J Comp Physiol B (2006) 176: 535–545
DOI 10.1007/s00360-006-0076-9

ORIGINAL PAPER

Julie P. Richmond · Jennifer M. Burns
Lorrie D. Rea

Ontogeny of total body oxygen stores and aerobic dive potential in Steller sea lions (*Eumetopias jubatus*)

Received: 28 September 2005 / Revised: 1 February 2006 / Accepted: 13 February 2006 / Published online: 3 March 2006

Abstract Two key factors influence the diving and hence foraging ability of marine mammals: increased oxygen stores prolong aerobic metabolism and decreased metabolism slows rate of fuel consumption. In young animals, foraging ability may be physiologically limited due to low total body oxygen stores and high mass specific metabolic rates. To examine the development of dive physiology in Steller sea lions, total body oxygen stores were measured in animals from 1 to 29 months of age and used to estimate aerobic dive limit (ADL). Blood oxygen stores were determined by measuring hematocrit, hemoglobin, and plasma volume, while muscle oxygen stores were determined by measuring myoglobin concentration and total muscle mass. Around 2 years of age, juveniles attained mass specific total body oxygen stores that were similar to those of adult females; however, their estimated ADL remained less than that of adults, most likely due to their smaller size and higher mass specific metabolic rates. These findings indicate that juvenile Steller sea lion oxygen stores remain immature for more than a year, and therefore may constrain dive behavior during the transition to nutritional independence.

Keywords Aerobic dive limit · Development · Oxygen stores · Pinniped · Steller sea lion

Communicated by I.D. Hume

J. P. Richmond · J. M. Burns
University of Alaska Anchorage, 3211 Providence Dr., Anchorage, AK, 99508, USA

L. D. Rea · J. P. Richmond
Alaska Department of Fish and Game, 525 W. 67th Ave., Anchorage, AK, 99518, USA

Present address: J. P. Richmond (✉)
University of Connecticut, Department of Animal Science, 3636 Horsebarn Rd Ext Unit 4040, Storrs, CT, 06269, USA
E-mail: julie.richmond@uconn.edu
Tel.: +1-860-4861068
Fax: +1-860-4864375

Abbreviations ADL: Aerobic dive limit · BMR: Basal metabolic rate · BV: Blood volume · cADL: Calculated aerobic dive limit · DLT: Diving lactate threshold · DMR: Diving metabolic rate · Hct: Hematocrit · Hb: Hemoglobin · Mb: Myoglobin · RBC: Red blood cell · RMR: Resting metabolic rate · PV: Plasma volume

Introduction

Species adapted for prolonged diving have certain physiological characteristics that promote aerobic metabolism while submerged. Most notable are increased blood and muscle oxygen stores, and the ability to suppress metabolic rate while diving (Castellini and Kooyman 1989; Butler and Jones 1997). To enhance the amount of oxygen available in the blood, marine mammals have an elevated mass specific plasma volume (PV), larger red blood cells (RBC), and more hemoglobin (Hb) per RBC than terrestrial mammals of similar size (Lenfant 1969; Lenfant et al. 1970; Kooyman 1985). Muscle myoglobin (Mb) concentrations are 10 to 20 times greater in diving mammals compared with terrestrial mammals (Castellini and Somero 1981; Kooyman 1998) and are proportional to dive capacity (Noren and Williams 2000). Increased Mb loads, in combination with enhanced oxidative capacity in their skeletal muscles, allow marine mammals to maintain aerobic metabolism under hypoxic conditions (Butler and Jones 1997; Kanatous et al. 1999; Davis et al. 2004). Peripheral vasoconstriction and bradycardia also help optimize the use of blood and muscle oxygen stores and therefore aid in extending aerobic metabolism and dive duration (Davis and Kanatous 1999; Davis et al. 2004).

Although these physiological adaptations are present in adults, previous research has revealed that young divers have oxygen stores significantly less than adults (aves: Ponganis et al. 1999; Noren et al. 2001; cetaceans: Dolar et al. 1999; Noren et al. 2001, 2002; otariids: Horning and Trillmich 1997; Costa et al. 1998; Sepulveda et al. 1999;

Baker and Donohue 2000; Fowler 2005; phocids: Thorson and LeBoeuf 1994; Burns and Castellini 1996; Clark 2004; Burns et al. 2005; Noren et al. 2005). Blood oxygen stores in juveniles are reduced due to their lower mass specific PV, hematocrit (Hct), Hb, and RBC counts compared with adults (aves: Ponganis et al. 1999; cetacean: Dolar et al. 1999; Noren et al. 2002; phocids: Clark 2004; Burns et al. 2005; otariids: Horning and Trillmich 1997; Costa et al. 1998; Sepulveda et al. 1999; Baker and Donohue 2000; Fowler 2005; Richmond et al. 2005). Muscle oxygen stores are also immature, with juveniles having lower myoglobin concentrations (Noren et al. 2001; Clark 2004; Burns et al. 2005; Noren et al. 2005; Fowler 2005) and an absence of differentiation in Mb concentration between primary swimming and non-swimming muscle types (Dolar et al. 1999; Kanatous et al. 1999).

Total body oxygen stores and rate of oxygen use are important in determining the aerobic potential of an animal. If oxygen becomes depleted during a dive lactic acid begins to accumulate (Kooyman et al. 1980). By definition, the aerobic dive limit (ADL) is empirically determined by measuring post dive lactate accumulation (Kooyman et al. 1980; Butler and Jones et al. 1997). Previous research has demonstrated that ADL can also be estimated by dividing total body oxygen stores by diving metabolic rate (DMR; Castellini et al. 1992; Ponganis et al. 1993). In pinnipeds, these calculated ADL (cADL) are similar to ADL determined by measured lactate accumulation (or diving lactate threshold, DLT; Ponganis et al. 1993, 1997; Burns and Castellini 1996; Butler and Jones 1997; Hurley and Costa 2001).

Steller sea lions provide a unique subject in which to study the development of aerobic dive potential because of their long developmental period and late onset of independent foraging (Calkins and Pitcher 1982; Merrick et al. 1988; Trites and Porter 2002). While young sea lions may not wean until one to 2 years of age, some evidence indicates supplemental feeding may occur after 5 months of age (Raum-Suryan et al. 2002). However, dive behavior and movement patterns continue to be limited in Steller sea lions until at least 1 year of age (Raum-Suryan et al. 2002, 2004; Loughlin et al. 2003; Pitcher et al. 2005). These results suggest that Steller sea lion pups are either physiologically or behaviorally constrained in their diving ability. This subject is especially relevant in light of the population decline of Steller sea lions and the hypothesis that suggests the decline may be due to a failure of juveniles to recruit into the adult population (York 1994). Physiological constraints in dive behavior could limit the prey resources available to newly independent juveniles and therefore limit nutrient intake and negatively influence survival.

To address whether physiological development limits the dive activity of young Steller sea lions, this study measured the components of tissue oxygen stores, and determined the cADL for individual sea lions ranging in age from 1 to 29 months of age. Oxygen stores and cADL values were then compared with adult cADL values and the dive behavior of similarly aged individuals to assess physiological limitations in young Steller sea lions and estimate potential impacts on foraging ability.

Methods

Animal collection and age determination

Between 2002 and 2003 Steller sea lions ($n = 53$) ranging in age from 1 month to 3 years were captured throughout their Alaskan range by the Alaska Department of Fish and Game (ADFG) and National Marine Mammal Laboratory (NMML). Sea lions from 4 months to 3 years of age were captured using an underwater capture method developed by the ADFG (Raum-Suryam et al. 2004). Pups found on rookeries were estimated to be 4 weeks of age (error ± 2 week) based on the average birth date of June 15 and lack of umbilicus (Pitcher et al. 2001). The age of older animals was assessed using date, body size, and degree of canine tooth eruption, or canine growth annuli (King et al. 2003; Laws 1962). Approximately 1–2 h post capture animals were anesthetized using isoflourane gas according to methods outlined in Heath et al. (1997).

