J Comp Physiol B (2007) 177:217–227
DOI 10.1007/s00360-006-0123-6

ORIGINAL PAPER

A longitudinal and cross-sectional analysis of total body oxygen store development in nursing harbor seals (*Phoca vitulina*)

Cheryl A. Clark · Jennifer M. Burns · Jason F. Schreer · Mike O. Hammill

Received: 29 June 2006 / Revised: 29 August 2006 / Accepted: 29 September 2006 / Published online: 7 November 2006

Abstract This study compared the efficacy of longitudinal and cross-sectional sampling regimes for detecting developmental changes in total body oxygen (TBO_2) stores that accompany behavioral development in free-ranging harbor seal pups. TBO_2 stores were estimated for pup ($n = 146$) and adult female ($n = 20$) harbor seals. Age related changes were compared between pups captured repeatedly during the lactation period (longitudinal dataset) and a second group of pups handled only once (cross-sectional dataset). At each handling, hematocrit, hemoglobin, red blood cell count, total plasma volume, blood volume, muscle myoglobin concentration, and blood and muscle oxygen stores were determined. Comparisons across age categories revealed newborn blood oxygen stores were initially elevated, declined to low values by early lactation, and increased through post-weaning. Muscle oxygen stores remained low and constant throughout lactation and only increased significantly post-weaning. Overall TBO_2 stores increased 17% during lactation, and weaned pups had TBO_2 stores that were 55% as large as those of adults. Thus, significant increases in TBO_2 stores must occur after weaning, as pups begin to forage independently. Results from the two sampling schemes did not differ, indicating that the logistically simpler cross-sectional design can be used to monitor physiological development in harbor seals.

Keywords Development · Harbor seals · Hematology · Myoglobin · Oxygen stores

Abbreviations

BV	Blood volume
Hct	Hematocrit
Hb	Hemoglobin
Mb	Myoglobin
RBC	Red blood cell
TBO_2	Total body oxygen

Introduction

The ability of air-breathing marine predators to forage successfully depends on their ability to remain submerged (Hindell et al. 2000). Dives that rely on aerobic metabolism are more efficient because animals can spend a greater proportion of their time underwater, rather than at the surface recovering from the lactic acidosis that results from anaerobic processes (Kooyman 1989; Castellini 1991). To maximize aerobic submergence

Communicated by I.D. Hume.

C. A. Clark · J. M. Burns
Department of Biological Sciences,
University of Alaska Anchorage,
3211 Providence Drive, Anchorage, AK 99508, USA

J. F. Schreer
Department of Biology, State University of New York
at Potsdam, Potsdam, NY 13676, USA

M. O. Hammill
Department of Fisheries and Oceans,
Institute Maurice-Lamontagne, 850 Route de la Mer,
C.P. 1000, Mont Joli, QC G5H 3Z4, Canada

C. A. Clark (✉)
Alaska Department of Fish and Game,
Wildlife Conservation, 525 W, 67th Ave,
Anchorage, AK 99518, USA
e-mail: cheryl_clark@fishgame.state.ak.us

times, marine mammals have adaptations that allow them to reduce the rate at which oxygen is consumed during dives. In addition, they have larger mass specific total body oxygen (TBO_2) stores (the sum of oxygen in the lungs, blood, and muscle) as compared to terrestrial mammals (Kooyman 1989; Castellini 1991; Thornson and Le Boeuf 1994; Butler and Jones 1997; Jørgensen et al. 2001; Noren et al. 2005). The increased oxygen stores are due to elevated blood volume (BV), high hemoglobin (Hb) levels, larger red blood cells (RBC) and greater muscle myoglobin (Mb) content (for a review, Butler and Jones 1997).

The importance of elevated TBO_2 stores in extending dive duration is evident in the strong correlation between average dive duration and mass specific TBO_2 stores (Costa et al. 2001, 2004). Deep, long diving species such as the northern elephant seal, *Mirounga angustirostris*, Australian sea lion, *Nephoca cinerea*, and New Zealand sea lion, *Phocarctos hookeri*, have much larger TBO_2 stores than species that generally make short, shallow dives such as the California sea lion, *Zalophus californianus*, and the Antarctic fur seal, (*Arctocephalus gazella*) (Lenfant et al. 1970; Snyder 1983; Kooyman 1989; Thornson and Le Boeuf 1994; Butler and Jones 1997; Costa 2001). Patterns of foraging activities used by different species may also influence oxygen use rates and affect how long an animal can remain submerged with otariids typically diving for shorter durations than phocids (Kooyman 1989; Butler and Jones 1997; Schreer and Kovacs 1997). Regardless of the species, the aerobic dive limit (ADL) is the maximum dive duration that can be attained before lactic acid begins to accumulate during the dive, and can be estimated by taking the ratio of TBO_2 stores to the diving metabolic rate (DMR) (Kooyman et al. 1980, 1983). This calculated ADL (cADL) is by no means a limit to diving ability, but a reference point to examine how often diving mammals remain within or exceed the ADL.

Total body oxygen stores, metabolic rate, and ADL have been determined for a variety of adult pinnipeds and cetaceans (Castellini 1991; Lydersen et al. 1992; Butler and Jones 1997; Schreer and Kovacs 1997; Sepulveda et al. 1999), with more recent work focused on juveniles (Burns and Castellini 1996; Horning and Trillmich 1997; Jørgensen et al. 2001; Noren et al. 2005; Burns et al. 2004, 2005; Richmond et al. 2006). These studies indicate that young divers have significantly lower oxygen stores than adults, in part due to lower mass specific plasma volume, hematocrit (Hct), RBC, and Hb concentration. Muscle myoglobin content was also lower than adults, suggesting immaturity in juvenile muscle development (Noren et al. 2001, 2005; Burns et al. 2005). Oxygen stores influence aerobic capacity, and because aerobic capacity influences diving and foraging patterns, understanding how TBO_2 stores develop in juvenile marine mammals may aid in interpreting how pups are able to make the transition to independent foraging.

Harbor seals (*Phoca vitulina*) are one of the most precocial phocids, swimming and diving only a few hours after birth and remaining active throughout the nursing period (Bigg 1969; Knudtson 1977; Boulva and McLaren 1979). This behavioral maturity suggests that the development of TBO_2 stores may occur more rapidly in harbor seals than in species with sedentary offspring. As previously reported, nursing harbor seal pups have increased cardiac control and extended terrestrial apneas compared to older pups (Greaves et al. 2004, 2005; Lapierre et al. 2004), suggesting physiological control at a young age. To investigate how physiological development impacts diving ability in young pups, we examined the development of blood and muscle oxygen stores from birth through weaning and compared pup values to adults.

