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Size and distribution of oxygen stores in harp and hooded seals from birth to maturity

J. M. Burns · K. C. Lestyk · L. P. Folkow · M. O. Hammill · A. S. Blix

Abstract Pinnipeds rely primarily on oxygen stores in blood and muscles to support aerobic diving; therefore rapid development of body oxygen stores (TBO_2) is crucial for pups to transition from nursing to independent foraging. Here, we investigate TBO_2 development in 45 harp (*Pagophilus groenlandicus*) and 46 hooded (*Cystophora cristata*) seals ranging in age from neonates to adult females. We found that hooded seal adults have the largest TBO_2 stores yet reported (89.5 ml kg^{-1}), while harp seal adults have values more similar to other phocids (71.6 ml kg^{-1}). In adults, large TBO_2 stores resulted from large blood volume (harp169, hood 194 ml kg^{-1}) and high muscle Mb content (harp 86.0, hood 94.8 mg g^{-1}). In contrast, pups of both species had significantly lower mass-specific TBO_2 stores than adults, and stores declined rather than increased during the nursing period. This decline was due to a reduction in mass-specific blood volume and the absence of an increase in the low Mb levels (harp 21.0, hood 31.5 mg g^{-1}). Comparisons with other phocid species suggests that the pattern of blood and muscle development in the pre- and post-natal periods varies with terrestrial period, and that muscle maturation rates may influence the length of the postweaning fast. However, final maturation of TBO_2 stores does not take place until after foraging begins.

Abbreviations

BV	Blood volume, l or ml kg^{-1}
cADL	Calculated aerobic dive limit
DMR	Diving metabolic rate
Hb	Hemoglobin, g dl^{-1}
Hct	Hematocrit, %
M_b	Total body mass, kg
M_{LBM}	Lean body mass, kg
Mb	Muscle myoglobin, mg g wet $muscle^{-1}$
MCHC	Mean corpuscular hemoglobin content
PV	Plasma volume, l or ml kg^{-1}
RCV	Circulating red cell volume, l
RMR	Resting metabolic rate
TBO_2	Total available body oxygen stores, ml O_2 kg^{-1}
TLV	Total lung volume, l

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Introduction

Dive duration among marine mammals is largely dependent on the amount of oxygen (O_2) that can be carried to depth, and the rate at which it is used. Large O_2 stores in the blood and muscle result from large blood volume, elevated hematocrit (Hct) and hemoglobin (Hb), large muscle mass, and high muscle myoglobin (Mb) concen-

trations (Scholander 1940; Kooyman 1989; Butler and Jones 1997). Diving metabolic rates (DMR) are minimized through selective down-regulation of metabolic rate (Ramirez et al. 2007), reduced body temperature (Kvadsheim et al. 2005), and cost efficient swimming patterns (Williams et al. 2004). Individual differences in the size of the O_2 reserves, and the rates at which O_2 is used result from evolutionary, developmental, and behavioral constraints: species that dive the longest have the largest O_2 stores, the lowest use rates, and tissue metabolic profiles that reflect an energy sparing strategy (Butler and Jones 1997; Mottishaw et al. 1999; Davis et al. 2004). For adult pinnipeds, maximal O_2 stores and minimal use rates place ultimate limits on diving activities, but have little impact on routine behaviors, as indicated by large differences between maximum and mean dive durations, and the relatively rare occurrence of anaerobic dives (Butler and Jones 1997).

In contrast, pinniped neonates are essentially terrestrial at birth, and do not possess the physiological adaptations necessary to sustain underwater diving activity. Newly independent pups are small, have elevated mass-specific metabolic rates (Donohue et al. 2000; Burns et al. 2005), reduced thermoregulatory abilities (Blix and Steen 1979), and poor diving heart rate control (Greaves et al. 2005). All these contribute to elevated DMR, at a time when mass-specific O_2 stores are substantially lower than those of adults (Burns and Castellini 1996; Burns et al. 2005; Noren et al. 2005; Richmond et al. 2006). Faced with these limitations, pups have three main options: to refrain from foraging before physiologically mature, to quickly develop the physiological traits necessary to support diving activity if rapid entry into the water is necessary, and/or perform dives that are significantly shorter and shallower than those of adults. As the age at which weaned pups begin foraging may depend on maturation of O_2 stores and use rates; studying how O_2 stores develop in species with different lactation and foraging strategies may provide insights into the interplay between physiology, foraging behavior, and life history.

To address these issues, we have studied the development of body oxygen stores in harp (*Pagophilus groenlandicus*) and hooded (*Cystophora cristata*) seals, two pelagic pack-ice-breeding species that differ in their life history and foraging strategies. Hooded seals have the shortest lactation period of any mammal (3.6 days) during which time pups gain a remarkable 7 kg day^{-1} (Bowen et al. 1987). Neonates are large (12.3% maternal mass, Lydersen et al. 1997), coordinated, and born with adult pelage and substantial subcutaneous blubber reserves, which serve as insulation and as an energy reserve (Blix and Steen 1979; Oftedal et al. 1991). Despite this, pups do not begin diving and foraging immediately postweaning,

but instead fast on the ice for approximately one month, during which time they lose approximately a third of the mass gained during the nursing period (Bowen et al. 1987).

In contrast, harp seal pups are smaller at birth (8% maternal mass, Schulz and Bowen 2004), born without a subcutaneous blubber layer or adult pelage, and instead rely on shivering and non-shivering thermogenesis to maintain core temperature until the blubber layer develops (Blix and Steen 1979). Because their neonatal lanugo coat is a very poor insulator when wet, harp seal pups typically refrain from diving until after they have molted into their adult pelage. The more altricial status of harp seals is also reflected in the longer period of maternal care (12 days) and a postweaning fast of 5–6 weeks (Sivertsen 1941). Thus, harp seal pups have a terrestrial period that is approximately 30% longer than hooded seals.

In addition to these life history differences, adult harp and hooded seals also differ in their diving behavior. Adult hooded seals perform long duration dives (max. > 52 min), which allow them to access deep prey resources (Folkow and Blix 1995, 1999), while harp seals are more active swimmers that generally make shorter dives (~5 min) in the upper water column (Folkow et al. 2004). These behavioral differences suggest that hooded seal adults should have larger total available body oxygen (TBO_2) stores than harp seal adults. While there is no published data on the diving behavior of young harp and hooded seal pups, the shorter terrestrial period of hooded seal pups suggest that their tissue oxygen stores should be more mature than harp seal pups at birth, and/or that changes should occur more quickly during the brief period before diving begins.

Thus, this project had three main goals: (1) to determine the size of the O_2 reserves in the blood, muscle, and lungs of adult hooded and harp seals, and to correlate this with diving behavior; (2) to characterize the developmental changes in TBO_2 stores during the nursing and fasting periods in harp and hooded seal pups; and (3) to determine whether, and to what extent, the temporal pattern of TBO_2 store development is correlated with life history strategies among the phocid lineage. To accomplish this last goal, we incorporate data from similar studies on other phocid species, performed by our lab and others.