Muscle collection and analysis

After anesthetization, two muscle biopsies of approximately 25 mg each were collected from live animals using a disposable sterile 6 mm biopsy punch. One biopsy was collected from the pectoralis, a primary swimming muscle in otariids, and the second from the latissimus dorsi, a superficial non-swimming muscle used for terrestrial locomotion and posture (Fig. 1). The biceps femoralis, also a non-swimming muscle, was used for analysis in three adult males where latissimus dorsi samples were not available. Previous analysis indicated no difference in Mb content of these two muscles (Richmond 2004). Three additional muscle samples were obtained, within 12 h of death, from animals collected for subsistence use by the Aleut Community of St. Paul, Alaska, and from two stranded adult animals necropsied by ADFG (deceased 3–5 days). Muscle samples ($n = 10$) from 1-month-old Steller sea lions were collected from deceased pups less than 6 h post mortem. Samples were frozen on dry ice and maintained at −80°C until analysis.

Frozen muscle samples were thawed, weighed and Mb concentration was determined using the methods described by Reynafarje (1962). Rat (*Rattus norvegicus*) vastus medialis (0.17 ± 0.02 Mb g%) and young elephant seal (*Mirounga angustirostris*) longissimus dorsi (2.46 ± 0.07 Mb g%) samples were used as low and high Mb controls, respectively, in each assay. Detailed sample handling and assay validations have been described previously (Richmond 2004).

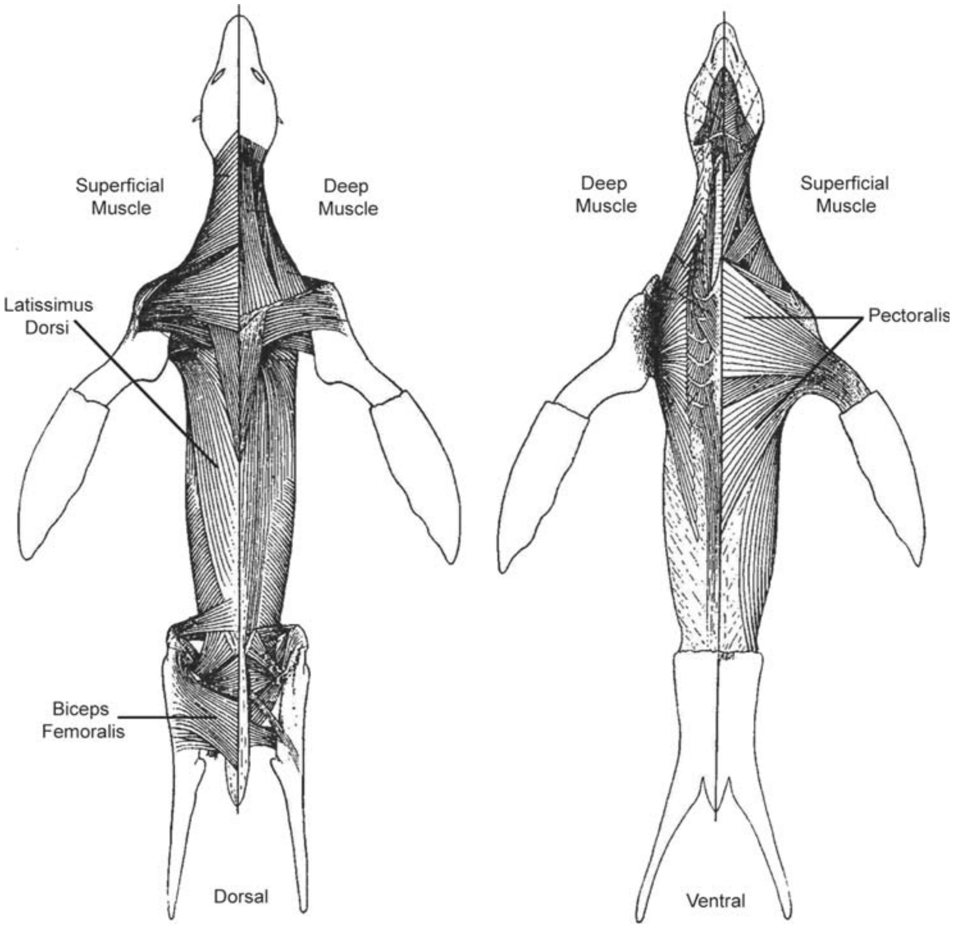

Fig. 1 Sea lion muscle diagram adapted from Howell (1929) showing muscles from which biopsy samples were taken

Blood collection and analysis

Once live captured sea lions were anesthetized, blood was collected from an interdigitial rear flipper vein or the caudal gluteal vein (Dierauf and Gulland 2001) into a heparanized vacuum tube. Blood was collected as soon as anesthesia took full effect to standardize protocol and minimize the effect of isoflourane on Hct (Castellini et al. 1996). Hematocrit was determined in duplicate using a standard clinical microhematocrit centrifuge (8 min at 14,000 G) and Hb was analyzed using the methanocyanide technique (Sigma kit 525–A).

To measure PV, Evan's blue dye (0.5 mg kg^{-1}) was injected through an intravenous catheter placed in an interdigital rear flipper vein. The catheter was then rinsed with approximately 3 ml of heparinized saline (1–5 IU ml^{-1}) to assure complete delivery of the dye. Three blood samples were collected into heparanized vaccutainers at 10, 20, and 30 min post injection. Blood was centrifuged for 8 min at 1,380 G, plasma removed and frozen on dry ice prior to storage at −80°C until processing. If samples were lipemic they were centrifuged at 14,000 G for 20 min at 0°C to separate lipid from plasma. Concentration of Evan's blue dye in the plasma was determined spectrophotometrically at $\lambda = 624$ and 740 nm (Foldager and Blomqvist 1991; El-Sayed et al. 1995). Serially collected plasma samples were fit to a regression line, and the instantaneous dilution volume determined from the *y*-intercept (El-Sayed et al. 1995). Individuals with positive slopes were removed from the analysis. Blood volume (BV) was calculated from hematocrit and PV measurements (Eq. 1; Kooyman et al. 1980; Ponganis et al. 1993):

$$\mathrm{BV} = \frac{\mathrm{PV}}{(100 - \mathrm{Hct})/100} \qquad (1)$$

Total body oxygen stores and aerobic dive limit

Total body oxygen stores were calculated as the sum of the available oxygen in lung, blood, and muscle. All components were measured and calculated for each individual unless otherwise mentioned. Diving lung volume was estimated as 55 ml kg^{-1} based on the measured lung volume in Steller sea lions from Lenfant et al. (1970), and the diving lung compression determined for California sea lions by Kooyman and Sinnett (1982). Lung oxygen content was estimated as 15% of diving lung volume (Eq. 2; Kooyman et al. 1983; Ponganis et al. 1993). These values were assumed to be constant with age on a mass specific basis.

$$\text{Lung ml O}_2 = \text{Mass} \times (55\ \text{ml kg}^{-1}) \times (0.15) \qquad (2)$$

Blood oxygen stores were estimated based on the sum of the available oxygen in arterial and venous blood compartments (Eqs. 3, 4; Ponganis et al. 1993). One-third of the total blood volume was considered arterial while the remaining two-thirds were venous. We assumed that arterial blood was 95% saturated at the start of a dive and 20% saturated at the end of the dive, and that venous blood contained 5 vol% less oxygen than the initial arterial saturation. The blood oxygen capacity was determined by multiplying the Hb content by the oxygen-binding capacity of Hb. Arterial oxygen content was 95% of the blood oxygen capacity. These calculations result in approximately 72% of the total oxygen capacity of the blood being available for maintenance of aerobic metabolism while diving.

$$\begin{aligned}\text{Arterial ml O}_2 = {} & 0.33 \times \text{BV} \\ & \times (0.95 - 0.20\ \text{saturation}) \\ & \times (1.34\ \text{ml O}_2\ g\ \text{Hb}^{-1}) \times (\text{g Hb})\end{aligned} \qquad (3)$$

$$\begin{aligned}\text{Venous ml O}_2 = {} & 0.67 \times \text{BV} \\ & \times (\text{arterial O}_2\ \text{content} - 5\ \text{vol\%})\end{aligned} \qquad (4)$$

Since no PV data was available for 1-month-old pups, blood oxygen stores were estimated using 5-month-old mass specific PV and the average Hct and Hb values measured in 1-month-old pups (Richmond et al. 2005). Blood oxygen stores were estimated for adult males using average mass specific BV and Hb values from adult females (M. A. Castellini, unpublished).