For this study we not only followed the development of harbor seal oxygen storage on a fine temporal scale, but we also compared two sampling methods: cross-sectional (i.e., one sample per individual) and longitudinal (i.e., multiple samplings per individual). Typically longitudinal sampling schemes benefit from lower sample sizes and their ability to characterize individual patterns, but suffer from the difficulty of required recapture of known individuals. In contrast, the logistically simpler cross-sectional design can prove inaccurate if not all individuals follow the same developmental pathway, and can require larger sample sizes to detect small changes among treatments. To our knowledge, this is the first direct comparison of ontogenetic development, terrestrial or marine, as assessed using two sampling schemes. The comparison presented here directly addresses the question of whether average values determined from a cross-sectional sampling design accurately represented the changes that were occurring within individuals, as assessed through a longitudinal sampling design.

Materials and methods

Animal captures and aging

Harbor seal pups were captured during May–July of 2000, 2001, and 2002 near two haul-out sites, Bic Island (48°24′N, 68°51′W) and Métis (48°41′N, 68°01′W), along the St. Lawrence River estuary in Quebec,

Canada. Seals were captured using a 5-m inflatable Zodiac and modified dip net (Dubé et al. 2003). Basic morphometric measurements (sex, length, girth, mass ± 0.5 kg) were taken then animals were sedated with an IV injection of Diazepam (0.3–0.8 mg kg^{-1}, Sabex Inc, Canada). To facilitate future recaptures and identification, pups were outfitted with uniquely numbered flipper tags (Jumbo Rototag, Dalton, England) and head tags (Hall et al. 2000). Pups in the cross-sectional study were captured only once; for the longitudinal sampling, pups were recaptured at approximately 1-week intervals throughout the 4-week lactation period. Whenever possible mothers were captured with their pups, sedated, and sampled identically to their pups.

At initial capture, seals were aged by mass and appearance. Pups were classified as newborn if they had umbilical remnants and uncoordinated swimming ability. Pups were considered weaned if they were difficult to capture and were never seen again with an adult female. For pups that fell between these two categories, age was estimated from mass following Dubé et al. (2003). All pups were placed into one of four age categories: newborn (0–4 days), early nursing (5–16 days), late nursing (17–27 days), and weaned pups (≥28 days).

Blood collection and analysis

Blood samples were taken from the extradural intravertebral vein (Geraci and Smith 1975) using a 1.5 in. 20G or a 3.5 in. 18G spinal needle into 10 ml Vacutainer® tubes. Following initial sample collection, an IV injection of Evans blue dye (0.5 mg kg^{-1}; Sigma E-2129) was administered to determine plasma volume (El-Sayed et al. 1995). Five additional 7 ml blood samples were taken at five, 10, 15, 20, 25, and 30 min post-injection to characterize the Evans blue dilution curve. The total volume of blood collected from any individual was <3% total blood volume. Blood samples were stored on ice until initial laboratory processing and analysis (within 8 h). After analysis, samples were stored at -20°C before being transferred to an ultra-cold −80°C freezer.

Hematocrit was measured from K_3-EDTA Vacutainers® by direct centrifugation. Hemoglobin concentration was measured from the same tubes using the cyano-methemoglobin photometric method (Sigma 525-A Kit). RBC counts were determined by direct counting using a hemacytometer and Unopettes (Becton Dickinson Vacutainer Systems). Manual RBC counts were performed immediately using a compound microscope or the prepared hemacytometer was digitally photographed (Leitz Diaplan compound microscope and Leica Image analysis system) and RBC digital images were counted later. Mean corpuscular hemoglobin content (MCHC) was calculated as MCHC = (Hb × 100)/Hct, mean corpuscular volume (MCV) as MCV = (Hct × 10)/(RBC), and mean cell hemoglobin (MCH) as MCH = (Hb × 10)/(RBC).

Plasma was separated from the blood by centrifugation, and the concentration of Evans blue dye in fresh plasma samples was determined spectrophotometrically (Beckman DU Series 600) at 624 and 740 nm following El-Sayed et al. (1995) with modifications by Foldager and Blomqvist (1991). Spectrophotometer readings were compared to standard dilution curves of Evans blue using pooled plasma from pups and adults. To determine instantaneous dilution volume, the concentration of Evans blue at the time of injection was calculated by fitting a regression line to serial samples and determining the intercept (SYSTAT V9.0). In cases where there was less than 6% loss of dye over 30 min, or in cases where the fitted regression line had a positive slope, the instantaneous dilution volume was calculated as the average concentration of all serial samples. Linear regression data were screened, and outliers (Studentized Residual was > |3|) removed. Only intercepts calculated for equations with $P < 0.10$ and $R^2 > 0.60$ were used. Blood volume (BV) was calculated using measured plasma volume (PV) and Hct as BV = (PV)/(1 − Hct).

Muscle collection and analysis

While the animals were sedated, a 15–25 mg muscle biopsy was taken from the longissmus dorsi, the major swimming muscle, for myoglobin determination. Muscle samples were collected with a sterile 6G muscle biopsy cannula (Bergstrom-Stille, Stockholm, Sweden) or a three mm sterile disposable biopsy punch (Miltex Instrument Company Inc). Muscle samples were immediately transferred into a liquid nitrogen dewar and stored at −80°C until analysis. Biopsy sites were monitored upon recapture and were barely visible to the eye or touch after 1–2 weeks.

Myoglobin content (Mb, g 100 g^{-1} muscle tissue) was determined following Reynafarje (1963). Briefly, muscle samples were placed in a 4 M potassium phosphate buffer (pH 6.6) at a ratio of 19.25 ml to 1 g of tissue and sonicated. Homogenates were centrifuged (50 min, 0°C, 28,000*g*) and the supernatants used for myoglobin estimates. Following centrifugation, the supernatant was bubbled with pure carbon dioxide and sodium dithionite was added to ensure complete reduction of myoglobin. Absorbances were read at 538 and

568 nm (Beckman DU Series 530 with multicell module) and final myoglobin content estimated from equation four of Reynafarje (1963). Buffer blanks and elephant seal muscle tissue of known myoglobin concentration (Castellini and Somero 1981; Thornson and Le Boeuf 1994) were used as assay controls.

Total body oxygen stores

Total body oxygen stores were calculated as the sum of blood, muscle, and lung oxygen stores following Lenfant et al. (1970) and Kooyman et al. (1983). Briefly, blood oxygen stores were calculated as: arterial oxygen (ml O_2) = (0.33 × BV) × (0.95 saturation to 0.20 saturation) × (Hb × 1.34 ml O_2 g^{-1} Hb) and venous oxygen (ml O_2) = (0.66 × BV) × (arterial saturation – 5 vol%) × (Hb × 1.34 ml O_2 g^{-1} Hb) and muscle oxygen stores (ml O_2) as: body mass × % muscle mass × (Mb) × 1.34 ml O_2 g^{-1} Mb. The assumptions for the oxygen binding capacity of muscle and hemoglobin are from Lenfant (1969). Muscle mass was assumed to be 19.2 and 28.8% for pups and adults, respectively, based upon measurements of muscle mass of hooded seals (*Crystophora cristata*, J.M. Burns, unpublished data). Lung oxygen stores were estimated as 2.6 ml O_2 kg^{-1} for pups and 12.2 ml O_2 kg^{-1} for adults (based on J.M. Burns, unpublished for *Crystophora cristata*). This assumed a fractional lung oxygen content of $FO_2 = 0.15$, and a diving lung volume of 50% total lung capacity (Kooyman 1989).

