Development of the Blood and Muscle Oxygen Stores in Gray Seals (*Halichoerus grypus*): Implications for Juvenile Diving Capacity and the Necessity of a Terrestrial Postweaning Fast

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ABSTRACT

To successfully transition from nursing to foraging, phocid seal pups must develop adequate diving physiology within the limited time between birth and their first independent foraging trip to sea. We studied the postpartum development of oxygen stores in gray seals (*Halichoerus grypus*, $n = 40$) to better understand the ontogeny of diving capacity in phocids. Hemoglobin (Hb), hematocrit (Hct), blood volume (BV), and myoglobin (Mb) levels in newborn (3 d postpartum [DPP]) and newly weaned (17 ± 0.4 DPP) pups were among the lowest measured across age classes. During the pups' terrestrial postweaning fast (PWF), Hb, Hct, mass-specific BV, and Mb increased by 28%, 21%, 13%, and 29%, respectively, resulting in a 35% increase in total body mass-specific oxygen stores and a 23% increase in calculated aerobic dive limit (CADL). Although Hb and Hct levels at the end of the PWF were nearly identical to those of yearlings, total body mass-specific oxygen stores and CADL of weaned pups departing for sea were only 66%–67% and 32%–62%, respectively, of those for yearlings and adult females. The PWF represents an integral component of the physiological development of diving capacity in phocids; however, newly independent phocids still appear to have limited diving capabilities at the onset of foraging.

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Introduction

Aquatic lung-breathing animals routinely experience prolonged periods of apnea while diving. During these periods, aerobic metabolic processes are supported by the use of onboard oxygen stores. The blood oxygen storage capacity in vertebrates is dependent on hemoglobin content and blood volume (Snyder 1983), while the muscle oxygen storage capacity is dependent on myoglobin content and muscle mass (Kooyman 1989). As a result, adult diving endotherms (i.e., aquatic and marine mammals and birds) have evolved elevated levels of blood hemoglobin (Lenfant et al. 1970; Snyder 1983; Kooyman 1989), blood volume (Snyder 1983; Kooyman 1989), and muscle myoglobin (Castellini and Somero 1981; Kooyman 1989), which confer greater mass-specific oxygen storage capacities than their terrestrial relatives (Snyder 1983; Kooyman 1989). Within marine mammals, species that dive the deepest and for the longest durations have the greatest oxygen carrying capacity in both the blood (Ridgway and Johnston 1966; Lenfant et al. 1970; Hedrick et al. 1986; Hedrick and Duffield 1991) and muscle (Castellini and Somero 1981; Noren and Williams 2000).

Although adult marine endotherms have elevated oxygen stores, recent studies suggest that neonates and juveniles have relatively low oxygen storage capacity in both the blood and muscle (Thorson 1993; Thorson and Le Boeuf 1994; Ponganis et al. 1999; Burns et al. 2000; Noren et al. 2001, 2002; Noren 2002; Clark 2004; Richmond 2004). In terrestrial and semiaquatic endotherms, the oxygen storage capacity in the blood and muscle also changes throughout life. For example, the hemoglobin content in humans (Rothstein 1993) and sheep (Potocnik and Wintour 1996) increases with age throughout development. Meanwhile, increases in myoglobin content in diving ducks, muskrats, geese, and voles have been associated with factors such as development, increased exposure to hypoxia, increased physical activity, and increased thermal demands (Morrison et al. 1966; Stephenson et al. 1989; MacArthur 1990; Saunders and Fedde 1991; MacArthur et al. 2001).

Neonatal pinnipeds (seals, fur seals, and sea lions) must shift from a terrestrial environment to an amphibious existence soon after birth or weaning. This transition requires that pups are physically prepared for the demands of swimming, diving, and foraging in the ocean in a relatively short period of time. In most true seals (family: Phocidae), the nursing period is short (4–50 d), and weaning occurs abruptly when the mother re-

turns to sea to forage and leaves her pup on the beach (Oftedal et al. 1987). The abandoned pups then undergo a terrestrial postweaning fast (PWF) of weeks or months during which they must rely on the large reserves of fat acquired during the suckling period (Bowen 1991). Although the adaptive significance of this PWF is not well understood, it may serve to prolong the pups' time on land so that the pups attain critical physiological traits for future diving and foraging at sea (Thorson 1993; Thorson and Le Boeuf 1994).