Methods

Animal handling

Eight harp seals and 25 hooded seals were captured between 23 March and 5 April 1999 in the West Ice off the Greenland Coast (~72°N, 15°W). Thirty-two harp and 21 hooded seals were captured between 3 March and 16

March 2005 in the Gulf of St Lawrence, Canada (~47°23′N, 61°52′W), and five harp seals were captured between 10 December 2005 and 8 February 2006. Captured seals were physically restrained, weighed (±0.5 kg), and chemically restrained either by i.v. injection of 0.2–0.3 mg kg^{-1} diazepam, or i.m. injection of 0.7–1.0 mg kg^{-1} tiletamine–zolazepam (Zoletil forte vet^{TM}). Following blood sampling (described below), seals were killed using methods approved for scientific collection by Canada and Norway. Animals were visually categorized as adults, yearlings, or pups. Hooded seal pups were categorized as neonatal (1 day), nursing (2–4 days), or weaned following Bowen et al. (1987). Yearling hooded seals were of similar size to weanlings, but had fully erupted dentition. Harp seal pups were aged based on appearance following Stewart and Lavigne (1980), and categorized as neonates (yellow coats and thin whites), nursing (fat whites), or weaned (ragged jackets and beaters).

For hooded seals handled in Norway, body composition was determined in two ways. Blubber mass, determined by direct dissection and weighing of the blubber without the skin, was converted to lipid mass by assuming that blubber was 90% lipid (Crocker et al. 2001), and lipid mass was estimated directly by i.v. injection of 0.5 g kg^{-1} D$_2$O (Cambridge Isotopes, 99.9%). Deuterium content in two post-equilibration blood samples (+2.0 and 2.5 h) was determined by IR spectrophotometry, and total body water (TBW) was determined from hydrogen-dilution space (Bowen and Iverson 1998). Lipid and lean body mass were estimated from TBW following Reilly and Fedak (1990). Results from the two techniques were compared using paired t-tests. Harp seal body condition was determined from blubber mass measurements.

Hematology and blood volume

Immediately following capture and restraint, blood was collected from the extradural vein into green (lyophilized lithium heparin), purple (liquid EDTA), or red (no additive) top vacutainers^{TM}. Within 6 h of sample collection, Hct (% red blood cells by volume) was determined by centrifugation, and Hb (g dL whole blood^{-1}) determined by the cyanomethemoglobin method (Sigma Kit 625A). Mean corpuscular hemoglobin content (MCHC; g dl RBC^{-1}) was calculated as 100 Hb Hct^{-1}.

Plasma volume (PV$_{EB}$) was determined in 18 harp and 34 hooded seals using the Evan's Blue methodology (ICSH 1973), in which a pre-weighed dose (~0.5 mg kg^{-1}) of dye was injected into the extradural vein, and up to six serial samples collected at 10-min intervals. Plasma optical density was determined at 624 and 740 nm in fresh and/or previously frozen (–80°C) samples, and PV$_{EB}$ calculated following Foldager and Blomqvist (1991) and El-Sayed

et al. (1995). Standard curves were run in parallel with all samples. Blood volume (BV$_{EB}$) was determined as PV$_{EB}$ (100 – HCT$_{max}$)/100. Because blood samples were collected immediately after animal restraint, and handling stress results in strong splenocontraction due to the alpha adrenergic stimulation by epinephrine (Cabanac et al. 1997), we are confident that we measured the maximum HCT for each animal. Furthermore, we visually confirmed that the spleen was contracted upon dissection of each animal. Both blood and plasma volumes are reported on an absolute (l) and mass-specific (ml kg^{-1}) basis. Paired t-tests were used to determine if there was a difference in the PV$_{EB}$ estimated from samples that were analyzed when fresh, and after freezing.

Circulating red cell volume (RCV) was determined for 14 hooded seals in 1999 using the ^{51}Cr technique (ICSH 1973). Because initial analysis revealed that the Evan's Blue method gave similar results (see below), this logistically intensive technique was not repeated in 2000 or 2005. For this analysis, ~30 ml of whole blood was added to a sterile Anticoagulant-Citrate–Dextrose solution (ACD, pH 6.0) at a concentration of 150 μl ACD·ml^{-1}. Labeled sodium chromate (Na$_2^{51}$CrO$_4$, Trace Sciences International, Ontario, Canada) was added to isolated red cells, the solution returned to normal Hct with saline, and incubated at 30°C for 30 min, after which rinsed, labeled, red cells were injected back into the animal at a dose of 1.2 μCi/kg. The final rinsate and a sample of the injectate were retained for analysis. Six post-injection blood samples were collected at 10-min intervals and frozen as whole blood. To determine the ^{51}Cr activity in the diluted injectate and post-injection samples, known masses and volumes of whole blood were counted on a Packard Cobra II Auto-Gamma Counter (240–400 keV; peak 320 keV, efficiency 2.7%) for 30 min. Counts were corrected for background, and expressed as counts·min^{-1} ml^{-1}, and activity at time zero calculated following Foldager and Blomqvist (1991) and Moritz et al. (1994). Blood volume (BV$_{51Cr}$) was determined as:

$$BV_{51Cr} = V_i \cdot C_s \cdot V_s \cdot C_{t0}^{-1}$$

where V_i = the injection volume, C_s = the corrected injectate activity, V_s = the standard's dilution factor (250), and C_{t0} = the extrapolated activity at time zero. Circulating RCV was calculated from BV$_{51Cr}$ by multiplying by the highest Hct measured, and blood volume additionally estimated as BV$_{RCV+PV}$ = RCV + PV$_{EB}$. Given the effect of handling stress and the observed contraction of the spleen, we believe labeled cells were diluted throughout the entire red cell pool. General linear ANOVA models were used to compare results among animals in which blood volume was determined in all three ways.

Muscle myoglobin and mass

Muscle samples (~5 g) were collected from the *M. longissimus dorsi*, and stored immediately in liquid nitrogen (Canada) or a –20°C freezer (Norway) until shipped to the US, where they were frozen at –80°C until analysis. Myoglobin content was determined within a year of collection following Reynafarje (1963) as modified by Castellini and Somero (1981), and samples reanalyzed if the c.v. for triplicates was more than 10%. The appropriate dilution factor for the assay was determined experimentally for pups and adults separately. Buffer blanks, lyophilized Mb, and muscle tissue from harbor seals (*Phoca vitulina*) and northern elephant seals (*Mirounga angustirostris*) with known Mb contents were used as assay controls. Total muscle mass was determined by complete dissection and weighing for all animals handled in Norway. Muscle mass for Canadian seals was estimated using allometric equation relating muscle mass to total body mass derived from the Norwegian seals.

Lung volume

The volume of air expelled through a Singer DTM-325 volumeter by passive recoil of excised lungs following positive pressure inflation to 30 cm H_2O (Leith 1976) was measured in triplicate for six adult females, two nursing and six weaned hooded seals in Norway. The relationship between lung volume and body mass was then fit with a power function, and the resultant allometric equation used to predict total lung volume for seals for which volume was not determined.

Total available body oxygen stores

For hooded and harp seals, TBO_2 were determined by adding the stores in lung, muscle and blood (Lenfant et al. 1970; Kooyman et al. 1983). Blood O_2 stores were determined using the individually measured Hct, Hb, and BV_{EB} following Kooyman (1989). Blood volume was taken to be one-third arterial and two-thirds venous, with arterial blood starting at 95% saturated and extracted to 20%. Venous blood oxygen content started at 5 vol% less than the starting arterial content, and was extracted to zero. Muscle O_2 stores were estimated by multiplying the measured Mb content by the O_2 binding capacity of Mb (1.34 ml O_2 g^{-1}), and the total muscle mass. Lung O_2 stores were calculated by assuming that the partial pressure of O_2 in the lung was 15%, and that diving lung volume as 50% of total lung capacity (Kooyman 1989). Mass-specific TBO_2 stores were calculated for all animals for which both muscle Mb and BV were measured. These values were then compared to those calculated from age-specific average BV, Hct, Hb, and Mb.