The calculated aerobic dive limit (cADL) was determined by dividing the TBO_2 store values collected from this study by diving metabolic rates estimated from resting metabolic rates (RMR) determined from previous harbor seal pup studies (Miller and Irving 1975; Miller et al. 1976). We used two estimates of DMR: a minimum value of 1× RMR is based on studies of diving, post-absorptive Weddell seals (Castellini et al. 1992), and an elevated value of 2× RMR, based on the average rate for diving, foraging Weddell seals (Williams et al. 2004) (Kooyman 1989; Schreer and Kovacs 1997). The cADL was calculated for both the longitudinal and cross-sectional animals based on their mass and measured TBO_2 stores (average TBO_2/MR based on average mass).

Statistical analysis

To test for age-related changes in blood parameters in the cross-sectional study, one-way ANOVA's were used. Bonferroni post hoc comparisons were used to identify significant differences between age categories. Statistical significance was assumed at $P < 0.05$. To track developmental changes in individual pups throughout lactation (longitudinal study), all data were analyzed using linear mixed-effects model. This model was used since it predicts how individual response trajectories change over time taking into account that data is not required for the same number of observations (i.e., it can predict missing values for individuals not captured in each age category; Fitzmaurice et al. 2004). To determine if there were differences between cross-sectional and longitudinal study parameters, longitudinal means in each age class were compared to cross-sectional means using two sample independent *t*-tests (with adjusted *P* values to account for multiple comparisons). All statistical analyses were performed using SPSS® (v11.5.0, SPSS Inc, Chicago, IL, USA).

Results

Animal captures

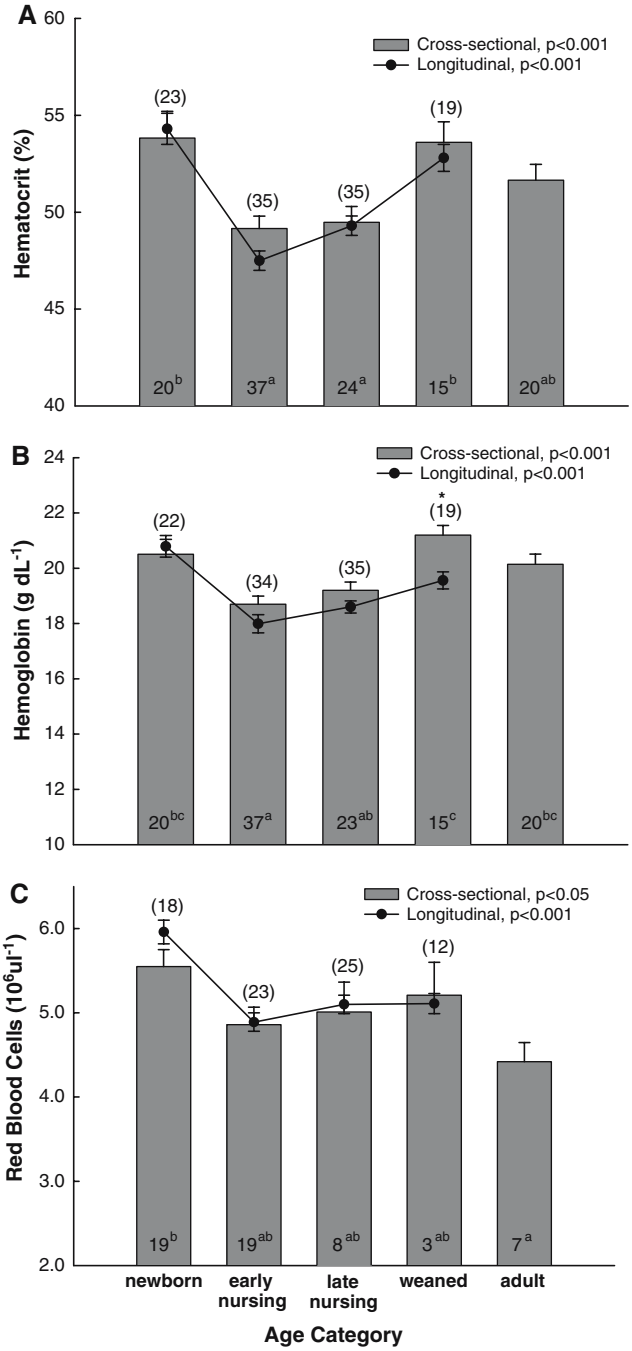
Over the 3 years of this study, 96 animals were captured as part of the cross-sectional study. For the comparative longitudinal study, an additional 50 pups were recaptured between two and four times in different age categories. No animals were included in both datasets. A total of 20 adult females were captured, and these same animals were used for comparative purposes in both the cross-sectional and longitudinal study. Growth rates determined for pups handled more than once (longitudinal study) were within the range of previous studies (0.50 ± 0.02 vs. 0.3–0.9 kg day^{-1}, Bowen et al. 2001; 0.39 ± 0.03 kg day^{-1}, Cottrell et al. 2002; 0.54 ± 0.14 kg day^{-1}, Dubé et al. 2003). The average mass and estimated age of pups in both the longitudinal and cross-sectional studies are shown in Table 1.

Blood parameters

In the cross-sectional study there were significant age-related changes in Hct, Hb, and RBC (one-way ANOVA: $F_{4,111} = 5.986$, $P < 0.001$; $F_{4,110} = 7.392$, $P < 0.001$; $F_{4,51} = 2.765$, $P < 0.05$, respectively). Bonferroni post hoc tests revealed that these parameters were elevated in newborn pups, declined in early nursing pups, and then increased until weaning, at which point pup values were similar to or higher than adult values (Fig. 1). These same effects were evident in the longitudinal study (linear mixed-effects model: Hct $F_{3,78.7} = 32.107$, $P < 0.001$; Hb $F_{3,82.7} = 20.286$, $P < 0.001$; RBC $F_{3,62.2} = 14.739$, $P < 0.001$, Fig. 1). When comparing the means between animals in the cross-sectional and longitudinal studies, there were no differences in Hct, Hb, or

Table 1 Estimated age (days) and body mass (mean ± SE) for harbor seal pups and adult females in the cross-sectional and longitudinal groups

Age category	Cross-sectional			Longitudinal		
	N	Estimated age (days)	Mass (kg)	*N*	Estimated age (days)	Mass (kg)
Newborn	20	1.3 ± 0.4	11.2 ± 0.3	23	1.5 ± 0.4	11.4 ± 0.5
Early nursing	37	10.5 ± 0.5	16.8 ± 0.3	35	10.8 ± 0.6	16.3 ± 0.4
Late nursing	24	20.8 ± 0.6	22.2 ± 0.4	35	21.0 ± 0.6	22.3 ± 0.4
Weaned	15	37.3 ± 1.3	26.9 ± 0.6	19	31.8 ± 0.9	25.6 ± 0.6
Adult female	20	–	65.8 ± 2.6			



RBC for any age class, with the exception that Hb in weaned pups was significantly higher in the cross-sectional study group (two sample independent *t*-test with Bonferroni correction, significant *P*-value = 0.003; Hb: $t_{32} = 3.488$, $P = 0.001$, all other *P*-values > 0.05). In neither the cross-sectional nor the longitudinal study was there a significant change with age in MCHC, MCH, or MCV (Table 2). In addition, there were no significant differences between the longitudinal and cross-sectional datasets in MCHC, MCH, and MCV (two sample independent *t*-tests, all *P*-values > 0.05).