Only a few studies have simultaneously measured the development of the blood and muscle oxygen stores in phocids or other marine mammals (northern elephant seal [*Mirounga angustirostris*]: Thorson 1993; Thorson and Le Boeuf 1994; hooded seal [*Cystophora cristata*]: Burns et al. 2000; bottlenose dolphin [*Tursiops truncatus*]: Noren et al. 2001, 2002; Noren 2002; harbor seal [*Phoca vitulina*]: Clark 2004; and Steller sea lion [*Eumetopias jubatos*]: Richmond 2004). Understanding the phylogenetic constraints and ecological correlates of the development of these physiological traits requires data on a wide number of species. The gray seal (*Halichoerus grypus*) is a large-bodied phocid seal whose pups nurse for only about 16 d (Boness et al. 1995) and undergo a 3–4-wk PWF (Davies 1949; Coulson and Hickling 1964). Only limited data are available on the oxygen stores of gray seals (Scholander 1940; Lapennas and Reeves 1982; Reed et al. 1994*a*, 1994*b*), and the development of these stores in this species is virtually unknown (Greenwood et al. 1971). Thus, gray seals serve as an excellent model for the study of oxygen storage development during the PWF. Our aim was to examine the development of the blood and muscle oxygen stores in gray seals from birth through the PWF, to compare these stores to those measured in yearlings and adults, and to investigate how this postnatal development may impact early diving capacity.

Material and Methods

Field Sampling

The Smithsonian's Conservation and Research Center Institutional Animal Care and Use Committee approved all experimental protocols used in this research. Fieldwork was conducted on gray seals (*Halichoerus grypus*) at Sable Island, Nova Scotia, Canada (43°55′N, 60°00′W) during the pupping season, December 2002 to February 2003. Ten yearlings and 10 mother-pup pairs at 3 d postpartum (DPP) were captured and sampled once for morphological and physiological measurements. Eighty additional mother-pup pairs were marked at parturition with dye, given a uniquely numbered hind flipper tag (Jumbo Rototag), and subsequently observed until the pups were weaned. Ten of these known-age pups (nursing period: 14–19 d; mean: 16.6 ± 0.5 d) were captured at weaning and held in a pen for longitudinal studies, during which they were sampled at 0, 12, and 24 d postweaning (DPW) and subsequently released.

At each sampling, seals were manually captured and sedated with a dose of diazepam, which was administered via the extradural vein at a dose of approximately 0.23, 0.23, 0.29, and 0.13 mg kg^{-1} for newborns (3 DPP), weaned pups, yearlings, and adult females, respectively. Dorsal body length, axillary girth, and body mass were measured for each individual. Samples were then taken for measurement of blood hemoglobin (Hb) content, hematocrit (Hct), plasma volume (PV), blood volume (BV), and muscle myoglobin (Mb) content as follows. An initial 10-mL blood sample ($T = 0$ min) was taken from the extradural vein into a heparinized tube (Vacutainer Brand) followed by an intravenous injection (2.5–4.5 mL) of Evans blue dye at a concentration of 4, 10, and 25 mg mL^{-1} for newborns (3 DPP), weaned pups and yearlings, and adults, respectively. Sequential 10-mL blood samples were taken as described above at 10, 20, and 30 min postinjection. A muscle sample was taken from the primary locomotor muscle, *longissimus dorsi*, at a location above the hip. The biopsy site was first shaved, cleansed with betadine, and infiltrated subcutaneously with 2.5 mL lidocaine containing epinephrine (Astra Pharmaceuticals). A 1-cm incision was made with a no. 11 scalpel blade through which a 6-mm sterile biopsy punch (Miltex) was inserted and a muscle sample was taken from beneath the blubber layer. Muscle biopsy samples averaged about 50 mg. All blood and muscle samples were immediately placed on ice and transported to the field lab several hours later.

Laboratory Analyses

At the field lab, duplicate 10-μL aliquots of whole blood from each $T = 0$ min tube were added to aluminum-foil-wrapped cryovials containing 2.5 mL of Drabkins Solution (Total Hemoglobin Sigma Kit 525A). The samples were stored in the dark at room temperature until analysis (within 2 mo of collection). Hb was determined from these samples using the cyanmethemoglobin technique following methods described in the total hemoglobin kit (Sigma Kit 525A) and adapted for phocid seals (Thorson 1993; Thorson and Le Boeuf 1994).