Statistical analyses

Before all analyses, data normality was assessed using probability plots, and data log-transformed as necessary to achieve normality. Differences in oxygen stores due to age, collection year, sample location, and animal sex were examined using a general linear ANOVA models (GLM in SPSS v 12.0) to test for all effects simultaneously. If data could not be normalized, Mann–Whitney U tests were used to compare between groups. Significance was assumed at $P < 0.05$, and Bonferroni post-hoc comparisons were used to identify age groups that differed significantly. To determine how lactation strategy influenced the rate of physiological development, we compared data on O_2 store development in seven phocid seals: harp, hooded, harbor, grey (*Halichoerus grypus*), Baikal (*Phoca sibirica*), Weddell (*Leptonychotes weddellii*), and northern elephant seals, using our own and previously published data. Regression analysis was used to examine the effect of the length of the terrestrial period (the duration of the lactation period and postweaning fast) on the relative maturity of O_2 stores (in blood, muscle, and total) at birth and at weaning for all above species. Data are presented as mean ± one SE unless otherwise noted.

Results

A total of 45 harp seals and 46 hooded seals, ranging in age from neonates (<2 days) to adult females were examined (Table 1). Due to ship and helicopter schedules, not all age classes were handled in both Norway and Canada, but we had a minimum of five animals in each age category when regions were combined. There was a notable difference in the size of adult females captured in the two regions: Canadian hooded seals were significantly heavier (274 ± 27 vs. 153 ± 6 kg, $t_{16} = 4.32$ $P < 0.001$) and longer than those from Norway (206 ± 5 vs. 177 ± 4 cm, $t_{12} = 4.24$ $P = 0.001$). In contrast, there were no significant differences in the mass or length of adult harp seals from the two regions, although those from Canada were slightly heavier (115 ± 5 vs. 99 ± 3 kg, $t_{13} = 2.03$ $P = 0.063$) but shorter (157 ± 2 vs. 162 ± 2 cm, $t_{13} = 1.29$ $P = 0.22$). When comparisons were possible, there were no differences in pup mass due to sex or region, although the power of this analysis was low due to limited sample size.

Body composition was determined by deuterium dilution and by dissection of the blubber for 14 hooded seals in 1999. The proportion of total body lipid estimated from dilution varied from 21.1 to 43.2% of total mass. Estimates obtained from dissection were strongly correlated with dilution estimates ($R^2 = 0.96$), but significantly underestimated total body lipid content by an average of 6.1 ± 1.0% (range –0.3 to –14.2%, paired $t_{13} = 6.08$, $P < 0.001$).

Table 1 Age, mass, and body condition of hooded and harp seals handled in Canada in 2005, and in Norway in 1999–2000. Body composition was estimated by weighing the blubber following dissection, and correcting for water content in the blubber, as described in the methods (% lipid, blubber) and, for a subset of hooded seals, from deuterium dilution (% lipid, D_2O)

Age Class	Canada		Norway			
	N (M, F)	Total mass (kg)	N (M, F)	Total mass (kg)	Lipid, blubber (%)	%Lipid, D_2O, (N)
Hooded seals						
Neonates (<2 days)	4, 1	21.9 ± 1.3	–	–	–	–
Nursing (2–4 days)	1, 2	38.5 ± 12.5	2,0	30.5 ± 0.5	25.6 ± 3.1	26.8 ± 2.3, (2)
Weaned/fasting (5–14 days)	2, 3	48.1 ± 2.0	5,6	36.5 ± 2.3	36.7 ± 1.8	40.5 ± 1.2, (6)
Yearling (12 months)	–		1,2	50 ± 1.7	24.4 ± 5.5	–
Adults	0, 8	252.1 ± 17.9	0,9	152.7 ± 6.2	20.7 ± 1.2	26.6 ± 2.9, (6)
Harp seals						
Neonates (<2 days)	4, 1	10.3 ± 1.1	–	–	–	–
Nursing (3–12 days)	6, 2	29.4 ± 1.1	3, 1	29.1 ± 0.6	41.9 ± 1.4	–
Weaned/fasting (14–28 days)	3, 2	36.6 ± 1.1	–	–	–	–
Yearling (10–11 mo.)	3, 2	29.3 ± 1.6	–	–	–	–
Adults	0, 14	115.4 ± 4.7	0, 4	98.8 ± 3.5	27.8 ± 1.1	–

Blood parameters

There was no difference in Hct, Hb, or MCHC due to either location or animal sex in either harp or hooded seals, so sexes and locations were pooled. For harp seals there was a significant effect of animal age on Hct ($F_{3,28} = 5.848$, $P = 0.003$) and Hb ($F_{3,28} = 5.685$, $P = 0.003$), but not MCHC. Nursing pups had Hct and Hb values that were significantly lower than either adults or weaned pups ($P < 0.05$). In contrast, there were no significant differences in Hct, Hb, or MCHC due to age in hooded seals, indicating that blood development had occurred in utero (Table 2).

There was no statistical difference between the plasma volume, as estimated using fresh or frozen plasma samples (paired $t_{13} = 1.11$, $P = 0.29$); because not all samples were able to be analyzed while fresh, data presented are from frozen samples only. There were no significant differences among the blood volume calculated from Evan's Blue

dilution, labeled red cell dilution, or the combined red cell and plasma volumes (repeated measures GLM, $F_{2,12} = 1.545$, $P = 0.253$, Table 3). All analyses presented here are from blood volumes estimated using the Evan's Blue technique.

There were no differences in PV_{EB} or BV_{EB} due to region or sex for either harp or hooded seals. However, hooded seals had mass-specific plasma (PV_{EB}) and blood (BV_{EB}) volumes that were higher than those of harp seals ($P > 0.05$ for all age classes where comparisons were possible). For harp seals, mass-specific PV_{EB} and BV_{EB} was lowest in nursing pups, and higher and similar for both neonates and adults ($PV_{EB,\%}$: $F_{2,11} = 11.73$, $P = 0.002$, $BV_{EB,\%}$: $F_{2,11} = 11.27$, $P = 0.002$, Fig. 1). Unfortunately, PV_{EB} measurements in weaned harp seal pups were not successful. In hooded seals, mass-specific PV_{EB} and BV_{EB} were high in neonates, declined during the nursing period, were lowest in weaned pups, and again high in adults ($PV_{EB,\%}$: $F_{3,24} = 39.24$, $P < 0.001$, $BV_{EB,\%}$: $F_{3,24} = 13.61$,

Table 2 Hematology parameters (mean ± SE) for harp and hooded seals, with sex and region combined. Superscripts indicate significant differences among age classes within a species

Age class	n	Max Hct, %	Hb (g dl whole blood^{-1})	MCHC (g dl RBC^{-1})
Hooded seals				
Neonates	5	56.4 ± 1.9	22.6 ± 0.3	40.2 ± 1.3
Nursing	5	54.8 ± 1.5	22.9 ± 0.7	41.7 ± 1.1
Weaned/fasting	15	58.9 ± 1.6	24.8 ± 0.8	42.1 ± 0.8
Adults	16	57.5 ± 1.4	23.3 ± 0.9	40.5 ± 1.2
Harp seals				
Neonates	5	54.5 ± 3.3 [a]	20.5 ± 1.1 [a]	37.8 ± 1.3
Nursing	11	46.9 ± 2.0 [b]	18.2 ± 0.9 [b]	37.8 ± 1.4
Weaned/fasting	5	58.6 ± 0.9 [a]	22.4 ± 0.7 [a]	38.8 ± 1.0
Adults	12	54.7 ± 1.5 [a]	22.6 ± 0.9 [a]	41.7 ± 1.2