Muscle oxygen stores were estimated from muscle mass and Mb load. We assumed that all oxygen in the muscle was available to the sea lions while diving. For animals less than 6 months of age, muscle mass was assumed to be 30% of total body mass, as determined by complete dissection of 1-month-old pups (Richmond 2004). Animals greater than 6 months of age were assumed to be 37% muscle, based on measurements from juvenile California sea lions (Ponganis et al. 1997). Total muscle mass was divided into swimming (52%) and non-swimming (48%) muscle types based on the complete dissection of 1-month-old sea lions (Richmond 2004). Muscle oxygen stores for adult female sea lions were calculated from the average Mb concentrations reported by Kanatous et al. (1999) for swimming and non-swimming muscle types. For all other age classes, muscle oxygen stores were calculated using the Mb concentration for the individual paired samples according to the following equation:

$$\begin{aligned}\text{Muscle ml O}_2 = {} & \text{Mass} \times \text{Mb} \times (1.34\ \text{ml O}_2\ \text{g}^{-1}\ \text{Mb}) \\ & \times (\%\ \text{muscle mass}) \\ & \times (\%\ \text{muscle type})\end{aligned} \qquad (5)$$

The ADL was determined by the total amount of available oxygen and the rate at which it was used. Resting metabolic rate (RMR) was calculated using basal metabolic rate (BMR) equations from Kleiber (1975) multiplied by age-specific scaling factors for Steller sea lions that account for growth (Winship et al. 2002). Estimates were comparable to RMR measured in free-ranging Steller sea lions of similar age and size (Hoopes et al. 2004).

$$\begin{aligned}\text{RMR}_{0-12}\ \text{months} = {} & 292.88 \times \text{Mass}^{0.75} \\ & \times (-0.083 \times \text{age in months} \\ & + 3.5)\end{aligned} \qquad (6)$$

$$\begin{aligned}\text{RMR}_{1-8}\ \text{years} = {} & 292.88 \times \text{Mass}^{0.75} \\ & \times (-0.071 \times \text{age in years} + 2.32)\end{aligned} \qquad (7)$$

A range of possible cADL were calculated based on three multipliers of RMR. The minimum rate of energy used during a dive was estimated by one times RMR (Ponganis et al. 1997; Hurley and Costa 2001). Multiples of two and four times resting represent estimates based on the minimum cost of transport and maximum observed field metabolic rates in Steller sea lions (Rosen and Trites 2002) and other otariids (Feldkamp 1987; Costa et al. 1989; Arnould and Boyd 1996; Donohue et al. 2000). To convert RMR to oxygen consumption a respiratory quotient of 0.76 (19.3 kJ l^{-1}O_2) was used. This assumes an equal portion of lipid verses protein fuel source (Schmidt-Neilsen 1997), and is intermediate between the lipid rich diet of nursing pups, and the protein rich diet of foraging animals (Adams 2000; Iverson et al. 2002).

Statistical analysis

Animals were grouped into 1 month age categories to determine if age had a significant influence on PV, BV, blood oxygen stores, muscle oxygen stores, total body oxygen stores, or ADL, using one-way ANOVA. The rate of change in total body oxygen store and ADL due to age was also analyzed using a linear regression, so that oxygen stores and cADL could be estimated for animals of known age. To determine if Mb concentration varied due to muscle type, age, or their interaction, a repeated measures ANOVA was used with muscle type as the within subject factor and age as the between subject factor. Tukey post-hoc was performed to evaluate mean differences among age categories.

Gender differences in adult muscle oxygen stores, total body oxygen stores, and ADL were analyzed using unpaired *T* test. Due to the small sample size within each age category, gender differences in young sea lions could not be evaluated. Gender difference in adult muscle myoglobin content was assessed using a Repeated Measures ANOVA of adult female Mb concentration measured by Kanatous (1997) and adult male Mb assayed in this study.

Prior to analyses, all variables were tested for normality using the Kolomogorov–Smirnov test. Statistical analyses were completed using SPSS 11.5 software package. Means are reported ± standard error (SE). Values were considered significant if $P \leq 0.05$.

Results

Blood and muscle development

Development of hematology variables in Steller sea lions was reported in detail by Richmond et al. (2005). Briefly, Hct and Hb values decreased after birth, reached the lowest point in animals of approximately 3 months of age, and then increased. A plateau in Hct and Hb increase occurred near the end of the first year of life.

There were significant age related changes in PV and BV on both an absolute (PV $F_{5,44} = 61.28$, $P < 0.001$; BV $F_{6,58} = 20.69$, $P < 0.001$), and mass specific basis (PV kg^{-1} $F_{5,44} = 7.07$, $P < 0.001$; BV kg^{-1} $F_{6,58} = 10.81$, $P < 0.001$). While absolute PV and BV increased linearly with age, mass specific PV and BV were similar among all age classes of young sea lions (Fig. 2). Post-hoc tests revealed that adult females had greater mass specific PV than young sea lions (9 and 17 months, $P < 0.001$; 21 months, $P = 0.003$; 29 months, $P = 0.030$) except 5 month olds ($P = 0.200$). There were no significant differences in mass specific BV among young sea lions ($P > 0.05$), but all ages were significantly less than adult female values (1–17 months, $P < 0.001$; 21 and 29 months, $P = 0.002$).

Myoglobin concentration in both swimming and non-swimming muscles increased significantly with age in juvenile sea lions ($F_{6,45} = 40.64$; $P < 0.001$). However, there were also significant effects of muscle type ($F_{1,45} = 287.33$; $P < 0.001$) and the interaction of type and age ($F_{6,45} = 33.04$; $P < 0.001$), with sea lions in all but the youngest age category having higher muscle myoglobin concentrations in their swimming muscles as compared with non-swimming muscles (Fig. 3). Adult males had greater Mb concentrations than adult females ($F_{1,11} = 22.01$; $P < 0.001$).

