile NES (Zeno et al., 2008). Indeed, some of our longest recorded dives came from non-pregnant females during the post-molt migration, which suggests that the fetus parasitizes maternal oxygen stores. Serial studies of blood volumes in the same seals throughout an annual cycle would be useful in better defining the effect of pregnancy on maternal blood volume in NES.

Mass

Over the reproductive cycle, NES females exhibit a 3-fold variation in body size. While it is not remarkable that oxygen stores scale positively with increased body mass, the strong effects of body mass on dive duration for NES (Fig. 3), independent of age or blood oxygen stores, have not been observed in previous studies (Le Boeuf, 1994). This pattern indicates that diving metabolism also scales strongly with body mass, conferring metabolic efficiency with increased size. Previous work on swimming speed across an order of magnitude size range in NES reported similar swimming speeds while diving and foraging, regardless of body size (Hassrick et al., 2007). A reduction in swimming effort (i.e. metabolic rate) due to the efficiency of transport associated with larger size likely accounts for some of the increase in dive duration as a function of increased mass. This has important implications for the evolution of large body size in marine mammals.

Oxygen stores

Blood and muscle oxygen stores were among the highest reported for any mammal. Plasma volume was approximately 9% of body mass (Table 1), slightly higher than previously reported values in NES (Simpson et al., 1970) and Weddell seals, which were approximately 7% of body mass (Ponganis et al., 1993). Blood volume was ~21% of body mass, the highest value reported for a pinniped.

The amount of Hb for transporting oxygen in a fixed blood volume varies mainly with Hct, the fraction of the total blood volume that is erythrocytes. MCHC is relatively constant in mammals at ~35–40% (Lenfant, 1969), although studies have revealed small effects of season (Villegas Amtmann, 2009), sex and physiological state (Rietkerk et al., 1994). Hct, which is quite plastic and dependent on the level of splenic contraction (Hurford et al., 1996), is nearly independent of body mass (Burke, 1966). While others have shown that deep-diving marine mammals have elevated Hct (Castellini et al., 1986; Hedrick and Duffield, 1991), in our model Hct exerted a significant influence on dive duration that was independent of plasma volume, suggesting that our measured variation reflected real differences in Hct and available blood oxygen stores during diving.

Elevated Mb concentrations in locomotor muscle are considered to be another hallmark of long-diving animals (Kooyman and Ponganis, 1998; Noren and Williams, 2000). Recent findings on hooded seals (*Cystophora cristata*) and harp seals (*Pagophilus groenlandicus*) (Burns et al., 2007) were thought to be the highest values ever reported in marine mammals (Aparicio et al., 2007; Burns et al., 2007). Mean values from our study (Table 2) were within this range and higher than previously reported for NES where samples had been taken using the same technique and biopsy site on the LD (Thorson, 1993). Within adult female NES from this study, Mb concentration did not vary significantly with body mass or age and ultimately did not have a significant influence on variation in dive duration. It is difficult to conclude from our skewed sample distribution whether the lack of variation in Mb results from a low representation of old seals. Well conserved values observed across all ages reinforce the importance of increased Mb concentrations for deep-diving species in general.

Aerobic diving

Seals with greater oxygen stores tended to exhibit longer dives (Fig. 4). While diving vertebrates are capable of using both aerobic and anaerobic metabolism, the majority of dives appear to rely primarily on aerobic metabolism (Dolphin, 1988; Kooyman, 1989; Kooyman et al., 1980; Kooyman et al., 1999; Ponganis et al., 1993). This is because diving aerobically increases foraging time by maximizing time underwater and minimizing extended recovery time at the surface to help clear accumulated lactate from anaerobic processes (Castellini et al., 1988). For NES, mean dive durations were below cADL (Table 3), indicating that the majority of dives were aerobic.

However, maximum dive durations were often more than twice cADL estimates, without changes in post-dive surface intervals (Fig. 5). Similar observations have been made with other species (Costa et al., 2004; Costa and Gales, 2003), but none of these studies has measured post-dive lactate levels, so the specific physiological processes are yet to be understood. The observed consistency of short surface intervals between sequences of long-duration dives (Fig. 5) suggests that anaerobic byproducts are either cleared over multiple surface intervals or that cADLs are too conservative. Long series of dives in great excess of cADL tend to discount the idea that lactic acid is being cleared as an oxidative substrate on

succeeding dives. If available oxygen from arterial blood were extended to 100% saturated at the beginning of a dive and extracted down to 10% of surface values over the dive cycle (Meir et al., 2009), calculations suggest an increase of mean total oxygen stores by 2 liters. This addition only extends mean cADL by 2 min, an insufficient quantity to explain dive durations extending over twice the cADL. It is more likely that seals are reducing their diving metabolic rate and thus our estimates of cADL are too low and do not reflect the range of aerobic diving achieved by metabolic adjustments. Studies in free-ranging Weddell seals, Steller sea lions (*Eumetopias jubatus*) and captive California sea lions have shown that diving metabolic rate can be quite variable and is reduced in prolonged dives (Fahlman et al., 2008; Hurley and Costa, 2001; Williams et al., 2004). Lower diving metabolic rates are likely to be achieved by reductions in heart rate, organ perfusion (Scholander, 1940) and with the use of prolonged gliding and intermittent swimming on descent to depth. These swimming modes have been widely recognized as energy-conserving behaviors to reduce metabolic demands (Crocker et al., 2001; Hassrick et al., 2007; Williams et al., 2000; Williams et al., 2004).

Conclusion

Our study shows that conditioning across a foraging trip, body mass and Hct interact in a manner that influences the diving behavior of females. Increases in diving ability with conditioning as a result of time at sea may help to counteract some loss of capacity due to age, allowing dive durations and associated foraging strategies to remain largely unchanged. Our data support the advantage of large body size for deep-diving air-breathers, with greater diving ability associated with increasing body mass. Important components of total oxygen stores, like plasma volume and muscle Mb concentrations, were relatively invariant among individuals but still important in facilitating high oxygen storage capacity and long dive durations. By contrast, variation in Hct directly influenced differences in diving performance. Our data suggest that individual physiological state variables and condition interact to determine breath-hold ability and should be considered together in life-history studies of foraging behavior and studies assessing demographic consequences of alterations in prey availability.

LIST OF SYMBOLS AND ABBREVIATIONS

ADL	aerobic dive limit
cADL	calculated aerobic dive limit
cAIC	calculated Akaike Information Criteria
DLT	diving lactate threshold
Hb	hemoglobin
Hct	hematocrit
LD	longissimus dorsii
Mb	myoglobin
MCHC	mean corpuscular hemoglobin concentration
NES	northern elephant seal
REML	restricted maximum likelihood
TDR	time-depth recorder
VIF	variance inflation factor
\dot{V}_{O_2}	rate of O_2 uptake

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