Hct was also determined from the $T = 0$ min samples using the microcentrifuge method. A small amount of blood from each sample was collected in duplicate into microhematocrit tubes and spun at 13,460 *g*/11,500 rpm for 5 min in a Micro-MB Microhematocrit/Microcentrifuge (ThermoIEC). Percent packed cell volume was then determined from a microcapillary reader (ThermoIEC).

The remaining blood from the 0-, 10-, 20-, and 30-min blood samples were spun at 1,000 rpm for 25 min in a desktop centrifuge. The supernatant from each sample was pipetted into a 15-mL tube (Corning) and frozen at 0°C in the field for several weeks and at −20°C thereafter. PV and BV were then determined from these samples (within 2 mo) using the Evans blue-dye method as described by Swan and Nelson (1971) and adapted by El-Sayed et al. (1995).

Muscle samples were frozen at 0°C in the field for several weeks and at −80°C thereafter. Mb content of the muscle was determined within 5 mo of collection following the methods of Reynafarje (1963) as described in detail by Noren and Williams (2000) and Noren et al. (2001).

Mean Corpuscular Hemoglobin Content (MCHC) and Aerobic Dive Limit (ADL) Calculations

To determine the average concentration of hemoglobin in a red blood cell, mean corpuscular hemoglobin content (MCHC) was calculated for each seal according to the equation

$$\text{MCHC} = (\text{Hb Hct}^{-1}) \times 100. \quad (1)$$

To estimate the absolute maximum dive duration supported by aerobic processes for each seal, the calculated aerobic dive limit (CADL) was determined by dividing the calculated total body oxygen store by metabolic rate following the methods described by Kooyman (1989). The total body oxygen store for each seal was determined by inputting their mass-specific Hb, BV, and Mb into the following equations adapted for phocids:

$$\text{blood oxygen store} = \text{arterial } O_2 + \text{venous } O_2, \quad (2)$$

$$\text{arterial } O_2 = (0.33 \times \text{BV} \times m) \times (\text{Hb} \times 1.34 \text{ mL } O_2 \text{ g Hb}^{-1}), \quad (3)$$

$$\text{venous } O_2 = (0.66 \times \text{BV} \times m) \times (\text{Hb} \times 1.34 \text{ mL } O_2 \text{ g Hb}^{-1}), \quad (4)$$

where 0.33 and 0.66 are the estimated proportions of arterial and venous blood, respectively (Lenfant et al. 1970), 1.34 mL O_2 g Hb^{-1} is the oxygen binding capacity of Hb (Kooyman 1989), m is body mass (kg), and arterial and venous saturation is 0.20 to 0.95 and 0.15 to 0.90 for equations (3) and (4), respectively (Kooyman 1989.

$$\text{muscle oxygen store} = (\text{Mb} \times 1.34 \text{ mL } O_2 \text{ g Mb}^{-1}) \times (m \times p), \quad (5)$$

where 1.34 mL O_2 g Mb^{-1} is the oxygen binding capacity of Mb (Kooyman 1989) and p is the estimated proportion of muscle mass in the body. We assumed that muscle mass represented 18.5% and 30.0% of total body mass for pups and yearlings/adults, respectively, according to previous data for phocid seals (Kooyman et al. 1983; Burns et al. 2000). The lung oxygen store was assumed to represent a constant proportion of body mass at 4.1 mL O_2 kg^{-1}, based on the estimated diving lung volume (27.3 mL kg^{-1}; Kooyman et al. 1970) and the expected proportion of oxygen extracted from the air in the lungs (0.15; Kooyman 1989).

It was beyond the scope of this study to measure the diving metabolic rate of gray seals. Thus, the CADL of the adult age class was based on the average metabolic rate measured for adult diving gray seals (5.2 mL O_2 min^{-1} kg^{-1}; Reed et al. 1994*a*), which approximated 1.9 times that of Kleiber (1975). This metabolic rate is similar to that which was used to calculate ADLs of adult elephant seals (Thorson 1993; Thorson and Le Boeuf 1994). Immature seals have relatively higher metabolic rates than adults because of additional costs associated with growth and development. Thus, because the resting metabolic rate of juvenile gray seals does not approach predicted values for adults until sometime after the first year of life (Boily and Lavigne 1997), the CADL of all immature seals was based on the mean daily energy expenditure measured for gray seal pups during the PWF (Reilly 1991), which approximated 2.7 times that of Kleiber (1975).