As pups grew, absolute (ml/animal) plasma and blood volume increased significantly (cross-sectional study: PV $F_{3,57} = 12.338$, $P < 0.001$; BV $F_{3,57} = 15.071$, $P < 0.001$; longitudinal study: PV $F_{3,54.8} = 18.717$, $P < 0.001$; BV $F_{3,53.5} = 23.059$, $P < 0.001$, Table 3). However, on a mass-specific (ml kg^{-1}) basis, there was a non-linear trend with age. Adults had higher mass-specific plasma volumes than pups of all ages (Fig. 2). In pups, mass-specific blood volume decreased by 37% from birth until late nursing (14.9 ± 01.4 to 9.4 ± 0.5 ml O_2 kg^{-1}), and then increased by 23% in weaned pups (16.6 ± 2.6 ml O_2 kg^{-1}, Fig. 2). There were no significant differences in the average values for pups in each age class across studies (two sample independent *t*-tests, all *P*-values > 0.05).

As expected, based on changes in Hct, PV, and Hb, blood oxygen storage capacity had significant age-related changes (cross-sectional: $F_{4,65} = 8.632$, $P <$

Fig. 1 The effects of age (mean ± SE) on **a** hematocrit, **b** hemoglobin, and **c** red blood cell counts in the cross-sectional and longitudinal datasets of harbor seals throughout lactation. Adult values are shown for reference. The cross-sectional dataset is represented with *shaded bars* and sample size for each age category is denoted in the bar with *superscripts of different letters* indicating statistically significant differences between age categories in the cross-sectional group (Bonferroni, $P < 0.05$). The longitudinal dataset is represented by the line graph (*filled circle*) and sample size for each age category is denoted in parentheses above the line graph. *P*-values for each dataset are denoted in the figure legend. There were no statistical differences found between Hct, Hb, and RBC, except for Hb in weaned pups (as denoted by the *) between longitudinal and cross-sectional samples. See text for statistics

Table 2 Effects of age on mean ± SE of mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), and mean cell hemoglobin (MCH) for cross-sectional and longitudinal groups of harbor seal pups and adult females

Age category	Cross-sectional[a]			Longitudinal[a]		
	MCHC (g dl^{-1})	MCV (fl)	MCH (pg)	MCHC (g dl^{-1})	MCV (fl)	MCH (pg)
Newborn	38.2 ± 0.5 (20)	98.8 ± 3.5 (19)	37.4 ± 1.2 (19)	36.8 ± 1.0 (23)	92.5 ± 2.8 (18)	33.7 ± 1.4 (18)
Early nursing	38.1 ± 0.5 (37)	103.5 ± 5.1 (19)	39.5 ± 2.3 (19)	37.7 ± 0.8 (34)	97.0 ± 2.5 (23)	36.3 ± 1.3 (23)
Late nursing	38.7 ± 0.5 (23)	100.7 ± 8.2 (8)	39.2 ± 3.6 (8)	37.7 ± 0.8 (35)	99.5 ± 2.4 (25)	36.9 ± 1.2 (25)
Weaned	39.8 ± 1.2 (15)	106.3 ± 9.7 (3)	40.5 ± 3.8 (3)	36.9 ± 1.1 (19)	102.7 ± 3.4 (12)	37.5 ± 1.7 (12)
Adult female	39.0 ± 0.5 (20)	116.4 ± 7.9 (7)	44.2 ± 3.04 (7)			

Sample size is shown in parentheses

[a] There were no statistical differences between MCHC, MCV, and MCH within cross-sectional and longitudinal dataset or between longitudinal and cross-sectional samples. See text for statistics

Table 3 Age-effects on absolute plasma and blood volume (mean ± SE) for the cross-sectional and longitudinal groups of harbor seal pups and adult females

Age category	Cross-sectional[d]			Longitudinal[d]		
	N	Plasma volume (l)	Blood volume (l)	*N*	Plasma volume (l)	Blood volume (l)
Newborn	7	0.81 ± 0.10[a]	1.78 ± 0.18[a]	13	0.71 ± 0.09[a]	1.62 ± 0.19[a]
Early nursing	23	0.94 ± 0.06[a]	1.91 ± 0.13[a]	31	0.93 ± 0.07[a]	1.79 ± 0.13[a]
Late nursing	16	1.05 ± 0.05[a]	2.08 ± 0.10[a]	33	1.12 ± 0.06[b]	2.32 ± 0.13[b]
Weaned	15	1.53 ± 0.13[b]	3.27 ± 0.25[b]	15	1.49 ± 0.09[c]	3.17 ± 0.17[c]
Adult female	10	5.05 ± 0.32[c]	10.13 ± 0.52[c]			

[a,b,c] Different superscripts within a column indicate statistically significant differences within cross-sectional and longitudinal groups (Bonferroni, $P < 0.05$)

[d] There were no significant differences found between absolute PV and BV between longitudinal and cross-sectional samples. See text for statistics

0.001; longitudinal: $F_{3,53.3} = 13.078$, $P < 0.001$, Table 4). Cross-sectional mass-specific blood oxygen stores decreased from birth to late nursing by 41%, then increased significantly in weaned pups by 30% such that blood oxygen stores in weaned pups were not different from those of newborn pups. However, even at weaning, blood oxygen stores were only 81% of adult values. A similar pattern was evident in the longitudinal study with blood oxygen stores of a weaned pup at 78% of adult values (Table 4). There were no significant differences across age categories between cross-sectional means and longitudinal means in oxygen storage capacity of blood (two sample independent *t*-tests, all *P*-values > 0.05).

Muscle parameters

There were significant age-related differences in muscle myoglobin content in both the cross-sectional and longitudinal studies, with the weaned pups having (Mb) values significantly higher than other pups, but lower than adult females (cross-sectional: $F_{4,673} = 105.906$, $P < 0.001$, longitudinal: $F_{3,44.6} = 10.291$, $P < 0.001$). However, even at weaning, myoglobin concentration was 58% lower than adults (Fig. 3). As expected from the changes in myoglobin content, muscle oxygen stores increased from newborn to weaned pups and weaned pup values were approximately one third of adults (Table 4). There were no significant differences across age categories between cross-sectional means and longitudinal means in muscle oxygen stores (two sample independent *t*-tests, all *P*-values > 0.05).