Total body oxygen store development

Mass specific blood, muscle, and total body oxygen stores were all significantly influenced by age (blood O_2 in ml kg^{-1} $F_{6,53} = 9.01$, $P < 0.001$; muscle O_2 in ml kg^{-1} $F_{7,52} = 76.19$, $P < 0.001$; total O_2 in ml kg^{-1} $F_{7,52} = 49.23$, $P < 0.001$; Table 1). However, the pattern of change with age differed between the store locations. Blood oxygen stores reached values similar to adults by 9 months of age ($P = 0.432$), while muscle oxygen stores were not similar to adult female values until 17 months of age ($P = 0.465$; Fig. 3), and never reached levels similar to those observed in adult males ($P < 0.001$). Mass specific total body oxygen stores were similar to adult female values by 21 months of age ($P = 0.928$), but remained less than adult males, even at 29 months ($P = 0.001$; Table 1). Adult females had significantly lower mass specific muscle oxygen stores ($t_{11} = -5.36$, $P = 0.006$) and total body oxygen stores ($t_{11} = -5.083$, $P < 0.001$) than adult males. In addition to the increase in mass specific total body oxygen stores observed throughout develop-

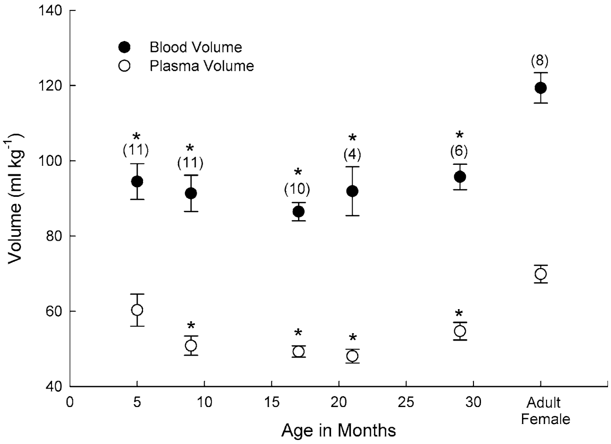

Fig. 2 Developmental change in mass specific plasma (*Open circle*) and blood volume (*filled circle*; mean ± SE). Sample size for each age category indicated above *symbol in parentheses*. Mass specific volumes that differ from adult females are identified by (*Asterisk*). Mass specific plasma and blood volume was similar in all animals less than 29 months of age. Adult female values were provided by M. A. Castellini (unpublished data)

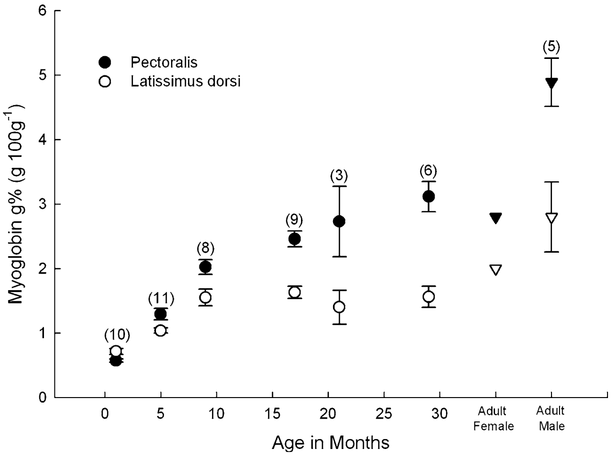

Fig. 3 Muscle myoglobin concentrations (mean ± SE) in Steller sea lions throughout development. *Solid symbols* represent swimming muscle type and *open symbols* represent non-swimming muscle type. Young sea lions are *circles* and adults are *triangles*. Sample size is indicated above *symbol in parentheses*. Average adult female myoglobin concentrations are from Kanatous et al. (1999). Rapid development in the first year of life is slowed during year two. Myoglobin concentrations in young sea lions did not reach adult male values within the time frame of this study

Table 1 Change in mass specific oxygen stores throughout development, and the relative percent of adult values

	Age in months	*N*	Mass (kg)	Blood O_2		Muscle O_2			Total body O_2		
				(ml of O_2 kg^{-1})	% of adult female	(ml of O_2 kg^{-1})	% of adult female	% of adult male	(ml of O_2 kg^{-1})	% of adult female	% of adult male
Young of the Year	1	10	25.1 ± 2.3	10.1^{a}	55	2.3 ± 0.1^{a}	16	10	20.6 ± 0.1^{a}	51	41
	5	11	62.2 ± 4.1	$12.4 \pm 0.7^{a,b}$	68	5.5 ± 0.3^{a}	39	23	26.2 ± 0.9^{b}	65	52
	9	8	98.8 ± 6.3	$15.5 \pm 1.0^{b,c,d}$	85	9.9 ± 0.6^{b}	71	41	32.9 ± 1.3^{c}	81	66
Yearlings	17	9	112.5 ± 4.7	$13.6 \pm 0.5^{a,b,c}$	75	$12.0 \pm 0.6^{b,c}$	86	50	33.7 ± 0.4^{c}	83	67
	21	3	131.6 ± 9.0	$16.2 \pm 2.2^{b,c,d}$	89	$13.3 \pm 2.7^{b,c}$	95	56	$37.5 \pm 5.5^{c,d}$	93	75
Juvenile	29	6	170.6 ± 15.8	$17.9 \pm 2.1^{c,d}$	98	15.2 ± 1.1^{c}	109	64	41.4 ± 2.3^{d}	102	83
Adults	Adult female	8	237.5 ± 9.2	$18.2 \pm 1.0\dagger^{d}$	100	$14.0\ddagger^{c}$	100	59	40.4 ± 1.0^{d}	100	81
	Adult male	5	650*	ND	ND	23.9 ± 1.8^{d}	171	100	$50.0 \pm 1.8{**}^{e}$	124	100

Total body oxygen stores are the sum of lung, blood, and muscle oxygen stores available while diving. See equations in text for calculations. Values shown are mean ± SE. Different letters within a column indicate statistical difference at $P < 0.05$

ND indicates no data available

†Adult female mass and blood data from M.A. Castellini (unpublished data)

‡Average adult female myoglobin from Kanatous et al. (1997) used to calculate muscle oxygen stores

*Adult male mass from necropsy of one adult male

**Adult male total body oxygen stores were calculated using mass specific adult female blood volume and hemoglobin concentration

ment, the site of primary oxygen storage also shifted from blood towards muscle as animals aged (Fig. 4).

Aerobic dive limit

Because the total body oxygen stores increased with age, the cADL of young sea lions also increased with age ($F_{7,52} = 543.85$, $P < 0.001$; Table 2). Adult males and females had cADL that were longer than all other age classes ($P < 0.001$), and differed due to gender ($t_{11} = -10.79$, $P < 0.001$). Assuming a diving metabolic rate of twice resting, the ADL ranged from 0.7 min in 1-month-old pups to 7.5 and 12.0 min in adult females and males, respectively. In addition, the scope of cADL based on the range of estimated diving metabolic rates increased with age from less than 1 min in 1-month-old pups to 18 min in adult males (Table 2).

Discussion

Few studies have measured both blood and muscle oxygen stores concurrently to calculate total body oxygen stores and estimate age related changes in aerobic dive capacity (otariid: Fowler 2005; phocids: Thorson and LeBoeuf 1994; Burns and Castellini 1996; Clark 2004; Noren et al. 2005; cetaceans: Noren et al. 2001, 2002). As with previous studies of blood or muscle development, young sea lions had oxygen stores that were less than adults (otariid: Costa et al. 1998; Fowler 2005; phocid: Thorson and LeBoeuf 1994; Burns and Castellini 1996; Clark 2004; Burns et al 2005; Noren et al. 2005; cetacean: Noren et al. 2002). This discussion will focus on interpreting the pattern of change and identifying the factors that may limit the rate of physiological or behavioral development in Steller sea lions.

Blood and muscle development

The pattern of oxygen storage capacity development in the blood was very different than that seen in muscle, in both timing and magnitude. Hematocrit and Hb decreased shortly after birth reaching a nadir at approximately 3 months of age, after which values increased reaching concentrations similar to adults at approximately 9 months of age (Richmond et al. 2005). This trend was in contrast to the consistently low mass specific PV and BV that never attained adult levels, and the absolute PV and BV that increased with age but still remained lower than adult values. The lack of increase in mass specific BV in young sea lions could be a result of inability of erythropoiesis to surpass growth rate. However, as hematology values were similar to adults around 9 months of age (Richmond et al. 2005), the failure of mass specific BV to increase to adult levels at this time can not be attributed to limited production of RBC, and must instead reflect differences in mass specific PV. Body composition may play a role in this apparent difference. If young animals have 20% greater fat reserves compared with adult females, then PV expressed on a lean body mass basis will be similar in young animals and adult females. Indeed, adult females in early lactation have approximately 10 to 15% body fat (R.W. Davis, unpublished data) while young sea lions (5–29 months of age) range from 29 to 47% body fat (Rea 2002). Similarly in harbor seals, PV and BV are not different by age when scaled to lean body mass verses total body mass (Burns et al. 2005).