Statistics

To assess developmental changes throughout the PWF, we used one-way repeated-measures ANOVA in combination with Tukey all pairwise comparisons tests to compare oxygen storage parameters (Hb, Hct, MCHC, PV, BV, and Mb), mass-specific oxygen stores (blood, muscle, and total body), and CADLs across the longitudinally sampled weaned pups (at 0, 12, and 24 DPW). We used one-way ANOVA in combination with Tukey all pairwise comparison tests to compare these same parameters across population age classes. These comparisons included the independent cross-sectional sampled animals (3-DPP neonates, yearlings, and adults) and only one data point from the longitudinally sampled weaned pups (24 DPW). Values are reported as mean ± SE. Findings were considered significant when $P < 0.05$.

Results

In pups sampled longitudinally, Hb content and Hct significantly increased on average by 28% and 21% over the PWF, respectively, while MCHC did not change (Fig. 1; Table 1). These results suggest that the number of red blood cells in the blood increased while the oxygen carrying capacity of individual red blood cells stayed consistent. As a result, mass-specific BV increased significantly throughout the PWF from 11.6% of body mass at weaning to 13.1% of body mass by 24 DPW (Fig. 2; Table 1). The consistent value for mass-specific PV over the PWF (Fig. 2; Table 1) provides evidence that the increased Hb and Hct at the end of the PWF were associated with the addition of red blood cells into the circulation and were not a result of dehydration. Although mean muscle Mb content appeared to increase on average by 29% over the PWF, the differences were not significant, a result potentially associated with the limited

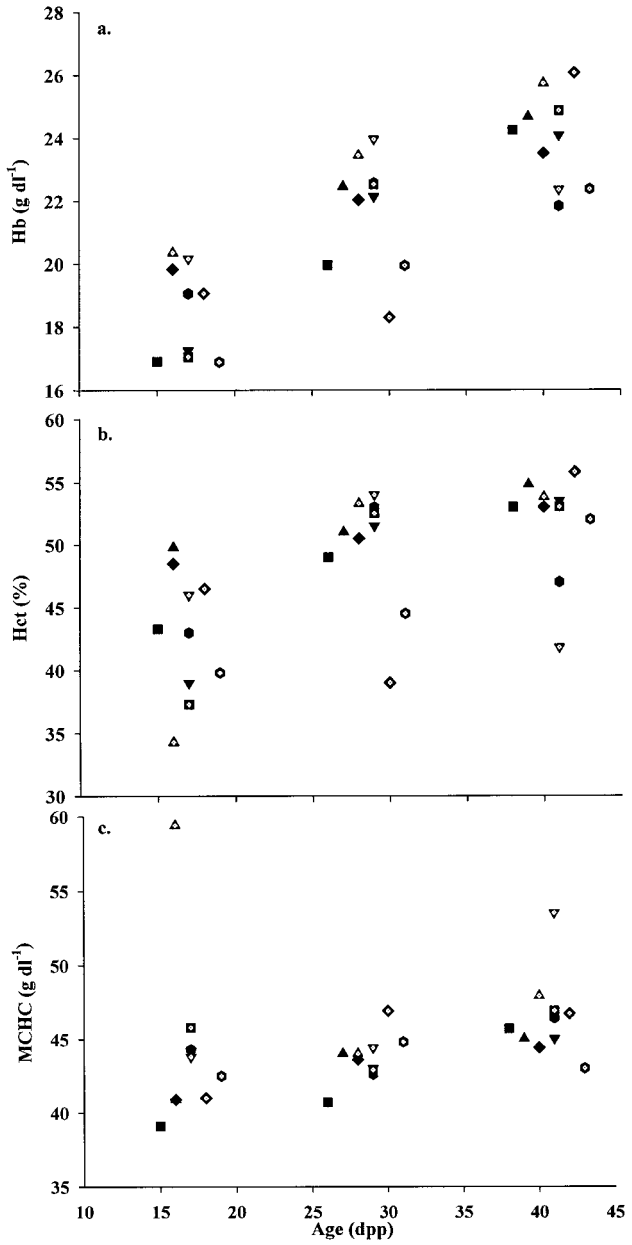

Figure 1. *a*, Hemoglobin content (Hb), *b*, hematocrit (Hct), and *c*, mean corpuscular hemoglobin content (MCHC) of gray seals during the postweaning fast (PWF) plotted as days postpartum (DPP). Each unique symbol represents an individual pup that was studied longitudinally during the PWF. Hb and Hct increased significantly during the PWF ($F_{2,29} = 31.505$, $P < 0.001$ and $F_{2,29} = 8.192$, $P = 0.003$, respectively), but MCHC did not change ($F_{2,29} = 1.716$, $P = 0.208$; repeated-measures ANOVA).

number of samples for the 0-DPW sample point (Fig. 3; Table 1).