Total body oxygen stores and calculated ADL

Total body oxygen stores were calculated by summing muscle and blood oxygen stores in the cross-sectional and longitudinal dataset (Table 4). There were significant differences due to age in both the cross-sectional and longitudinal data sets: from birth to late nursing, mass-specific TBO_2 stores decreased significantly; they then increased significantly in weaned pups, but remained lower than in adult females (cross sectional: $F_{4,58} = 37.093$, $P < 0.001$; longitudinal: $F_{3,35.5} = 9.979$; $P < 0.001$, Table 4). At weaning, pups from the cross-sectional dataset had 54–55% of the TBO_2 stores of adults. There were no significant differences in the average oxygen store values at any age between the two study groups (two sample independent *t*-tests, all *P*-values > 0.05). Investigation into the relative distri-

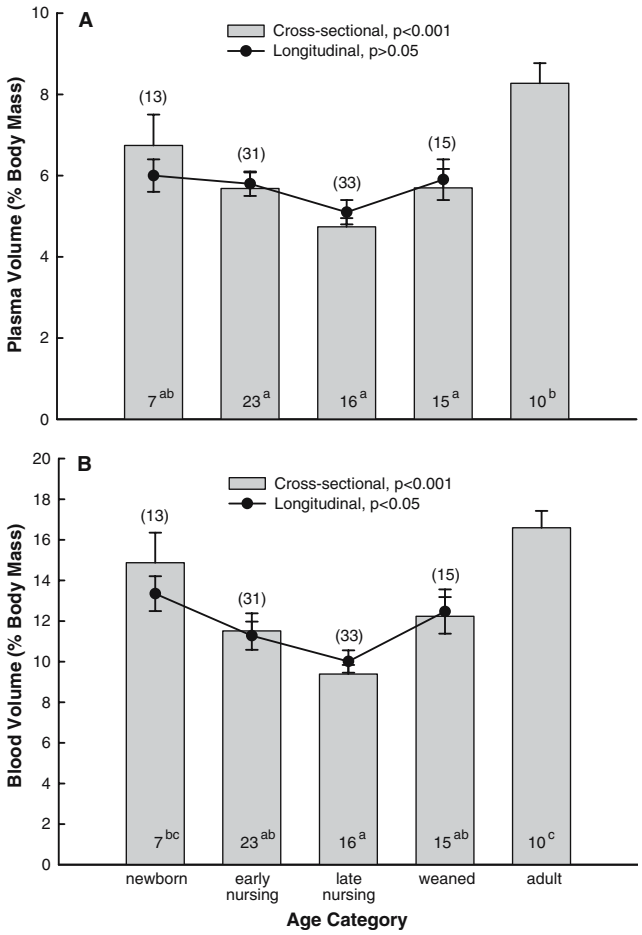


Fig. 2 The effects of age on patterns of change (mean ± SE) in **a** plasma volume and **b** blood volume, calculated from hematocrit expressed as a percentage of body mass for the cross-sectional and longitudinal harbor seal datasets. The cross-sectional dataset is represented with *shaded bars* and sample size for each age category is denoted in the bar with *superscripts of different letters* indicating statistically significant differences between cross-sectional age categories (Bonferroni, $P < 0.05$). The longitudinal dataset is represented by the line graph (*filled circle*) and sample size for each age category is denoted in parentheses above the line graph. *P*-values for each dataset are denoted in the figure legend. There were no statistical differences found between %PV and %BV between longitudinal and cross-sectional samples. See text for statistics

bution of oxygen among the different stores (blood, muscle) throughout the nursing period indicated that pups stored 73–84% of TBO_2 in the blood, and 10–17% in the muscle, while adults stored 53% of TBO_2 in the blood and 37% in the muscle (Table 4).

Not unexpectedly, there were significant differences in the cADL among age classes (cross-sectional: $F_{4,58} = 353.021$, $P < 0.001$; longitudinal: $F_{3,35.3} = 25.549$, $P < 0.001$), but not between the two study groups (Table 5). Because neonates had very large blood oxygen stores, neonatal cADL's were elevated in comparison to older pups. By the time pups were weaned, their cADL's were significantly higher (41%) than younger pups, but still only 30% of adult cADL's.

Discussion

This study documented and compared the development of tissue oxygen stores in harbor seal pups from birth to weaning in two different sampling regimes (longitudinal vs. cross-sectional). The fine temporal scale of analyzing physiological development was necessary to detect patterns of change throughout the lactation period, however, the cross-sectional and longitudinal sampling methods yielded similar results. Of all the parameters compared between the two studies, only Hb in weaned pups from the longitudinal was significantly lower than the cross-sectional weaned pups. While this might be due to anemia induced by multiple blood collections in a 4-week period, the absence of differences in any other hematological parameters, and the relatively small volume drawn at each handling argues against this interpretation. Therefore, our results strongly support the conclusion that appropriately designed cross-sectional studies are equally able to characterize developmental changes in physiological parameters as the more logistically intensive longitudinal studies.

For marine mammals, blood is an important site of oxygen storage (Kooyman 1985, 1989). The pattern of blood development shown in young harbor seals is not qualitatively different from that of other mammalian neonates, although the absolute values reflected adaptations for increased oxygen storage (Fowler 1986; Kooyman 1989). For example, harbor seal neonates had elevated Hct, Hb concentration, and RBC counts followed by an immediate decrease in early nursing pups before an increase in weaned pups. This pattern is characteristic of terrestrial mammalian blood development (Matoth et al. 1971; Schalm et al. 1975; Spensley et al. 1987; Potocnik and Wintour 1996), and has also been documented in variety of marine mammal species (Bryden and Lim 1969; Kooyman 1989; Thornson and Le Boeuf 1994; Horning and Trillmich 1997; Sepulveda 1999; Noren et al. 2002; Burns et al. 2005). Thus, it appears that the adaptations for diving that lead to increased oxygen stores have not altered the basic pathways by which blood develops (Hochachka and Mottishaw 1999).