Given that there was little change in mass specific BV, the age related increase in mass specific blood oxygen stores were primarily due to increases in the Hct and Hb content in the blood. By 9 months of age, both Hct and Hb were similar to those in adult females (Richmond et al. 2005), and as a result blood oxygen stores were

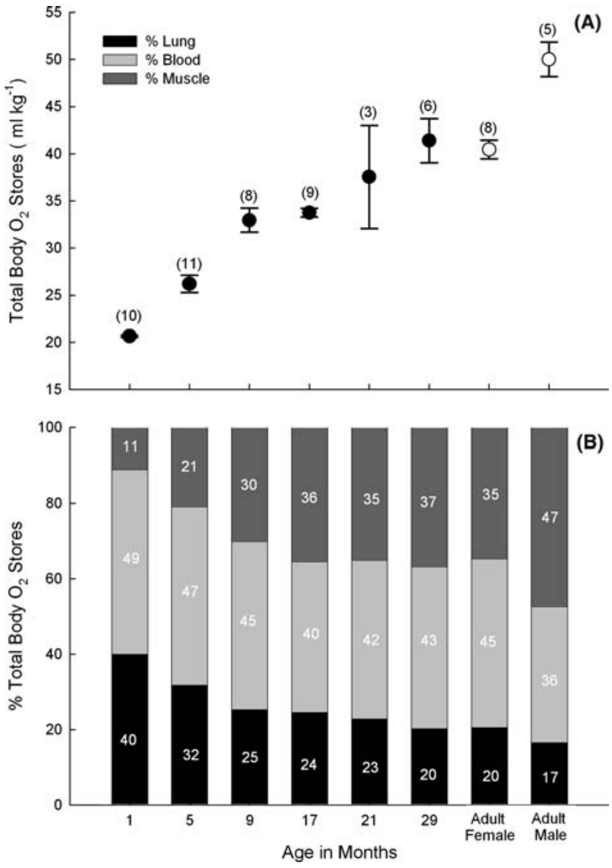

Fig. 4 Developmental trend in **a** mass specific total body oxygen stores and **b** the percentage of oxygen stored in lung, blood, and muscle. *Closed circles* are young sea lions and *open circles* indicate adult animals. Total body oxygen stores increased linearly from 1 to 29 months of age according to the equation Total Body $O_2 = 0.707 \times (\text{age in months}) + 22.271$, $R^2 = 0.736$, $P < 0.001$

85% of adult values. Several other studies have shown that juvenile blood oxygen stores approach adult values near the onset of independent foraging (Horning and Trillmich 1997; Noren et al. 2002, 2005; Clark 2004; Burns et al. 2005), suggesting that Steller sea lions could be weaned near the end of their first year of life.

Similar to other marine mammals, Steller sea lions were born with muscle Mb content that is very different than adults (Lenfant et al. 1970; Thorson and LeBoeuf 1994; Dolar et al. 1999; Ponganis et al. 1999; Noren et al. 2001; Clark 2004; Fowler 2005). At birth, Mb stores were low (1-month-old pups 0.5 g% vs. adult males 4.9 g%), and there was little difference in Mb concentration between muscle types in contrast to adults where swimming muscles have a greater concentration of Mb compared with non-swimming muscles. The rapid increase in Mb concentration seen in early development slowed in the second year of life, even though Mb concentrations were still considerably less than adults (Richmond 2004). Steller sea lion growth rates also slowed in the second year of life from 0.25 to 0.13 kg day^{-1} (Winship et al. 2001; Fadely et al. 2004). The reduced growth rate and rate of muscle development may reflect diminished energy intake associated with weaning and the initiation of independent foraging (Calkins and Pitcher 1982; Merrick et al. 1988; Fadely et al. 2004; ADFG, unpublished data). In many species, this period of newly independent foraging is associated with high mortality and reduced growth (Le Boeuf et al. 1994; Winship et al. 2001; Bowen et al. 2003). Northern elephant seal pups actually lose mass during their first independent foraging trip, and the increase in Mb concentration over this period is much slower than during previous months (Thorson and Le Boeuf 1994). Thus, changes in the pattern of muscle development in Steller sea lions may indicate a shift towards nutritional independence.

While both blood and muscle oxygen stores increased with animal age, they did not increase at the same rate, with blood stores attaining values similar to adults at a younger age compared with muscle. As a result, the relative proportion of oxygen stored in blood and muscle changed with age. While all age classes stored the majority of oxygen in the blood, the ratio of oxygen in blood to muscle changed from 5:1 to 3:2 as young sea lions matured, indicating a growing reliance on muscle oxygen stores. In addition, since muscle oxygen stores were calculated based on the relative mass of swimming versus non-swimming muscles in neonates, it is likely

Table 2 Change in calculated aerobic dive limit (cADL) with development in free-ranging Steller sea lions

Age in months	MAX cADL (1× RMR)	MID cADL* (2× RMR)	MIN cADL (4× RMR)	MAX–MIN difference
1	1.3 ± 0.03	0.7 ± 0.02	0.3 ± 0.01	1.0
5	2.2 ± 0.10	1.1 ± 0.05	0.6 ± 0.02	1.6
9	3.7 ± 0.18	1.8 ± 0.09	0.9 ± 0.04	2.8
17	4.7 ± 0.05	2.3 ± 0.28	1.2 ± 0.01	3.5
21	5.5 ± 0.93	2.7 ± 0.46	1.4 ± 0.22	4.1
Sub-adult	6.6 ± 0.38	3.3 ± 0.18	1.6 ± 0.09	5.0
Adult female	15.0 ± 0.40	7.5 ± 0.19	3.7 ± 0.09	11.3
Adult male	23.9 ± 0.88	12.0 ± 0.44	6.0 ± 0.22	17.9

Total body oxygen stores divided by three estimates of diving metabolic rate calculated from resting metabolic rate (RMR). Values shown are mean ± SE

*Linear regression equation of mid-range cADL for animals 1–29 months of age
$\text{cADL} = 0.094 \times (\text{age in months}) + 0.684$, $R^2 = 0.907$, $P < 0.001$

that the values reported here for older animals underestimate the actual value. If this is the case, then older animals may rely even more heavily on muscle oxygen stores than indicated here.

Increasing reliance on muscle oxygen stores with age may be related to the development of cardiovascular control. Young marine mammals generally do not possess the ability to regulate their cardiovascular system to the same extent as adults (Castellini et al. 1994; Thorson and Leboeuf 1994; Greaves et al. 2004; Noren et al. 2004). As a result, blood supplies oxygen to tissues throughout the course of a dive (or apnea event). In contrast, as animals develop cardiovascular control, diving heart rate drops, blood is redistributed away from the periphery and muscles, and blood oxygen is conserved for core tissues (Blix et al. 1983; Cherepanova et al. 1993). Consequently, muscles must rely more heavily on endogenous oxygen stores while diving (Cherepanova et al. 1993; Davis and Kanatous 1999). Thus, muscle oxygen stores become very important in maximizing aerobic potential once cardiovascular control is well established. In harbor seals, a precocial species that begins diving within hours of birth, cardiovascular control develops gradually over the first 20 days of life (Greaves et al. 2004; Lapierre et al. 2004) corresponding to Mb concentrations that nearly double from birth to weaning (Clark 2004; Burns et al. 2005). If Mb concentrations remain low once cardiovascular control is established, reduced blood flow to the periphery would require muscles to rely more heavily on anaerobic metabolism, therefore increasing recovery times, and reducing dive efficiency (Butler and Jones 1997). In addition, increased muscle oxygen stores serve to prolong dive durations because less oxygen is removed from blood stores, and both Hb and Mb can be depleted at the same rate (Davis and Kanatous 1999).