Cross-sectional comparisons across the population demonstrated that there were significant differences among age classes in Hb content, Hct, mass-specific PV and BV, and muscle Mb content (Table 1). For example, adult mass-specific BV and Mb are 1.3–1.8 and 1.9–2.4 times, respectively, those for newborn and weaned pups. Meanwhile, MCHC remained relatively constant among groups (Table 1).

As a result of the ontogenetic changes in the blood and muscle, the oxygen storage of the body increased with a concomitant increase in CADL throughout development. The mass-specific oxygen stores in the blood and in total differed significantly across longitudinal sample points and increased by 46% and 35%, respectively, over the PWF (Table 2). Although mass-specific muscle oxygen stores appeared to increase by 33% over the PWF, the differences were not significant, a result potentially associated with the limited number of Mb samples for the 0-DPW sample point (Table 2). Cross-sectional comparisons across the population demonstrated that there were also significant differences among age classes in mass-specific oxygen stores in blood, in muscle, and in total (Table 2). Furthermore, assuming that the oxygen storage in the lung represented a constant proportion of body mass, the relative contribution of each of the three storage sources to total oxygen stores changed ontogenetically. The relative contribution from both lung and blood storage appeared to decline from birth through adulthood (Table 2). In contrast, the relative contribution of muscle oxygen storage appeared to increase from 12% of total stores at birth to 16% near the end of the PWF and to 21%–27% in yearlings and adults (Table 2). Concurrent with the increasing total body mass-specific oxygen stores during the PWF and thereafter, CADL increased significantly by 23% during the PWF and increased across age classes such that the CADL of adult females is 4.0, 3.1, and 2.0 times that of newborn pups (3 DPP), weaned pups (24 DPW), and yearlings, respectively (Table 2). Furthermore, the actual aerobic dive limits of young foraging gray seals may be even lower than the CADLs presented here. The CADLs of the immature gray seals were calculated with a metabolic rate measured from fasting gray seal pups (Reilly 1991). The fast undoubtedly elicited depressed metabolic rates. Once the pups initiate foraging, a rapid increase in metabolic rate is expected, which would further decrease aerobic dive limit.

Discussion

Of the marine mammal groups, phocid seals have the greatest mass-specific oxygen storage capacity (Kooyman 1989). In this family, the blood is the most important oxygen store (generally about 65%), followed by the muscle (30%) and the lung (5%; Kooyman 1989). Oxygen storage in the lungs is negligible due to lung collapse at 40 m (Kooyman et al. 1972; Falke et al. 1985), which prevents gas exchange with the circulatory system (Kooyman et al. 1970). Like other adult phocids, adult gray seals have high mass-specific Hb, Hct, BV, and Mb levels (Reed et al. 1994*a*, 1994*b*; Table 1) and similar contributions to total

Table 1: Blood and muscle properties of gray seal age classes

Class (age)	Hb (g dL^{-1})	Hct (%)	MCHC (g dL^{-1})	PV (mL kg^{-1})	BV (mL kg^{-1})	Mb (g 100 g $muscle^{-1}$)
Newborn (3 DPP)	19.9 ± .3[a]	45.4 ± .6[a]	43.9 ± .5	86.6 ± 8.9[b]	159.0 ± 17.3[b]	1.7 ± .1[b,c]
0 DPW (17 ± .4 pp)	18.7 ± .5[d,e]	42.8 ± 1.6[d,e]	44.2 ± 1.8	65.8 ± 1.8	115.7 ± 4.1[e]	2.1 ± .3
12 DPW (29 ± .5 DPP)	21.7 ± .6[e,f]	49.8 ± 1.5[f]	43.7 ± .5	60.6 ± 1.8	121.8 ± 4.9	2.5 ± .3
24 DPW (41 ± .5 DPP)	24.0 ± .5[b,d,f,g]	51.8 ± 1.3[b,f,g]	46.5 ± .9	63.2 ± 1.7[b,c]	131.2 ± 2.6[b,c,f]	2.7 ± .3[b]
Yearling (1 yr)	23.0 ± .9[b]	50.4 ± 1.9	45.8 ± .7	99.3 ± 3.2[a]	201.5 ± 7.5[a]	3.2 ± .3[g]
Adult (>6 yr)	19.6 ± 1.5[a,c]	44.9 ± 2.1[a]	43.1 ± 1.8	116.6 ± 9.5[a,g]	213.2 ± 17.2[a,g]	4.0 ± .3[a,g]