An examination of RBC characteristics suggests how blood oxygen stores develop. Immediately after birth, Hct, Hb concentration, and RBC counts declined as plasma volume expanded. Because there were no age-related changes in RBC characteristics (MCH,

Table 4 Oxygen stores in the blood and muscle of harbor seal pups and adult females

Age category	Cross-sectional[d]						Longitudinal[d]					
	N	Blood (ml O_2 kg^{-1})	*N*	Muscle (ml O_2 kg^{-1})	*N*	Total body (ml O_2 kg^{-1})	*N*	Blood (ml O_2 kg^{-1})	*N*	Muscle (ml O_2 kg^{-1})	*N*	Total body (ml O_2 kg^{-1})
Newborn	7	32.8 ± 2.7^{bc} (84%)	10	4.0 ± 1.4^{a} (9%)	7	39.0 ± 3.0^{b}	13	29.7 ± 1.8^{b} (80%)	6	4.6 ± 0.5^{a} (13%)	6	38.0 ± 2.5^{b}
Early nursing	23	22.9 ± 1.9^{ab} (78%)	21	4.3 ± 1.4^{a} (16%)	20	29.1 ± 2.2^{ab}	30	20.8 ± 1.2^{a} (74%)	18	4.3 ± 0.3^{a} (16%)	18	26.7 ± 1.5^{a}
Late nursing	15	19.0 ± 1.0^{a} (73%)	15	4.3 ± 1.2^{a} (17%)	14	25.5 ± 1.0^{a}	33	20.1 ± 1.2^{a} (70%)	22	5.3 ± 0.3^{a} (20%)	22	26.7 ± 1.4^{a}
Weaned	15	27.0 ± 2.0^{bc} (75%)	14	6.0 ± 1.6^{a} (17%)	14	35.2 ± 2.4^{b}	15	26.0 ± 1.6^{b} (71%)	9	6.8 ± 0.4^{b} (20%)	9	34.3 ± 2.0^{b}
Adult female	10	33.2 ± 1.5^{c} (53%)	18	21.5 ± 4.2^{b} (37%)	8	64.1 ± 1.5^{c}						

Values in parentheses represent what percentage of blood and muscle comprise total body oxygen stores for the given age categories

[a,b,c] Different superscripts within a column indicate statistically significant differences within cross-sectional and longitudinal groups (Bonferroni, $P < 0.05$)

[d] There were no significant differences found between blood, muscle, total body oxygen stores, and percent blood and muscle oxygen stores between longitudinal and cross-sectional samples. See text for statistics

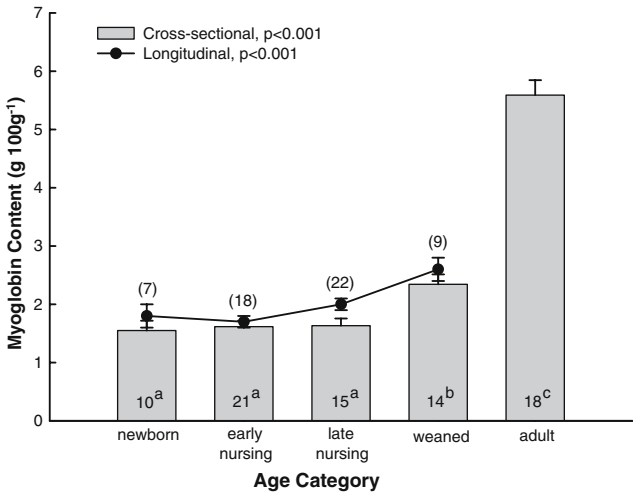


Fig. 3 Effects of age on the development of muscle myoglobin content (mean ± SE) in harbor seals for cross-sectional and longitudinal datasets. The cross-sectional dataset is represented with *shaded bars* and sample size for each age category is denoted in the bar with *superscripts of different letters* indicating statistically significant differences between age categories in the cross-sectional group (Bonferroni, $P < 0.05$). The longitudinal dataset is represented by the line graph (*filled circle*) and sample size for each age category is denoted in parentheses above the line graph. *P*-values for each dataset are denoted in the figure legend. There were no significant differences found in (Mb) between longitudinal and cross-sectional samples. See text for statistics

MCV, etc) the initial decline likely results from dilution of the RBC pool by increases in absolute plasma volume. However, subsequent increases in Hct, Hb and cell counts are associated with slight increases in cell size and hemoglobin content, suggesting the addition of new cells with elevated oxygen carrying capacity to the pool. An increase in RBC production is necessary and expected as plasma volume expands and likely results from the increase in circulating erythropoietin (the glycoprotein that stimulates the production of RBCs) concentration that occurs at this time (Clark et al. 2006). Similar increases in RBC production during early development have been documented in juvenile bottlenose dolphins, *Tursiops truncatus*, and Steller sea lions, *Eumetopias jubatus* (Noren et al. 2002 and Richmond et al. 2005, respectively).

In combination, these findings suggest that the development of blood oxygen stores follows a two-stage process in neonatal harbor seals. From birth through mid-lactation, mass-specific blood oxygen stores fall, largely due to a decline in mass-specific blood volume. During this period, the dilution effect predominates, as increased cell production fails to keep pace with the increases in plasma volume and body mass. The inability of blood to expand as quickly as mass is gained was previously noted in harbor seals (Jørgensen et al. 2001; Burns et al. 2005) and foals (Spensley et al. 1987). However, by late lactation, blood production is increasing and the produced cells have slightly elevated oxygen storage capacity. This allows both blood volume and storage capacity to increase through weaning as growth slows and independence is achieved. Because blood oxygen stores are such a large component of total oxygen stores, it is this final stage of blood development that leads to the increased oxygen storage seen in weaned pups, and that likely enables young, naïve divers to become independent foragers. Because these changes are apparent even when changing body condition is controlled for (Burns et al. 2005), increases in oxygen stores can be attributed to animal age.

Table 5 Mean ± SE calculated aerobic dive limit (cADL) as calculated using total body oxygen (TBO_2) stores for animals in each age category divided by resting metabolic rate (RMR) for the age category

Age category	Cross-sectional[d]				Longitudinal[d]		
	TBO_2 (ml O_2 kg^{-1})	RMR (ml O_2 kg^{-1} min)	cADL RMR (min)	cADL 2× RMR (min)	TBO_2 (ml O_2 kg^{-1})	cADL RMR (min)	cADL 2× RMR (min)
Newborn	39.0 ± 3.0^{b}	13.3[e]	2.9 ± 0.2^{a}	1.5 ± 0.1^{a}	38.0 ± 2.5^{b}	2.8 ± 0.2^{ab}	1.4 ± 0.1^{ab}
Early nursing	29.1 ± 2.2^{ab}	12.3[f]	2.4 ± 0.2^{a}	1.2 ± 0.1^{a}	26.7 ± 1.5^{a}	2.2 ± 0.1^{a}	1.1 ± 0.1^{a}
Late nursing	25.5 ± 1.0^{a}	10.2[h]	2.5 ± 0.1^{a}	1.3 ± 0.1^{a}	26.7 ± 1.4^{a}	2.6 ± 0.1^{ab}	1.3 ± 0.1^{ab}
Weaned	35.2 ± 2.4^{b}	8.3[e]	4.3 ± 0.3^{b}	2.1 ± 0.1^{b}	34.3 ± 2.0^{b}	4.1 ± 0.2^{c}	2.1 ± 0.1^{c}
Adult females	64.1 ± 1.5^{c}	4.6[g]	13.9 ± 0.3^{c}	7.0 ± 0.2^{c}			

[a,b,c] Different superscripts within a column indicate statistically significant differences within cross-sectional and longitudinal groups (Bonferroni, $P < 0.05$)