Table 3 Plasma and red cell volumes as determined simultaneously in hooded seals by the Evan's Blue and ^{51}Cr techniques, and the resulting estimates of blood volume as determined from measured plasma volume and hematocrit (BV_{EB}), by adding the plasma and red cell volumes (BV_{RCV+PV}), and by dividing the estimated RCV by Hct (BV_{51Cr}). There were no significant differences in the blood volume estimated using the different techniques

Age class	n	Plasma volume (l)	Red cell volume (l)	Blood Volume l (BV_{EB})	Blood Volume l (BV_{RCV+PV})	Blood Volume l (BV_{51Cr})
Nursing	2	2.43 ± 0.02	2.63 ± 0.25	5.29 ± 0.01	5.06 ± 0.27	4.69 ± 0.36
Weaned/fasting	6	1.76 ± 0.13	2.49 ± 0.22	4.65 ± 0.29	4.24 ± 0.30	3.93 ± 0.31
Adults	6	14.05 ± 0.69	16.81 ± 1.32	28.37 ± 1.32	30.86 ± 1.16	30.11 ± 1.17

$P < 0.001$, Fig. 1). This pattern of declining mass-specific fluid volume with age in both harp and hooded seals was due to a more rapid acquisition of large lipid reserves than expansion of blood reserves. On an absolute basis both PV_{EB} and BV_{EB} increased only slightly with age in young harp seals ($PV_{EB,L}$ from 0.95 ± 0.04 to 1.42 ± 0.05L, $F_{1,5} = 9.15$, $P = 0.029$; $BV_{EB,L}$ from 1.92 ± 0.06 to 2.8 ± 0.08L, $F_{1,5} = 7.25$, $P = 0.043$). In hooded seals, there was no statistically significant change in fluid volume with age, although the same trend was present.

Lung volume

There was no significant difference in the lung volume, as a percent of body mass for nursing (29.5 ± 2.8 ml kg^{-1}) or weaned pups (39.5 ± 3.4 ml kg^{-1}), but adults had significantly larger lung volumes (81.4 ± 5.8 ml kg^{-1}, Mann–Whitney $U = 3.098$, $P = 0.001$). Overall, total lung volume (TLV, L) was estimated for all animals in which it was not directly measured as TLV $= 0.007M_b^{1.48}$ ($F_{1,13} = 391.64$, $P < 0.001$; $R^2 = 96.8$).

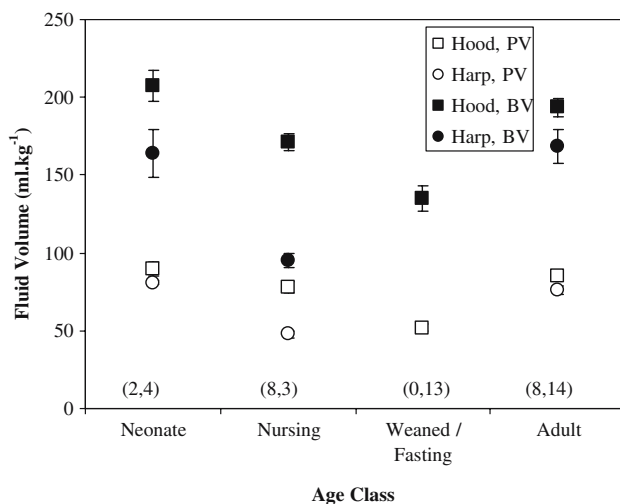**Fig. 1** Mean (±SE) plasma volume (ml kg^{-1}, as measured by Evan's blue dye dilution) and blood volume (ml kg^{-1}) in pups and adult harp and hooded seals. Harp seals are shown as *circles*, hooded seals as *squares*. Sample sizes are indicated (harp, hooded), and come from animals handled in both Canada and Norway

Muscle parameters

Muscle mass was determined for 33 seals captured in Norway (8 harp seals, 25 hooded seals). On both percent total body mass and lean body mass (determined by subtracting blubber mass, corrected for water content, from total body mass) by age class, there were significant differences in absolute and mass-specific muscle mass due to age (total mass $F_{1,26} = 13.118$, $P < 0.001$, lean body mass $F_{1,24} = 5.886$, $P = 0.002$), but not due to species or sex. Adults had the highest percent muscle, and neonates the lowest. There were no significant differences among the pup age categories (neonates, nursing, weaned), or between yearlings and adults (Fig. 2). The allometric equation that best predicted muscle mass (in kg) as a function of total mass was $Y = 0.070M_b^{1.275}$ ($F_{1,31} = 954$, $P < 0.001$, $r^2 = 0.969$). The relationship between muscle mass and lean body mass (kg) was $Y = 0.241M_{LBM}^{1.099}$ ($F_{1,29} = 2,170$, $P < 0.001$, $r^2 = 0.987$). In both cases, the scaling coefficient was significantly different from unity.

The concentration of Mb in the *M. longissimus dorsi* was determined in 45 harp seals and 46 hooded seals ranging in age from neonatal pups to adult females. Recovery rates for lyophilized Mb were 96.9 ± 3.7%, and Mb values determined for tissue control were similar to those previously reported: adult northern elephant seal 71.9 ± 5.8 vs. 67 ± 15 mg g wet muscle^{-1} (Thorson 1993), adult harbor seal 56.1 ± 2.6 vs. 59.0 ± 7.0 mg g wet muscle^{-1} (Clark et al. 2006a), and northern elephant seal pup 23.5 ± 1.9 vs. 24 ± 2 mg g wet muscle^{-1} (Noren and Williams 2000). In harp seals, adult Mb was 51% higher than in yearlings, and approximately four times higher than that in neonatal pups, and there was no difference in Mb among pup age classes ($F_{4,34} = 72.3$, $P < 0.001$). In hooded seals, adult Mb was 24% greater than in yearlings, and three times higher than in pups ($F_{4,39} = 192.8$, $P < 0.001$). While there was a trend towards increasing Mb with age in pups of both species, this trend was never significant (post-hoc comparisons, $P > 0.05$). There were no significant differences in Mb between species in any age class (Fig. 3), although hooded seals tended to have higher levels than harp seals.
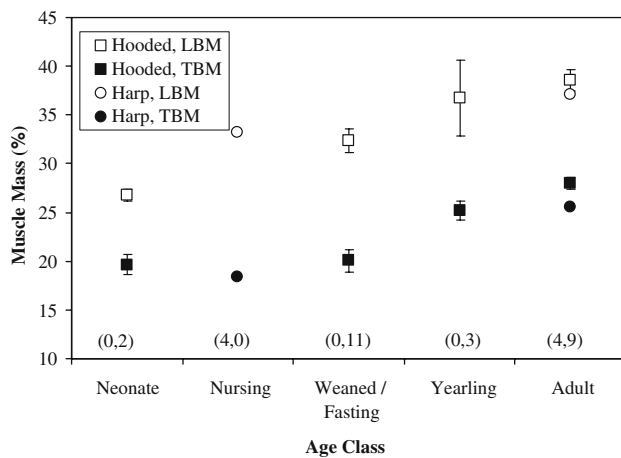
Fig. 2 Mean (±SE) muscle mass, as a percent of total body mass (TBM) and lean body mass (LBM) for harp and hooded seals handled in Norway. Muscle mass (kg) was determined by complete dissection, and lean body mass (kg) was determined by subtracting blubber mass, corrected for water content, from total body mass. Sample sizes are indicated (harp, hooded)