Note. Values are mean ± SE for hemoglobin (Hb), hematocrit (Hct), mean corpuscular hemoglobin content (MCHC), mass-specific plasma volume (PV), mass-specific blood volume (BV), and muscle myoglobin (Mb) content. Ten seals were sampled in each age class, except for newborn Mb ($n = 9$) and 0 d postweaning (DPW) Mb ($n = 7$). DPP = days postpartum. Significant differences across postweaning (PW) longitudinal sample points (repeated-measures ANOVA on PW longitudinal samples: 0, 12, and 24 DPW) were found for Hb ($F_{2,29} = 31.505$, $P < 0.001$), Hct ($F_{2,29} = 8.192$, $P = 0.003$), and mass-specific BV ($F_{2,29} = 5.965$, $P = 0.010$). Mass-specific PV ($F_{2,29} = 3.274$, $P = 0.061$) and MCHC ($F_{2,29} = 1.716$, $P = 0.208$) values did not differ significantly across longitudinal sample points during the fast. Although the mean value for Mb appeared to increase throughout the PW period, the differences across sample points were not significant ($F_{2,26} = 0.764$, $P = 0.483$). Significant differences across population age classes (ANOVA on cross-sectional population samples: newborn, 24-DPW pup, yearling, and adult) were found for Hb ($F_{3,39} = 6.044$, $P = 0.002$), Hct ($F_{3,39} = 4.769$, $P = 0.007$), mass-specific PV ($F_{3,39} = 11.111$, $P < 0.001$), mass-specific BV ($F_{3,39} = 8.773$, $P < 0.001$), and Mb ($F_{3,38} = 11.477$, $P < 0.001$). MCHC ($F_{3,39} = 2.041$, $P = 0.125$) values did not differ significantly across population age classes.

[a] Values different than 24 DPW (Tukey all pairwise comparison across cross-sectional population samples at $P < 0.05$).

[b] Values different than adult (Tukey all pairwise comparison across cross-sectional population samples at $P < 0.05$).

[c] Values different than yearling (Tukey all pairwise comparison across cross-sectional population samples at $P < 0.05$).

[d] Values different than 12 DPW (Tukey all pairwise comparison across postweaning longitudinal samples at $P < 0.05$).

[e] Values different than 24 DPW (Tukey all pairwise comparison across postweaning longitudinal samples at $P < 0.05$).

[f] Values different than 0 DPW (Tukey all pairwise comparison across postweaning longitudinal samples at $P < 0.05$).

[g] Values different than newborn (Tukey all pairwise comparison across cross-sectional population samples at $P < 0.05$).

oxygen stores from blood (66%), muscle (27%), and lung (7%; Table 2). These large oxygen stores correspond with extended periods of time at sea, characterized by continuous diving and a high percentage of time spent submerged (Reed et al. 1994*a*; Beck et al. 2003). Adult female gray seals dive along the bottom of the ocean and are capable of reaching depths greater than 250 m for maximum durations of 22 min (Beck et al. 2003).

In contrast to adult values, gray seal pups had relatively underdeveloped oxygen stores in the blood and muscle both at birth (3 DPP) and at weaning (at about 17 DPP; Table 1). This resulted in low total oxygen stores (Table 2). With such a brief nursing period, the pups were still very young at weaning and thus perhaps necessitate a PWF to develop the physiology that supports diving. The total mass-specific oxygen stores of the pups increased by 35% after 24 d of the PWF (at about 41 DPP) and were 66% of those in yearlings. At the end of the PWF, gray seal pups at Sable Island depart to sea to forage for the first time at approximately 40 DPP (S. R. Noren, unpublished observation). Although the dive capacity of the pups increased during the PWF, it was still limited compared to that of yearlings and adults.