[d] There were no significant differences found between cADL's between longitudinal and cross-sectional samples. See text for statistics

[e] Miller and Irving (1975), [f] Miller et al. (1976), [g] Davis et al. (1985)

[h] Metabolic rate calculated based upon linear regression [MR = −1.7134 (age in weeks) + 15.3] of values for newborn, early nursing and weaned pups

In contrast to blood, which develops during the nursing period, muscle development proceeds at a much slower pace. Muscle oxygen stores are not well developed at birth and this persists through lactation. Although muscle myoglobin increases slightly at weaning, mass-specific muscle oxygen stores in weaned pups are much lower than blood oxygen stores relative to adult values (28 vs. 81%, respectively). Similar work on other diving vertebrates (Ponganis et al. 1999; Burns et al. 2000, 2005; Noren et al. 2001; McIntyre et al. 2002; Fowler 2005; Richmond et al. 2006) has shown that muscle myoglobin values in dependent neonates can be as much as 90% lower than adults. Although changes in myoglobin production in harbor seals could be associated with the classical exercise response (Schmidt-Nielson 1997), where increased activity demands more oxygen in the muscle, this is not a likely explanation for the results found here as harbor seal pups are active soon after birth. Similarly, dolphins swim at birth and also show a delayed development of muscle myoglobin (Noren et al. 2001). It is more likely that myoglobin production, like post-natal myogenesis, is regulated by a suite of regulatory factors (Weller et al. 1986; Garry et al. 1996; Patel et al. 2002). Still, it is important to note that while muscle development lagged behind that of blood in harbor seal pups, its contribution to TBO_2 stores is higher than terrestrial mammals (Snyder 1983; Kooyman 1989).

The limited data on diving behavior of pinniped pups indicates that they are behaviorally immature at birth (Thornson and Le Boeuf 1994; Corpe 1996; Horning and Trillmich 1997; Merrick and Loughlin 1997). During the nursing period, harbor seal pups from this study population generally remain within the shallow waters of their whelping areas (Bekkby and Bjørge 2000; Greaves et al. 2005), and have conservative dive depths (2.1 ± 0.1 m) that are constant with age (Greaves et al. 2005). As young swimmers, nursing harbor seal pups follow their mothers throughout lactation, spending from 30 to 70% (Bekkby and Bjørge 2000; Greaves 2002) of their time underwater, depending on habitat. Once weaned, however, pups increase dive performance by increasing depth and duration of dives (Bowen et al. 1999; Bekkby and Bjørge 2000; Frost et al. 2001; Lowry et al. 2001). This shift in behavior is accompanied by an increase in the cADL, likely a result of increased TBO_2 stores. While not modeled here, cardiac control also increases with pup age (Greaves et al. 2005), suggesting that mass-specific diving metabolic rate may decline as pups age, further increasing aerobic dive capacity. Overall, the cADL's in this study are similar to those estimated in previous studies for nursing pups (2.6–3.1 min) and weaned pups (4.6 min) (Bowen et al. 1999; Ashwell-Erickson and Elsner 1981; Burns et al. 2005). When compared to average dive durations of 0.57–1.5 min (Greaves et al. 2005; Bowen et al. 1999, respectively), these findings suggest that young harbor seal pups remain within their physiological limits throughout the nursing period, and dives that exceed the cADL are rare, even in weaned pups.

In summary, despite their precocial behavior, harbor seal pups are not physiologically mature at birth or even at the time of weaning. Throughout the nursing period, it was clear that blood and muscle matured at different rates. Blood development followed a non-linear pattern with elevated Hct, Hb concentration, RBC counts and blood oxygen stores in newborn pups declining to low values in early nursing pups and subsequently increasing through weaning. In contrast, muscle development lagged that of blood, with increases in myoglobin concentration evident only in weaned pups. These fine scale details have not been previously

reported in harbor seal developmental studies, and could be detected in both the longitudinal and cross-sectional datasets.

Acknowledgments Many thanks to the 2000–2002 field crews for their hard work and enthusiasm, most-notably: P. Carter, D. Dion, Y. Dubé, J. Gosselin, D. Greaves, J. Greig, J. Lapierre, S. Turgeon, and G. Yunker. Thank you to L. Measures for providing essential laboratory space and equipment while in the field. C. Beck, C. Neal, and I. van Tets provided valuable advice on statistical analysis. Thank you to L. Clark for editing earlier drafts of this manuscript and to two anonymous reviewers for further improvements to this paper. Funding for this work was provided by the Department of Fisheries and Oceans, Canada, the Natural Sciences and Engineering Research Council of Canada through an Operating Grant to JFS, the University of Waterloo, the Environment and Natural Resource Institute-Alaska, the University of Alaska Anchorage, and EPSCoR IAEP Fellowship provided by grant #NSF EPS-0092040 for 2002–2004. Research was authorized by the Animal Care Committees of: University of Alaska Anchorage, the University of Waterloo, and MMPA Permit # 1003-1646-00.

References

Ashwell-Erickson S, Elsner RW (1981) The energy cost of free existence for Bering Sea harbor and spotted seals. In: Hood DW, Calder JA (eds) The eastern Bering Sea shelf: oceanography and resources. University of Washington Press, Seattle, pp 869–899

Bekkby T, Bjørge A (2000) Diving behaviour of harbour seal *Phoca vitulina* pups from nursing to independent feeding. J Sea Res 44:267–275

Bigg MA (1969) The harbour seal in British Columbia. Bull Fish Res Can 172:1–33

Boulva J, McLaren IA (1979) Biology of the harbor seal (*Phoca vitulina*) in eastern Canada. Bull Fish Res Can 200:1–24

Bowen WD, Boness DJ, Iverson SJ (1999) Diving behaviour of lactating harbour seals and their pups during maternal foraging trips. Can J Zool 77:978–988

Bowen WD, Ellis SL, Iverson SJ, Boness DJ (2001) Maternal effects on offspring growth rate and weaning mass in harbour seals. Can J Zool 79:1088–1101

Bryden MM, Lim GHK (1969) Blood parameters of the southern elephant seal (*Mirounga leonina*) in relation to diving. Comp Biochem Physiol 28:139–148

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

Burns JM, Blix AS, Folkow LP (2000) Physiological constraint and diving ability: a test in hooded seals, *Cystophora cristata*. FASEB J 14(4):A440

Burns JM, Clark CA, Richmond JR (2004) The impact of lactation strategy on independent foraging. Int Congr Ser 1275:341–350

Burns JM, Costa DP, Frost K Harvey JT (2005) Development of body oxygen stores in harbor seals: effects of age, mass, and body composition. Physiol Biochem Zool 78(6):1057–1068

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

Castellini MA (1991) The biology of diving mammals: behavioral, physiological and biochemical limits. In: Gilles R (ed) Advances in comparative and environmental physiology. Springer, Berlin Heidelberg New York, pp 105–134