Fig. 3 Mean (±SE) myoglobin (Mb) concentration (mg g^{-1} wet tissue weight) in the *M. longissimus dorsi* muscle, and mean (±SE) hemoglobin (Hb) concentration (g dl^{-1} whole blood) for harp and hooded seals from both Canada and Norway. Sample sizes are indicated (Mb harp, hooded; Hb harp, hooded)

Total available body oxygen stores

There were significant changes in mass-specific TBO$_2$ stores with age in both harp and hooded seals (Table 4, harp $F_{2,12} = 55.61$, $P < 0.001$, hood $F_{3,30} = 44.18$, $P < 0.001$), but no effect of region or sex. Overall, the longer diving, more precocial, hooded seals had significantly larger TBO$_2$ stores at all ages than harp seals. In both species, mass-specific TBO$_2$ stores were highest in adults, and lowest in weaned pups. The decline in mass-specific TBO$_2$ stores as pups aged was due to the decline in BV$_{EB}$ which, in combination with relatively constant Hct and Hb, caused a decline in mass-specific available blood O$_2$ stores (harp $F_{2,14} = 10.74$ $P = 0.001$, hooded $F_{3,30} = 4.26$, $P = 0.013$). Mass-specific muscle O$_2$ stores did not change significantly with age during the nursing period in either harp or hooded seal pups, but pup values were significantly lower than those of yearlings and adults (harp $F_{3,25} = 183.2$, $P < 0.001$; hooded $F_{4,35} = 242.7$, $P < 0.001$). As a result, the relative distribution of O$_2$ changed with age, with neonatal pups storing the majority of their O$_2$ in the blood and little in the muscle, and adults storing roughly equivalent amounts in blood and muscle (harp $F_{2,12} = 35.31$, $P < 0.001$, hooded $F_{3,30} = 130.20$ $P < 0.001$).

Comparative data

The U-shaped pattern of age related changes in the relative maturity of mass-specific TBO$_2$ stores seen in harp and hooded seals was also present in all other phocids for which such data exists (Fig. 4). In addition, there was a strong ne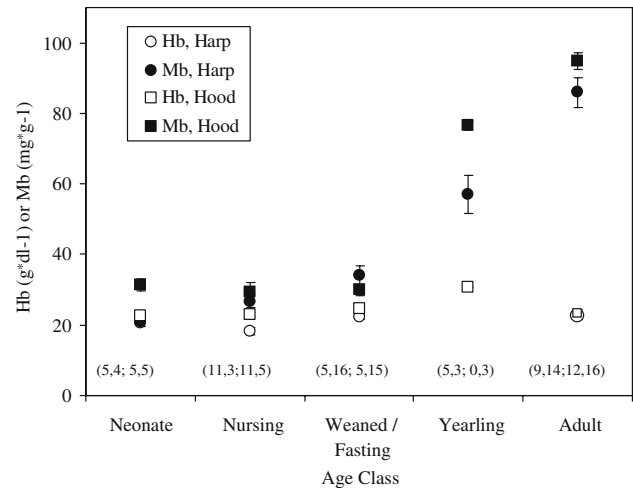gative relationship between the relative maturity of mass-specific TBO$_2$ stores and mass-specific blood O$_2$ stores at birth, expressed as a percentage of the adult store size, and the length of the terrestrial period, in days (% Adult TBO$_2$ = 75.88 − 0.407 days, $R^2 = 0.85$, $F_{1,5} = 33.76$, $P = 0.002$, % Adult BO$_2$ = 125.72 − 0.963 days, $R^2 = 0.81$, $F_{1,5} = 26.96$, $P = 0.003$), but no such correlation with respect to mass-specific muscle O$_2$ stores (Fig. 5a). There was no significant relationship between the length of the terrestrial period and the relative maturity of mass-specific blood O$_2$ or TBO$_2$ stores at weaning, but there was a positive correlation between the length of the terrestrial period and the relative maturity of mass-specific muscle O$_2$ stores at weaning (% Adult MO$_2$ = 17.29 + 0.384 days, $R^2 = 0.56$, $F_{1,5} = 8.72$, $P = 0.032$, Fig. 5b).

Discussion

There are three main findings from this research. First, we found that harp and hooded seal adults have large TBO$_2$ stores, due primarily to very high concentrations of muscle Mb. In fact, to our knowledge, hooded seals have the highest known myoglobin concentration of all marine mammals. Second, we found neonatal pups lacked the large O$_2$ stores of adults, primarily due to much reduced muscle mass and Mb, as has been previously reported in other pinnipeds (Burns et al. 2005; Noren et al. 2005; Richmond et al. 2006). Finally, our comparative analysis revealed a strong negative correlation between the size of the O$_2$ stores at birth and the length of the terrestrial period, suggesting that the temporal pattern of O$_2$ store development is correlated with phocid life history strategies, and

Table 4 Mass-specific oxygen stores in blood, muscle, and lung for hooded and harp seals of different ages. Blood volume (BV) and myoglobin (Mb) were measured directly; muscle mass and lung volume were either measured directly, or extrapolated from allometric equations, as indicated in the methods. Total oxygen stores were calculated for individuals for which both BV and Mb were measured directly (†), or from average values for each age class (‡). The relative distribution of oxygen in blood and muscle reserves is shown. Superscripts indicate significant differences among age classes within a species

Age class	n (blood, muscle, both)	Blood O_2 ml kg^{-1}	Muscle O_2 (ml kg^{-1})	Lung O_2 (ml kg^{-1})	Total O_2 (ml kg^{-1})†	Total O_2 (ml kg^{-1})‡	% O_2 blood	% O_2 muscle
Hooded seals								
Neonates	4,4,4	48.6 ± 3.2 [a]	6.9 ± 0.5 [a]	2.3 ± 0.1	57.7 ± 2.7 [a]	57.8	83.9 ± 1.5 [a]	12.1 ± 1.2 [a]
Nursing	3,3,3	41.8 ± 0.5 [a]	7.3 ± 0.3 [a]	2.8 ± 0.2	51.7 ± 0.3 [a]	51.9	80.7 ± 0.5 [a,b]	14.2 ± 0.6 [a,b]
Weaned/fasting	13,16,13	35.0 ± 2.6 [b]	8.1 ± 0.6 [a]	3.1 ± 0.1	46.6 ± 2.9 [a]	46.2	74.6 ± 1.2 [b]	18.5 ± 1.0 [b]
Yearling	0,3,0	–	25.8 ± 1.3 [b]	3.4 ± 0.1	–	75.1*		
Adults	14,14,14	45.9 ± 2.6 [a]	37.2 ± 1.0 [c]	6.8 ± 0.3	89.5 ± 3.0 [b]	89.9	50.8 ± 1.3 [c]	41.9 ± 1.2 [c]
Harp seals								
Neonates	2,5,2	33.8 ± 8.7 [a]	3.7 ± 0.1 [a]	1.6 ± 0.1	39.4 ± 8.7 [a]	39.1	84.9 ± 3.2 [a]	10.5 ± 2.1 [a]
Nursing	8,10,4	18.8 ± 1.4 [b]	6.5 ± 0.6 [a]	2.7 ± 0.1	28.8 ± 1.9 [a]	28.0	65.1 ± 1.6 [b]	25.3 ± 1.4 [b]
Weaned/fasting	0,5,0	–	8.6 ± 0.6 [a]	3.0 ± 0.1	–	30.4**		
Yearling	0,5,0	–	19.0 ± 1.8 [b]	2.7 ± 0.1	–	59.6*		
Adults	8,9,6	37.9 ± 3.5 [a]	29.7 ± 1.2 [c]	5.0 ± 0.1	71.6 ± 3.4 [b]	72.6	49.0 ± 3.1 [c]	44.0 ± 3.0 [c]

* Assumes blood volume, Hb, and muscle mass (harps only) are equivalent to adult values

** Assumes blood volume equal to that in nursing pups

that the postweaning fast serves as an important period for the maturation of blood and muscle O_2 stores in preparation for aquatic life. Still, final maturation of TBO_2 stores does not take place until after foraging begins, indicating that naïve foragers are likely constrained by lower aerobic capacity.