Interspecific comparison

The few studies that have addressed the development of total body oxygen stores have mainly focused on phocids, which have a much shorter lactation period, typically dive much deeper and longer than Steller sea lions, and therefore have much greater mass specific total body oxygen stores (Thorson and Leboeuf 1994; Burns and Castellini 1996; Noren et al. 2002; Clark 2004; Burns et al. 2005; Fowler 2005). These studies have suggested that marine mammals with short maternal dependency periods (e.g. harbor seal, elephant seal) should develop oxygen stores at a faster rate than species with long lactation (e.g. Steller sea lion, Australian sea lion, and bottlenose dolphin), regardless of the absolute store size. Steller sea lions fit this pattern, with juveniles not achieving oxygen stores similar to adults until the end of their second year. These studies have also suggested that pups begin independent foraging at similar relative oxygen store size, compared with adult values (Thorson and LeBoeuf 1994; Noren et al. 2002; Clark 2004; Richmond 2004; Fowler 2005; Noren et al. 2005). For example, at 1 month of age (the end of maternal support) harbor seals and elephant seals have mass specific total body oxygen stores that are approximately 50 to 60% adult values, and this increases to 73% of adult values at the onset of independent foraging (3 months of age) in northern elephant seals (Thorson and LeBoeuf 1994; Clark 2004). If it is assumed that Steller sea lions begin independent foraging at total body oxygen stores that are of similar proportion to adult stores, comparable with other marine mammal species (approximately 75% of adult values; Thorson and LeBoeuf 1994; Noren et al. 2002, 2005; Clark 2004), then this data suggests that Steller sea lions could wean at the end of the first year of life. This conclusion is supported by the plateau in development of total body oxygen stores in the second year of life, similar to observations after weaning in other marine mammals with long or short maternal dependence strategies (Thorson and Le Beouf 1994; Noren et al. 2002; Richmond 2004). If Steller sea lions continue to nurse through the second year of life, their mass specific total body oxygen stores at weaning are equivalent to adult female values, and therefore no longer constraining of aerobic capacity. Thus, nursing through year two may provide both nutritional and physiological advantages.

Aerobic dive limit

In addition to reduced oxygen stores, young animals also have a greater mass specific metabolic rate and additional energy requirements for growth (Schmidt-Nielsen 1997). Young divers are also unable to maintain heart rate suppression while diving to the extent reported in adult animals (Castellini et al. 1994; Thorson and Leboeuf 1994; Greaves et al. 2004; Lapierre et al. 2004; Noren et al. 2004), and are therefore unlikely to regulate use of oxygen stores as efficiently during breath-hold (Davis et al. 2004).

Typically only 5 to10% of dives in aquatic birds and mammals exceed ADL, because anaerobic metabolism is energetically less efficient than aerobic metabolism, and requires an extended surface interval to remove accumulated lactic acid to restore normal pH values to the system (Kooyman et al. 1980; Butler 2001; Costa et al. 2001). Juvenile animals may exceed their ADL more frequently while diving than adults (Kooyman et al. 1983; Burns 1999). Diving beyond the ADL has been correlated with reduced foraging efficiency in marine mammals, due to the increased time required to process byproducts of anaerobic metabolism (Boyd 1997).

It is important to recognize that ADL is not a fixed number, but a fluid value that reflects the extent of oxygen loading before a dive, and the rate of oxygen use during a dive, both of which can vary (Wilson 2003; Davis et al. 2004). Even though cADL rely on minimum estimates of DMR and maximum oxygen stores, they still offer an acceptable metric for estimating aerobic capacity for most diving mammals, and were used in this study to investigate the development of aerobic dive potential in Steller sea lions.

The developmental changes in cADL demonstrate that all young Steller sea lions had a reduced aerobic dive capacity compared with adults, due both to lower oxygen stores and greater use rates. When cADL were compared with diving pattern of young sea lions and adults, it became clear that the relationship between behavior and physiology differed by age class. For example, the mean dive durations for young of the year Steller sea lions (0.8 min: Merrick and Loughlin 1997; Loughlin et al. 2003; 0.9 min: Rehberg 2005) and yearling animals (1.7 min, Rehberg 2005) were less than the cADL estimated using a DMR of twice RMR, and 95% of dives were shorter than the cADL estimated using RMR. Although, if young sea lions have a DMR four times RMR, then most dives are longer than the cADL, suggesting that highly energetic dives would require anaerobic metabolism. In contrast, adult females seem to be diving well within aerobic threshold, while juveniles appear to be consistently diving at the upper range of their aerobic scope. The median dive duration for adult females (2.0 min) was similar to the cADL estimated using a DMR of four times RMR, and even the maximum reported dive duration (8 min; Merrick and Loughlin 1997) was less than cADL estimated with a DMR of twice RMR. This suggests that adult animals have more flexibility in how they manage their oxygen stores, and that physiology has the potential to constrain juvenile behavior in young animals (Burns and Castellini 1996; Horning and Trillmich 1997; Costa et al. 1998; Burns 1999; Noren et al. 2002).

The potential for physiology to limit diving behavior and foraging efficiency of juvenile Steller sea lion is critical in light of the hypothesis that one of the causes of the decline in population numbers is a reduction in juvenile survivorship as a result of reduced prey availability (York et al. 1996; Adams 2000). This hypothesis assumes that juveniles, with lower diving capacity and limited behavioral options, would be more susceptible to shifts in prey availability than adults. This study has demonstrated that the cADL of Steller sea lions less than 29 months of age was lower than that of adults, and that young animals likely dive closer to their cADL than do adults, as would be expected if foraging was more difficult (Costa et al. 1989; Horning and Trillmich 1999; Burns 1999). However, reduced dive capacity is only a disadvantage if young sea lions are foraging on the same resources as adults, and cannot shift to prey that are easier to capture or found in shallower waters. Unfortunately, with limited information on the location and composition of juvenile Steller sea lions prey species, and no data on foraging efficiency, it is difficult to determine if observed behavioral differences would affect survival. It is clear that juveniles are indeed limited in their aerobic capacity, and that this limitation persists through the first 2 years of life. As a result, newly weaned Steller sea lions, regardless of age at weaning (1 or 2 years of age), cannot as easily access all the prey resources available to adults, and therefore may be more vulnerable to shifts in prey abundance or distribution.

Acknowledgements This research was made possible through funds provided by CIFAR (NA17RJ1224) and co-operative agreement with NOAA and ADFG (NA17FX1079). All research conducted under Marine Mammal Protection Act Permit: no. 358-1564 (ADFG), no. 782-1532 (NMML), and 481-1623 (LGL). Protocols were reviewed and approved by Institutional Animal Care and Use committees at UAA (no. 2002Burns02, 2003Burns06) and ADFG DWC (03-2002). This work would not be possible without the help of numerous people from the past and present Steller sea lion research teams of ADFG and NMML. We would like to thank M. Castellini and R. Davis for the use of unpublished data, D. Pfeiffer, S. Zinn and two anonymous reviewers for constructive comments on the manuscript, and C. Clark for help with the laboratory analysis.