These results are consistent with other immature phocids studied to date, which have low mass-specific BV and Mb levels at the onset of independent foraging (Thorson 1993; Thorson and Le Boeuf 1994; Burns et al. 2000; Clark 2004; this study). Yet there is undoubtedly a minimum level of oxygen stores required at independence to ensure that immature phocids are competent predators. Consequently phocids with short postpartum terrestrial periods (nursing and PWF periods combined) should obtain enhanced mass-specific levels of Hb, BV,

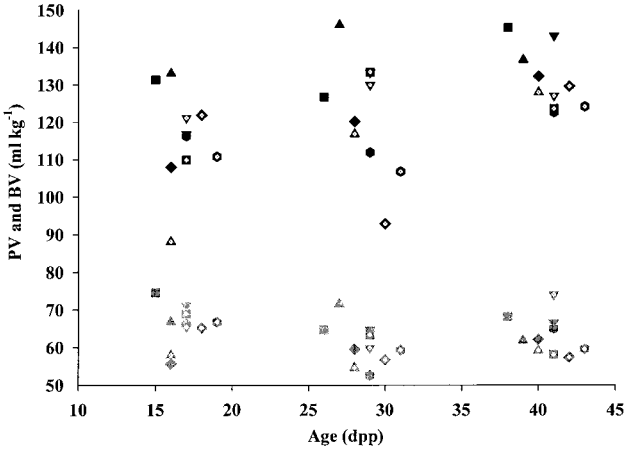

Figure 2. Mass-specific plasma volume (PV) and blood volume (BV) of gray seal pups during the postweaning fast (PWF) plotted as days postpartum (DPP). Each unique symbol represents an individual pup that was studied longitudinally during the PWF. Mass-specific PV (*gray symbols*) did not change ($F_{2,29} = 3.274$, $P = 0.061$), while mass-specific BV (*black symbols*) increased significantly during the PWF ($F_{2,29} = 5.965$, $P = 0.010$; repeated-measures ANOVA).

and Mb earlier in life than those with longer postpartum terrestrial periods. For instance, northern elephant seal pups have low mass-specific Hb, BV, and Mb at birth and throughout their 28-d nursing period (Thorson 1993; Thorson and Le Boeuf 1994). After weaning, elephant seal pups experience a terrestrial PWF period (Le Boeuf et al. 1972), which lasts on average for 65 d (Noren et al. 2003). During this time, Hb, BV, and Mb levels increase with a concomitant rise in total mass-specific oxygen stores (Thorson 1993; Thorson and Le Boeuf 1994). At the end of the PWF, 90-d-old elephant seal pups have mature Hb levels and 68% and 76% of adult mass-specific BV and Mb, respectively (Thorson 1993; Thorson and Le Boeuf 1994). In comparison, gray seal pups at Sable Island are less than half the age of elephant seal pups at the onset of independent foraging (40 DPP on average; S. R. Noren, unpublished observation). At this stage, gray seal pups have mature Hb levels and 62% and 68% of adult mass-specific BV and Mb, respectively (Table 1). Furthermore, at the time of departure, both northern elephant and gray seal pups exhibit a similar percentage of the total mass-specific oxygen store found in their adult female counterpart, 73% (Thorson 1993; Thorson and Le Boeuf 1994) and 67% (Table 2), respectively. Interestingly, bottlenose dolphins and Steller sea lions, which both have protracted nursing periods of >1 yr, demonstrate a similar level of physiological development at weaning, approximately 81% (calculated from Noren et al. 2002) and 83% (Richmond 2004) of adult total mass-specific oxygen stores, respectively. The convergence of obtaining greater than half of adult mass-specific oxygen storage capacity across phylogenetically diverse species at the onset of independence at sea, regardless of days postpartum, suggests that this oxygen store level may have an adaptive advantage for preliminary diving and hence foraging activity. Nevertheless, the reduced oxygen stores of immature marine mammals invariably set the limits to their diving behavior and probably shape their prey selection.

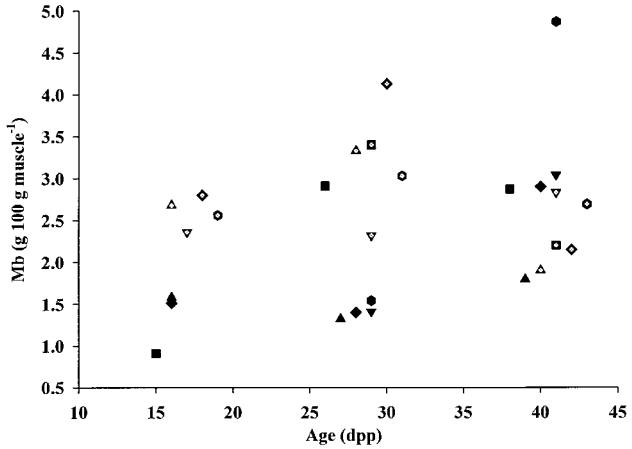

Figure 3. Myoglobin content (Mb) of the *longissimus dorsi* of gray seals during the postweaning fast (PWF) plotted as days postpartum (DPP). Each unique symbol represents an individual pup that was studied longitudinally during the PWF. Although Mb appeared to increase during the PWF, the differences across sample points were not significant ($F_{2,26} = 0.764$, $P = 0.483$; repeated-measures ANOVA).