Adult O_2 stores and behavior

Like other long-diving phocids, hooded seal adults store large amounts of oxygen in their blood and muscle, and their TBO_2 stores are slightly larger than Weddell seals (86 ml O_2 kg^{-1}, Ponganis et al. 1993) and northern elephant seals (85 ml O_2 kg^{-1}, Thorson 1993). In contrast, the shorter-diving harp seal adults have TBO_2 stores similar to many phocid species, but larger than in all otariids and the walrus (*Odobenus rosmarus*) (Fig. 6). Like other pinnipeds, both harp and hooded seals have achieved their large TBO_2 stores by elevating both their blood and muscle oxygen stores, relative to terrestrial species (Kooyman 1989; Mottishaw et al. 1999). The Hct, Hb, and MCHC values reported here are similar to those earlier reported for harp (Ronald 1970; Geraci 1971; Boily et al. 2006) and hooded seals (Clausen and Ersland 1968; Keiver et al. 1987; Cabanac 2000; Boily et al. 2006) as well as for other phocid species (Bossart et al. 2001). Similarly, blood volumes for both harp and hooded seal adults fall within the range reported for other phocid species (Costa et al. 1998). Our estimate of BV in adult harp seals (168 ± 11 ml kg^{-1})

is similar to that reported by Ronald (1970; 160 ml kg^{-1}), and our estimate of BV in hooded seal adults is similar to that reported for hooded seals by Keiver et al. (1987; PV = 85 ml kg^{-1}, BV = 20.8 ml kg^{-1}, if the trapped cell correction is omitted, ICSH 1973), as well as to that seen in other long diving phocids (northern elephant, Weddell, and Baikal seals, see Fig. 6). Our hooded seal values are substantially higher than those reported by Cabanac (2000), but we discount Cabanac's findings because his Evan's Blue dosage was less than half that typically used for pinnipeds, and because the reported fluid volumes (PV 43.7 ml kg^{-1}, BV 130 ml kg^{-1}) are lower than in any other phocid (Costa et al. 1998). Further, the close agreement between our independent BV_{EB} and BV_{51Cr} estimates supports the higher values. Thus, while both harp and hooded seal adults have large blood oxygen stores as compared to terrestrial animals, these stores are not remarkable relative to other phocids, and fit with observed differences in dive behavior.

In contrast, our Mb values for both adult female harp (85.9 ± 12.5 mg g^{-1}) and hooded seals (94.8 ± 8.9 mg g^{-1}) are, to our knowledge, the highest yet recorded in any marine mammal. While our value for harp seals is higher than that previously reported (70.3 ± 11.0 mg g^{-1}, George et al. 1971), we are confident in our results due to the large sample size and rigorous assay validation. Higher Mb values are found in marine mammals that make longer dives (Noren and Williams 2000; Kanatous and Garry 2006) thus, the higher Mb in the major swimming muscles
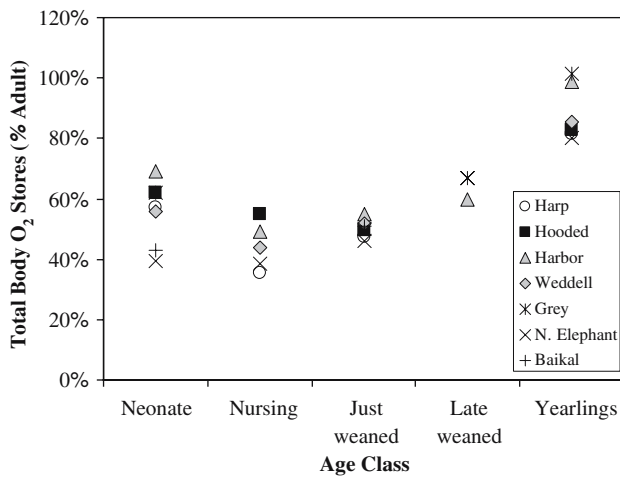
Fig. 4 Age related changes in mass-specific total available body oxygen stores, as a percent of adult value, for seven phocid species. Data sources as follows: harp, hooded, harbor (*Phoca vitulina*, Burns et al. 2005; Clark et al. 2006a), grey (*Halichoerus grypus*, Noren et al. 2005), Baikal (*Phoca sibirica*, Ponganis et al. 1997; Shoshenko et al. 1999), Weddell (*Leptonychotes weddellii*, Kooyman et al. 1983; Burns and Castellini 1996; Burns, unpub), and northern elephant seals (*Mirounga angustirostris*, Thorson 1993). All authors followed Kooyman (1989) in their calculations of TBO$_2$ stores. Muscle oxygen stores for northern elephant seal pups were recalculated using values for mass-specific muscle mass determined for southern elephant seal pups of similar age (Bryden 1972). In the original work, muscle mass was assumed to be 30%, this recalculation assumes 20% for nursing pups, 22.5% for weaned pups, and 30% for adults. The muscle mass of Baikal seals was set equal to that of harp seals

of hooded seals fits with observed behavioral patterns (Folkow and Blix 1995, 1999), and their large blood volume, high Hb, and large spleen (Cabanac et al. 1999). If a blood volume of approximately 20% by mass represents a physiological limit (Costa et al. 1998; Wright and Davis 2006), then it may be that the high Mb in hooded seals represents increases in the only other available O$_2$ storage site, the muscle. Why we observed similarly elevated Mb in the major swimming muscles of harp seals is less clear since their dives are not as long as those in hooded seals (Folkow et al. 2004). However, since the optimal Mb for given aerobic dive duration increases as muscle metabolic rate increases (Wright and Davis 2006), harp seals might have elevated stores due to their more active locomotory patterns. Indeed, the highest previously reported Mb values is also from a relatively small, active, arctic species, the ribbon seal (80.7 mg g^{-1}, Lenfant et al. 1970).