References

Adams TC (2000) Foraging differences and early maternal investment in adult female Alaskan Steller sea lions (Eumetopias jubatus). PhD Dissertation Texas A&M University

Arnould JPY, Boyd IL (1996) The relationship between foraging behaviour and energy expenditure in Antarctic fur seals. J Zool 239:769–782

Baker JD, Donohue MJ (2000) Ontogeny of swimming and diving in northern fur seal (*Callorhinus ursinus*) pups. Can J Zool 78:100–109

Blix AS, Elsner R, Kjekshus JK (1983) Cardiac output and its distribution through capillaries and A-V shunts in diving seals. Acta. Physiol. Scand. 118:109–116

Boyd IL (1997) The behavioural and physiological ecology of diving. TREE 12:213–217

Bowen WD, Ellis SL, Iverson SJ, Boness DJ (2003) Maternal and newborn life-history trends: implications for explaining the decline of harbour seals (*Phoca vitulina*), on Sable Island. J Zool Lond 261:155–163

Burns JM (1999) The development of diving behavior in juvenile Weddell seals: pushing physiological limits in order to survive. Can J Zool 77:737–747

Burns JM, Castellini MA (1996) Physiological and behavioral determinants of the aerobic dive limit in Weddell seal (*Leptonychotes weddellii*) pups. J Comp Physiol 166:473–483

Burns JM, Costa DP, Frost KJ, Harvey JT (2005) Development of body oxygen stores in harbor seals: effects of age, mass, and body composition. Physiol Biochem Zool 78:1057–1068

Butler PJ (2001) Diving beyond the limits. News Physiol Sci 16:222–227

Butler PJ, Jones DR (1997) Physiology of diving of birds and mammals. Physiol Rev 77:837–899

Calkins DG, Pitcher KW (1982) Population assessment, ecology and trophic relationships of Steller sea lions in the Gulf of Alaska. In: Environmental assessment of the Alaska continental shelf. U.S. Department of Commerce and U.S. Department of Interior 19:447–546

Castellini JM, Meiselman HJ, Castellini MA (1996) Understanding and interpreting hematocrit measurements in pinnipeds. Mar Mamm Sci 12:251–264

Castellini MA, Somero GN (1981) Buffering capacity of vertebrate muscle: correlations with potentials for anaerobic function. J Comp Physiol 143:191–198

Castellini MA, Kooyman GL (1989) Behavior of freely diving animals. Undersea Biomed Res 16:355–362

Castellini MA, Kooyman GL, Ponganis PJ (1992) Metabolic rates of freely diving Weddell seals: correlations with oxygen stores, swim velocity and diving duration. J Exp Biol 165:181–194

Castellini MA, Rea LD, Sanders JL, Castellini JM, Zenteno-Savin T (1994) Developmental changes in cardiorespiratory patterns of sleep-associated apnea in northern elephant seals. Am J Physiol 267:R1294–R1301

Cherepanova V, Neshumova T, Elsner R (1993) Muscle blood flow in diving mammals. Comp Biochem Physiol 106A:1–6

Clark CA (2004) Tracking changes: postnatal blood and muscle oxygen store development in harbor seals (*Phoca vitulina*). MSc Thesis University of Alaska Anchorage

Costa DP, Croxall JP, Duck CD (1989) Foraging energetics of Antarctic fur seals in relation to changes in prey availability. Ecology 70:596–606

Costa DP, Gales NJ, Crocker DE (1998) Blood volume and diving ability of the New Zealand sea lion, *Phocartos hookeri*. Physiol Zool 7:208–213

Costa DP, Gales NJ, Goebel ME (2001) Aerobic dive limit: how often does it occur in nature? Comp Biochem Physiol A 129A(4):771–783

Davis RW, Kanatous SB (1999) Convective oxygen transport and tissue oxygen consumption in Weddell seals during aerobic dives. J Exp Biol 202:1091–1113

Davis RW, Polasek L, Watson R, Fuson A, Williams TM, Kanatous SB (2004) The diving paradox: new insights into the role of the dive response in air-breathing vertebrates. Comp Biochem Physiol A 138:263–268. DOI:10.1016/j.cbpb.2004.05.003)

Dierauf LA, Gulland MD (2001) CRC handbook of marine mammal medicine. CRC, Boca Raton

Dolar MLL, Suarez P, Ponganis PJ, Kooyman GL (1999) Myoglobin in pelagic small cetaceans. J Exp Biol 202:227–236

Donohue MJ, Costa DP, Goebel ME, Baker JD (2000) The ontogeny of metabolic rate and thermoregulatory capabilities of northern fur seal, *Callorhinus ursinus*, pups in air and water. J Exp Biol 203:1003–1016

El-Sayed H, Goodall SR, Hainsworth R (1995) Re-evaluation of Evans blue dye dilution method of plasma volume measurement. Clin Lab Haem 17:194

Fadely BS, Gelatt TS, Rea LD, King JC, Loughlin TR (2004) Regional variation of juvenile Steller sea lion (*Eumetopias jubatus*) growth rates in Alaska. Conference Abstract Sea Lions of the World. Anchorage AK. p 21

Feldkamp SD (1987) Swimming in the Californai sea lion: morphometrics, drag, and energetics. J Exp Biol 131:117–135

Foldager N, Blomqvist CG (1991) Repeated plasma volume determination with the Evans blue dye dilution technique: the method and a computer program. Comput Biol Med 21:35–41

Fowler SL (2005) Ontogeny of diving in the Australian sea lion. PhD Thesis University of California Santa Cruz

Greaves DK, Hughson RL, Topor Z, Schreer JF, Burns JM, Hammill M (2004) Changes in heart rate variability during diving in young harbor seals, *Phoca vitulina*. Mar Mamm Sci 20:861–871

Heath RB, DeLong R, Jameson V. Bradley D, Spraker T (1997) Isoflurane anasthesia in free ranging sea lion pups. J Wild Dis 33:206–210

Hoopes LA, Rea LD, Rosen DAS, Worthy GAJ (2004) Effects of body condition on resting metabolism in captive and free-ranging Steller sea lions (*Eumetopias jubatus*). Symp Comp Nutr Soc 5:79–82

Horning M, Trillmich F (1997) Development of hemoglobin, hematocrit, and erythrocyte values in Galapagos fur seals. Mar Mamm Sci 13:100–113

Horning M, Trillmich F (1999) Lunar cycles in diel prey migrations exert a stronger effect on the diving of juveniles than adult Galapagos fur seals. Proc Royal Soc Lon B 266:1127–1132

Howell A, Brazier (1929) Contribution to the comparative anatomy of the eared and earless seals (Genera Zalophus and Phoca). Washington, Smithsonian Institution United States National Museum. In: Proceedings of the United States National Museum 2736, pp 142

Hurley JA, Costa DP (2001) Standard metabolic rate at the surface and during trained submersions in adult California sea lions (*Zalophus californianus*). J Exp Biol 204:3273–3281

Iverson SJ, Frost K, Lang SLC (2002) Fat content and fatty acid composition of forage fish and invertebrates in Princ William Sound, Alaska: factors contributing to among and within species variability. Mar Ecol Prog Ser 241:161–181

Kanatous SB (1997) High aerobic capacities in the skeletal muscles of seals, sea lions and fur seals: adaptations to diving hypoxia. PhD Dissertation Texas A&M University

Kanatous SB, DiMichele LV, Cowan DF, Davis RW (1999) High aerobic capacity in the skeletal muscles of pinnipeds: adaptations to diving hypoxia. J App Physiol 86:1247–1256

King J, Gelatt T, Pitcher K (2003) A field based method for estimating age in wild juvenile Steller sea lions (*Eumetopias jubatus*). Conference Abstract Biology of Marine Mammals. Greensboro NC. p 84

Kleiber M (1975) The fire of life. R. E. Krieger Publishing Company, Huntington

Kooyman GL (1985) Physiology without restraint in diving mammals. Mar Mamm Sci 1:166–178

Kooyman GL, Castellini MA, Davis RW, Maue RA (1983) Aerobic diving limits of immature Weddell seals. J Comp Physiol 151:171–174

Kooyman GL, Ponganis PJ (1998) The physiological basis of diving to depth: birds and mammals. Ann Rev Physiol 60:19–32