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

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

Clark CA, Burns JM, Schreer JF, Hammill MO (2006) Erythropoietin concentration in developing harbor seals (*Phoca vitulina*). Gen Comp Endocrinol 147:262–267

Corpe HM (1996) The behavioural ecology of young harbour seals in the Moray Firth, NE Scotland. Thesis/Dissertation. Aberdeen University

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

Costa DP, Kuhn CE, Weise MJ, Shaffer SA, Arnould JPY (2004) When does physiology limit the foraging behaviour of freely diving mammals? Inter Congr Ser 1275:359–366

Cottrell PE, Jeffries S, Beck B, Ross PS (2002) Growth and development in free-ranging harbor seal (*Phoca vitulina*) pups from southern British Columbia, Canada. Mar Mamm Sci 18:721–733

Davis RW, Williams TM, Kooyman GL (1985) Swimming metabolism of yearling and adult harbor seals (*Phoca vitulina*). Physiol Zool 58(5):590–596

Dubé Y, Hammill MO, Barrette C (2003) Pup development and timing of pupping in harbour seals (*Phoca vitulina*) in the St. Lawrence River estuary, Canada. Can J Zool 81:188–194

El-Sayed H, Goodall SR, Hainsworth FR (1995) Re-evaluation of Evans blue dye dilution method of plasma volume measurement. Clin Lab Haem 17:189–194

Fitzmaurice G, Laird N, Ware J (2004) Applied longitudinal analysis. Hoboken, NJ, pp 501

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

Fowler ME (1986) Zoo and wild animal medicine, 2nd edn. WB Saunders Company, Philadelphia, pp 1127

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

Frost KJ, Simpkins MA, Lowry LF (2001) Diving behavior of subadult and adult harbor seals in Prince William Sound, Alaska. Mar Mamm Sci 17:813–834

Garry DJ, Bassel-Dubay R, Richarson JG, Neufer PD, Williams RS (1996) Postnatal development and plasticity of specialized fiber characteristics in the hindlimb. Dev Gen 19:146–156

Geraci JR, Smith TG (1975) Functional hematology of ringed seals (*Phoca hispida*) in the Canadian arctic. J Fish Res Can 32:2559–2564

Greaves DK (2002) Ontogeny of diving heart rate and behaviour of harbor seal pups, *Phoca vitulina*. MSc Thesis. University of Waterloo, pp 1–86

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

Greaves DK, Schreer JF, Hammill MO, Burns JM (2005) Diving heart rate development in postnatal harbour seals, *Phoca vitulina*. Physiol Biochem Zool 78(1):9–17

Hall A, Moss S, McConnell B (2000) A new tag for identifying seals. Mar Mamm Sci 16:254–257

Hindell MA, Lea MA, Morrice MG, MacMahon CR (2000) Metabolic limits on dive duration and swimming speed in the southern elephant seal *Mirounga leonine*. Phys Biochem Zool 73:790–798

Hochachka PW, Mottishaw PD (1999) Evolution and adaptation of the diving response: phocids and otariids. In: Portner HO, Playle RC (eds) Cold ocean physiology. Cambridge University Press, Oxford, pp 391–431

Horning M, Trillmich F (1997) Ontogeny of diving behavior in the Galapagos fur seal. Behaviour 134:1211–1257

Jørgensen C, Lydersen C, Kovacs KM (2001) Diving development in nursing harbour seal pups. J Exp Biol 204:3993–4004

Knudtson PM (1977) Observations on the breeding behavior of the harbor seal, in Humboldt Bay, California. Calif Fish Game 63:66–70

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

Kooyman GL (1989) Diverse divers. Springer, Berlin Heidelberg New York

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

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

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

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. Respir Physiol 9:277–286

Lowry LF, Ver Hoef J, DeLong RL (2001) Movements of satellite-tagged subadult and adult harbor seals in Prince William Sound, Alaska. Mar Mamm Sci 17:835–861

Lydersen C, Ryg MS, Hammill MO, O'Brien J (1992) Oxygen stores and aerobic dive limit of ringed seals (*Phoca hispida*). Can J Zool 70:458–461

Matoth Y, Zaizov R, Varsano I (1971) Postnatal changes in some red cell parameters. Acta Paediatr Scand 60:317–323

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

McIntyre IW, Campbell KL, MacArthur RA (2002) Body oxygen stores, aerobic dive limits and diving behaviour of the star-nosed mole (*Condylura cristata*) and comparisons with non-aquatic talpids. J Exp Biol 205:45–54

Miller K, Irving L (1975) Metabolism and temperature regulation in young harbor seals *Phoca vitulina richardi*. Am J Physiol 229:506–511

Miller K, Rosenmann M, Morrison P (1976) Oxygen uptake and temperature regulation of young harbor seals (*Phoca vitulina richardi*) in water. Comp Biochem Physiol 54A:105–107

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

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

Noren SR, Iverson SJ, Boness DJ (2005) Development of blood and muscle oxygen stores in gray seals (*Halichoerus grypus*): implications for juvenile diving capacity and the necessity of terrestrial postweaning fast. Physiol Biochem Zool 78(4):482–490

Patel K, Christ B, Stockdale FE (2002) Control of muscle size during embryonic, fetal, and adult life. In: Brand-Saberi B (ed) Vertebrate myogenesis. Springer, Berlin Heidelberg New York, pp 163–186

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

Potocnik SJ, Wintour EM (1996) Development of the spleen as a red blood cell reservoir in lambs. Reprod Fertil Dev 8:311–315

Reynafarje B (1963) Simplified method for the determination of myoglobin. J Lab Clin Med 61:138–145

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 Endocrinol 141:240–247

Richmond JP, Burns JM, Rea LD (2006) Ontogeny of total body oxygen stores and aerobic dive potential in Steller sea lions (*Eumetopias jubatus*). J Comp Physiol B 176:535–545

Schalm OW, Jain NC, Carroll EJ (1975) Veterinary hematology, 3rd edn. Lea and Fibiger, Philadelphia, pp 807

Schmidt-Nielson K (1997) Animal physiology: adaptation and environment, 5th edn. Cambridge University Press, Cambridge, pp 91–125

Schreer JF, Kovacs KM (1997) Allometry of diving capacity in air-breathing vertebrates. Can J Zool 75:339–358

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

Snyder GK (1983) Respiratory adaptations in diving mammals. Respir Physiol 54:269–294

Spensley MS, Carlson GP, Harrold D (1987) Plasma, red blood cell, total blood, and extracellular fluid volumes in healthy horse foals during growth. Am J Vet Res 48(12):1703–1707

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

Weller PA, Price M, Isenberg H, Edwards YH, Jeffreys AJ (1986) Myoglobin expression: early induction and subsequent modulation of myoglobin and myoglobin mRNA during myogenesis. Mol Cell Biochem 6:4539–4547

Williams TM, Fuiman LA, Horning M, Davis RW (2004) The cost of foraging by a marine predator, the Weddell seal *Leptonychotes weddellii*: pricing by the stroke. J Exp Biol 207:973–982