The lung oxygen stores of harp and hooded seals are small, like in other phocid seals (Kooyman 1989). Moreover, during diving, lungs collapse at depths of 25–50 m (Falke et al. 1985), making the little oxygen present unavailable to the organism. Therefore, lung oxygen stores are likely unimportant, except in very shallow dives.
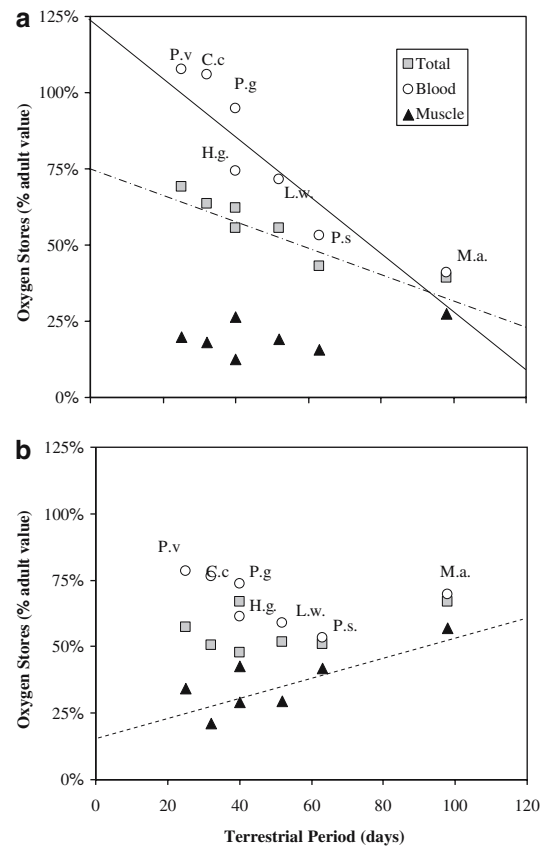

Fig. 5 The relative maturity of mass-specific blood, muscle, and total available body oxygen stores in pups, as a percent of adult values at **a** birth, and **b** as close to the end of the terrestrial period as possible, relative to the length of the terrestrial period. The fitted regression lines for the relationship between mass-specific TBO$_2$ at birth as a percent adult TBO$_2$ and the length of the terrestrial period (*dot-dash line*); mass-specific blood O$_2$ stores at birth as a percent adult mass-specific blood O$_2$ stores and the length of the terrestrial period (*solid line*); and mass-specific muscle O$_2$ stores at weaning as a percent adult mass-specific muscle O$_2$ stores and the length of the terrestrial period (*dotted line*) are shown; equations are provided in the text. Data are from harbor (*P.v.*), hooded (*C.c.*), harp (*P.g.*), grey (*H.g.*), Weddell (*L.w.*), Baikal (*P.s.*) and northern elephant seals (*M.a.*). Information on the duration of the lactation and postweaning fast periods were taken from the following sources: harp (Sivertsen 1941), hooded (Bowen et al. 1987), grey (Noren et al. 2005), harbor (Muelbert and Bowen 1993), Weddell (Rea et al. 1997), Baikal (Atkinson 1997), and northern elephant seals (Thorson 1993)

If we derive the calculated aerobic dive limit (cADL) by dividing measured TBO$_2$ stores by a diving metabolic rate (DMR) estimated to be $1.7 \times$ RMR$_{Kleiber}$ (Williams et al. 2004), the cADL for adult female harp and hooded seals is $12.1 \pm 1.7_{(SD)}$ and $17.2 \pm 2.7_{(SD)}$ min, respectively. While the cADL of the harp seal is similar to that of other phocids of similar size, the estimated cADL for hooded seals is relatively long, as it is similar to both the cADL and the ADL derived from post-dive lactate levels for adult Weddell seals, which are twice their mass (Ponganis et al. 1993;
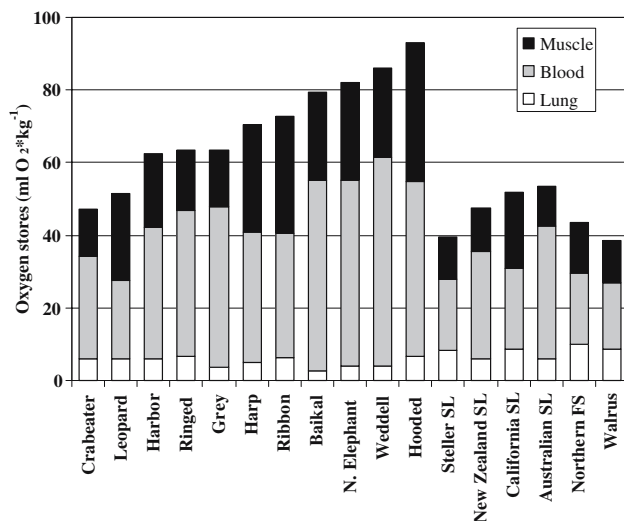
Fig. 6 Mass-specific total available body oxygen stores (ml O_2 kg^{-1}) for a variety of adult pinnipeds, with relative distribution in blood, muscle, and lungs shown. Data are from sources identified in Fig. 4, or as follows: crabeater seal (Burns, unpub), leopard seal (Kuhn et al. 2005), ringed (Lydersen et al. 1992), ribbon (Lenfant et al. 1970), Baikal (Ponganis et al. 1997), Steller sea lion (Richmond et al. 2006), California sea lion (Weise 2006), New Zealand sea lion (Costa et al. 1998), Australian sea lion (Kuhn et al. 2006), northern fur seal and walrus (Lenfant et al. 1970). Values reported in Lenfant et al. (1970) and Lydersen et al. (1992) were recalculated following the methods in Kooyman (1989) so that values could be directly compared across all species

Wright and Davis 2006). The only other small phocid with such a long (lactate) ADL is the Baikal seal, which relies both on large TBO_2 stores and low DMR to extend its submergence limits (Ponganis et al. 1997). These calculations suggest that the elevated O_2 stores in both blood and muscles of hooded seals allow adults to dive to great depths and durations without having to rely significantly on anaerobic metabolism.

Developmental pattern

Overall, the developmental pattern in harp and hooded seals fit with that previously observed in pinnipeds (Thorson 1993; Burns and Castellini 1996; Shoshenko et al. 1999; Burns et al. 2005; Noren et al. 2005; Richmond et al. 2006; Kuhn et al. 2006). Neonates had significantly lower TBO_2 stores than adults when examined on a mass-specific basis, and there was a slight decline, rather than increase in the size of the mass-specific oxygen stores during the nursing period. This pattern was apparent in both species, but harp seals were slightly less mature at birth, and the decline in their mass-specific TBO_2 stores was more pronounced than in the more precocial hooded seals. Lower mass-specific stores in neonates can be attributed primarily to lower muscle mass and Mb load, while lower stores at weaning are due both to

immature tissue reserves, and to the decline in mass-specific PV and BV that results from a massive gain in lipid reserves. It is important to note, however, that there are increases in the absolute amount of oxygen stored in tissues with age during the terrestrial period, due to small increases in absolute muscle mass, Mb load, and fluid volumes. We observed a similar pattern in blood development in both harbor seals and Steller sea lions (*Eumetopias jubatus*), and attributed the delay in BV expansion to the time required for production of new red blood cells in response to erythropoietin levels that increased only after birth (Burns et al. 2005; Richmond et al. 2005; Clark et al. 2006b).