Kooyman GL, Sinnett EE (1982) Pulmonary shunts in harbor seals and sea lions during simulated dives to depth. Physiol Zool 55:105–111

Kooyman GL, Wahrenbrock EA, Castellini MA, Davis RW, Sinnett EE (1980) Aerobic and anaerobic metabolism during voluntary diving in Weddell seals: evidence of preferred pathways from blood chemistry and behavior. J Comp Physiol 138:335–346

Lapierre JL, Schreer JF, Burns JM, Hammill MO (2004) Developmental changes in cardiorespiratory patterns associated with terrestrial apnoeas in harbour seal pups. J Exp Bio 207:3891–3898. DOI 10.1242/jeb.01222

Laws RM (1962) Age determination of pinnipeds with special reference to growth layers in the teeth. Zeit Fur Saug 27:129–146

LeBoeuf BJ, Morris P, Reiter J (1994) Juvenile survivorship of northern elephant seals. In: LeBoeuf BJ, Laws RM (eds) Elephant seals: population ecology, behavior, and physiology. University of California Press, Berkley, pp 121–136

Lenfant C (1969) Physiological properties of blood of marine mammals. In Andersen HT (ed) The biology of marine mammals. Academic, New York. pp. 95–116

Lenfant C, Johansen K, Torrance JD (1970) Gas transport and oxygen storage capacity in some pinnipeds and the sea otter. Resp Physiol 9:277–286

Loughlin TR, Sterling JT, Merrick RL, Sease JL, York AE (2003) Diving behavior of immature Steller sea lions (*Eumetopias jubatus*). Fish Bull 101:566–582

Merrick RL, Gearin PJ, Osmek S, Withrow DE (1988) Field studies of northern sea lions at Ugamak Island, Alaska during the 1985 and 1986 breeding seasons. U.S. Department of Commerce, NOAA Technical Memorandum NMFS F/NWC-143, pp 60

Merrick RL, Loughlin TR (1997) Foraging behavior of adult female and young-of-the-year Steller sea lions in Alaska waters. Can J Zool 75:776–786

Noren SR, Cuccurullo V, Williams TM (2004) The development of diving bradycardia in bottlenose dolphins (*Tursiops truncatus*). J Comp Physiol B 174:139–147. DOI 10.1007/s00360–003–0398–9

Noren SR, Iverson SJ, Boness DJ (2005) Development of the Blood and muscle oxygen stores in gray seals (*Halichoerus grypus*): implications for juvenile diving capacity and the necessity of a terrestrial postweaning fast. Physiol Biochem Zool 78(4):482–90

Noren SR, Lacave G, Wells RS, Williams TM (2002) Development of blood oxygen stores in bottlenose dolphins (*Tursiops truncatus*): implications for diving capacity. J Zool 258:105–113. DOI 10.1017/S0952836902001243

Noren SR, Williams EE (2000) Body size and skeletal muscle myoglobin of cetaceans: adaptations for maximum dive duration. Comp Biochem Physiol A 126:181–191. DOI 10.1016/S1095–6433(00)00182–3

Noren SR, Williams TM, Pabst DA, McLellan WA, Dearolf JL (2001) The development of diving in marine endotherms: preparing the skeletal muscles of dolphins, penguins, and seals for activity during submergence. J Comp Physiol B 171:127–134. DOI 10.1007/s003600000161

Pitcher KW, Burkanov VN, Calkins DG, Le Boeuf BJ, Mamaev EG, Merrick RL, Pendleton GW (2001) Spatial and temporal variation in the timing of births of Steller sea lions. J Mamm 82:1047–1053

Pitcher KW, Rehberg MJ, Pendleton GW, Raum-Suryan KL, Gelatt TS, Swain UG, Sigler MF (2005) Ontogeny of dive performance in pup and juvenile Steller sea lions in Alaska. Can J Zool 83:1214–1231

Ponganis PJ, Kooyman GL, Castellini MA (1993) Determinants of the aerobic dive limit of Weddell seals: analysis of diving metabolic rates, postdive end tidal PO_2s, and blood and muscle oxygen stores. Physiol Zool 66:732–749

Ponganis PJ, Kooyman GL, Winter LM, Starke LN (1997) Heart rate and plasma lactate responses during submerged swimming and trained diving in California sea lions, *Zalophus californianus*. J Comp Physiol B 167:9–16

Ponganis PJ, Starke LN, Horning M, Kooyman GL (1999) Development of diving capacity in emperor penguins. J Exp Biol 202:781–786

Raum-Suryan KL, Pitcher KW, Calkins DG, Sease JL, Loughlin TR (2002) Dispersal, rookery fidelity, and metapopulation structure of Steller sea lions (*Eumetopias jubatus*) in an increasing and a decreasing population in Alaska. Mar Mamm Sci 183:746–764

Raum-Suryan KL, Rehberg MJ, Pendleton GW, Pitcher KW, Gelatt TS (2004) Development of dispersal, movement patterns, and haulout use by pup and juvenile Steller sea lions (*Eumetopias jubatus*) in Alaska. Mar Mamm Sci 20:832–850

Rea LD (2002) Indices of condition in Steller sea lions (*Eumetopias jubatus*). In: DeMaster D, Atkinson S (eds) Steller sea lion decline: is it food II. University of Alaska Sea Grant Fairbanks AK, pp 29–33

Rehberg MJ (2005) Pattern matters: changes in the organization of swimming and diving behavior by Steller sea lion juveniles in Alaska. MSc Thesis University of Alaska Anchorage

Reynafarje B (1962) Simplified method for the determination of myoglobin. J Lab Clin Med 61:138–145

Richmond JP (2004) Ontogeny of total body oxygen stores and aerobic dive potential in the Steller sea lion (*Eumatopias jubatus*). MSc Thesis University of Alaska Anchorage

Richmond JP, Burns JM, Rea LD, Mashburn KL (2005) Postnatal ontogeny of erythropoietin and hematology in free-ranging Steller sea lions (*Eumetopias jubatus*). Gen Comp Endo 141:240–247. DOI 10.1016/j.ygcen.2005.01.004

Rosen DAS, Trites AW (2002) Cost of transport in Steller sea lions, *Eumetopias jubatus*. Mar Mamm Sci 18(2):513–524

Schmidt-Nielsen K (1997) Animal physiology adaptation and environment. Cambridge University Press Cambridge

Sepulveda MS, Ochoa-Acuna H, Homer BL (1999) Age-related changes in hematocrit, hemoglobin, and plasma protein in Juan Fernandez fur seals (*Arctocephalus philippii*). Mar Mamm Sci 15:575–581

Thorson PH, LeBoeuf BJ (1994) Developmental aspects of diving in northern elephant seal pups. In: LeBoeuf BJ, Laws RM (eds) Elephant seals: population ecology, behavior, and physiology. University of California Press Berkley, pp 271–289

Trites AW, Porter BT (2002) Attendance patterns of Steller sea lions (*Eumetopias jubatus*) and their young during winter. J Zool Lond 256:547–556

Wilson RP (2003) Penguins predict their performance. Mar Ecol Prog Ser 249:305–310

Winship AW, Trites AW, Calkins DG (2001) Growth in body size of the Steller sea lion (*Eumetopias jubatus*). J Mamm 82:500–519

Winship AW, Trites AW, Rosen DAS (2002) A bioenergetic model for estimating the food requirements of Steller sea lions *Eumetopias jubatus* in Alaska. Mar Ecol Prog Ser 229:291–312

York AE (1994) The population dynamics of the northern sea lions, 1975–85. Mar Mamm Sci 10:38–51

York AE, Merrick RL, Loughlin TR (1996) An analysis of the Steller sea lion metapopulation in Alaska. In: McCullough DR, (ed) Metapopulations and wildlife conservation. Island Press Washington DC, pp 259–292