Perhaps to compensate, species with relatively short terrestrial periods, such as hooded and harp seals are born with Hct, Hb, and MCHC values that are more similar to those of adults than are species with longer terrestrial periods (Geraci 1971; Worthy and Lavigne 1982; Thorson 1993; Horning 1997; Noren et al. 2005; Richmond et al. 2005). Indeed, in the extremely precocial hooded seal, there were no differences in HCT, Hb, or MCHC due to age, and we are unaware of any other pinniped in which this occurs. For precocial species, being born with mature hematology minimizes the impact of the reduction in mass-specific blood O_2 stores that accompanies the rapid mass gain during the nursing period. In addition, if *absolute* RCV and PV volumes do not decline as mass is lost during the post-weaning fast, *mass-specific* PV and BV will increase—without the need for new cell production. Indeed, provided that no red cells are destroyed during the fast, the *mass-specific* BV of hooded seal pups should be equivalent to that of adults by the end of the postweaning fast. Alternatively, destruction of some red blood cells would make heme available for Mb synthesis; stochiometrically four Mb molecules could be synthesized from the heme reclaimed from a single catabolized Hb molecule (Jandl and Katz 1963). Thus, the mass loss associated with the postweaning fast may allow pups to increase the size of their *mass-specific* TBO_2 stores and/or redistribute heme from blood to muscle prior to the initiation of diving activity (Burns et al. 2004). Unfortunately, ice conditions and ship schedules prevented us from obtaining blood and tissue samples from pups at the end of the post-weaning fast.

In contrast to blood oxygen stores, muscle mass and Mb loads in harp and hooded seal pups were much lower than that of adults at birth and at weaning, but not by 1 year of age. The more precocial hooded seal neonates had higher absolute values than harp seal pups, and the difference between neonatal and adult values was lower in hooded seals than in harp seals. These findings indicate that while Mb develops very slowly prior to the onset of foraging, it increases more rapidly once foraging begins. Since this pattern is also evident in harbor seals and cetaceans which begin to dive right after birth (Noren et al. 2001; Burns

et al. 2005; Clark et al. 2006a), the absence of Mb increases during the terrestrial period in harp and hooded seals can not be attributed solely to lack of underwater activity. Conversely, Mb increases with age in fledging penguins, in the absence of diving activity (Ponganis et al. 1999), indicating that neither work nor hypoxic stress is necessary for Mb production. However, while Mb development proceeded slowly in harp and hooded seals during the terrestrial period, the fact that neonates have Mb > 20 mg g^{-1} indicates quite extensive prenatal development. In terrestrial mammals Mb production expression is limited or absent in utero, but increases significantly in the postnatal period (Weller et al. 1986; Patel et al. 2002), and even otariids have much lower neonatal Mb levels (<1 mg g^{-1} at birth, Richmond et al. 2006).

The mismatch between O_2 store development in blood and muscle has implications for the diving behavior of pups because efficient utilization of O_2 reserves requires that both blood and muscle O_2 stores be depleted simultaneously (Davis and Kanatous 1999; Wright and Davis 2006). Among adults, elevated Mb levels facilitate longer dives by allowing a stronger dive response and lower DMR. For young seals, which lack significant Mb reserves and the cardiovascular control necessary to sustain vasoconstriction (Greaves et al. 2005), the O_2 demands of the working muscles must be provided by blood O_2 stores. Thus, the early maturation of large blood O_2 stores may facilitate diving in young animals by providing O_2 required for aerobic metabolism to muscles that lack endogenous Mb reserves (Burns et al. 2004). Still, reduced Mb loads significantly limit the cADL of weaned pups, which is approximately one-third that of adults (harp 4.3 min, hooded 6.0 min). In addition, if muscle perfusion is sustained during diving, DMR are likely much higher than in adults, further reducing cADL. Therefore, delayed Mb maturation provides another possible explanation for the postweaning fast: pups may remain terrestrial until Mb increases sufficiently to allow them to efficiently use both blood and tissue O_2 reserves. Support for this hypothesis comes from the slight increase in Mb values observed during terrestrial period in all phocids studied (Thorson 1993; Shoshenko et al. 1999; Burns et al. 2005; Noren et al. 2005; Clark et al. 2006a; Burns, unpub). Alternatively, if pups do begin diving prior to the increase in Mb, then their behaviors will likely reflect reduced mass-specific O_2 stores and elevated body and muscle metabolic rates. Unfortunately, the only extant data on diving behavior in either of these species comes from adult animals.

Comparative patterns

Among the phocid lineage there are two main developmental pathways (Boness and Bowen 1996; Schulz and Bowen 2004): long lactation periods with early aquatic activity and short (or no) aquatic postweaning fasts (bearded, ringed, Weddell, and harbor seals), or brief lactation period followed by longer terrestrial (or ice based) postweaning fasts with little aquatic activity (elephant, grey, harp, and hooded seals). Despite the different strategies, it is rare for pups to begin independently foraging before 1 month of age, and this duration is independent of whether pups enter the water during the nursing period, or wait until the end of a postweaning fast. Even after foraging begins, it can be days or weeks before pups forage well enough to meet their energy demands, making it difficult to pinpoint when pups become nutritionally independent (Muelbert and Bowen 1993; Rea et al. 1997). The comparative data presented here suggests that the rate of physiological development is under strong selective pressure both in utero, and after birth, but that the underlying pattern is similar for all species (Mottishaw et al. 1999). Across both groups, the relative maturity of mass-specific TBO$_2$ stores at birth was strongly and negatively correlated with the length of the terrestrial period, such that species such as harbor, harp, and hooded seals that begin foraging at a younger age are physiologically more mature at birth than those species that have longer nursing periods, or more protracted postweaning fasts. This pattern was even evident in the comparison between harp and hooded seals, for hooded seals have both a shorter terrestrial period and slightly more mature oxygen stores than harp seals. In contrast, there was broad similarity in the relative maturity of mass-specific TBO$_2$ stores at the end of the terrestrial period, with pups of all species beginning to forage once mass-specific TBO$_2$ stores are approximately two-thirds those of adults. This suggests that there is a minimum threshold of maturity, below which foraging cannot be efficiently sustained.

The similarity in total store size at independence was not reflected in the distribution of O_2 in blood and muscle. Pups of species that forage at a younger age store a larger proportion of their O_2 in the blood, while longer terrestrial periods appear to allow for greater muscle maturation. As a result, pups with longer terrestrial periods begin foraging with O_2 distribution more similar to that of adults. This suggests that the pattern of blood and muscle development may reflect resolution of two different trade-offs: one by mother, and one by pup. From the maternal perspective, the length of the lactation period is influenced by body size, capital reserves, and the relative stability of the breeding substrate (Kovacs and Lavigne 1986; Boness and Bowen 1996; Schulz and Bowen 2004). The very short lactation period in hooded seals maximizes the proportion of maternal energy reserves transferred to the pup while minimizing metabolic overhead (Kovacs and Lavigne 1992; Lydersen et al. 1997). In addition, it increases the

likelihood that hooded seal pups will be successfully weaned despite unstable and fluid nature of pack ice breeding substrate. However, early foraging activity by pups that are weaned at a young age may not be optimal, for the reasons discussed earlier. Instead, these pups may remain ashore until blood and muscle O_2 reserves, and perhaps other physiological processes such as cardiovascular control, have matured sufficiently to support underwater foraging activities (Greaves et al. 2005; Noren et al. 2005). These findings suggest that the postweaning fast represents a critical period for physiological development, and is not simply a period when pups wait for food to become available. However, since final maturation of muscle only occurs postweaning, it likely requires some combination of prey intake, physical work, and hypoxic challenge (Patel et al. 2002; Brooks et al. 2005). Thus, while this comparative dataset suggests that the rates of physiological development pre and post-natally are labile, clearly there are limits to how fast development can occur, and final maturation of TBO$_2$ stores does not occur for weeks to months after foraging begins. These limitations on early development may negatively influence pup foraging efficiency and survival rates, if ongoing patterns of climate change cause earlier breakup of the sea ice used as a platform during the postweaning fast (Kelly 2001; Stirling 2005; Johnston et al. 2005).

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