CADLs provide an estimation of how physiological development influences the diving capabilities for individuals. The increase in oxygen storage capacity from birth to the end of the PWF in gray seals was associated with a 27% increase in CADL. Although this stage of development corresponds with the pups' first foraging trip at sea, continued physiological development and growth promote an additional 50% and 61% increase in total oxygen stores and CADL, respectively, during the pups' first year at sea (Table 2). However, while the majority of oxygen store development was complete by the end of the first year of life (i.e., similar to that of adults), yearlings still had significantly lower CADLs than adult females. Adult females had a 95% greater CADL than yearlings (Table 2). This change in CADL is attributed to the attainment of a mature metabolic rate after the first year of life (Boily and Lavigne 1997) and an increase in body size, with the associated relative decrease in mass-specific oxygen utilization rates (Kooyman et al. 1983).

The dramatic differences between the CADLs of weaned pups heading out to sea, yearlings, and adults suggest that actual dive times should differ across these segments of the population. The CADL (11.9 ± 0.9 min) determined for adult female gray seals in this study and in a previous study (9.6 min; Reed et al. 1994*a*) are consistent with the diving behavior of free-ranging adult gray seals, where 90% of dives were less than 8 min (Beck et al. 2003). Although there are no published accounts of the diving behavior of free-ranging immature gray seals for comparison, direct measurements of the diving behavior of other juvenile phocid seals (northern elephant: Thorson 1993; Thorson and Le Boeuf 1994; ringed [*Phoca hispida*]: Lydersen and Hamill 1993; bearded [*Erignathus barbatus*]: Lydersen et al. 1994; harbor: Bowen et al. 1999; and Weddell [*Leptonychotes weddellii*]: Burns 1999) confirm that juvenile phocids have shorter dive durations, dive to shallower depths, and dive for a lower percentage of time compared to adults. Differences in diving ability are also probably associated with interage class variation in diet. Hence, prey choice may serve as an indication of the reduced diving capability of juvenile gray seals. Gray seals less than a year old demonstrate a greater reliance on silver hake and consume less deep-dwelling squid than that demonstrated for gray seals 1 yr old or older (Bowen et al. 1993).

In summary, the results of our study confirm that across phylogenetically diverse groups of marine mammals, postnatal development is required to attain mature mass-specific Hb, Hct, BV, and Mb levels. Like previous studies (Thorson 1993; Thorson and Le Boeuf 1994; Noren 2002; Clark 2004; Richmond 2004), our study demonstrates that the development of Mb is protracted in comparison to the development of Hb. Further-

Table 2: Body mass, estimated mass-specific oxygen stores, and calculated aerobic dive limits (CADL) of gray seal age classes

Class (age)	Mass (kg)	Blood O_2 (mL kg^{-1})	Muscle O_2 (mL kg^{-1})	Total O_2 (mL kg^{-1})	CADL (min)
Newborn (3 DPP)	21.3 ± .8	30.8 ± 3.6[a] (76 ± 2%)	4.2 ± .1[a,b] (12 ± 1%)	38.1 ± 3.7[a,b]	3.0 ± .3[a,b]
0 DPW (17 ± .4 DPP)	52.8 ± 1.4	21.0 ± .9[c,d] (70 ± 2%)	5.1 ± .7 (17 ± 2%)	30.7 ± 1.1[c,d]	3.1 ± .1[d]
12 DPW (29 ± .5 DPP)	44.4 ± 1.3	25.8 ± 1.5[d,e] (72 ± 3%)	6.1 ± .8 (17 ± 2%)	36.1 ± 1.2[d,e]	3.4 ± .1[d]
24 DPW (41 ± .5 DPP)	40.1 ± 1.3	30.6 ± .9[a,c,e] (74 ± 2%)	6.8 ± .7[a,b] (16 ± 2%)	41.4 ± .8[a,b,c,e]	3.8 ± .1[a,b,c,e]
Yearling (1 yr)	51.6 ± 2.7	45.3 ± 2.6[f,g] (72 ± 2%)	12.9 ± 1.3[f,g] (21 ± 2%)	62.3 ± 3.2[f,g]	6.1 ± .3[b,f,g]
Adult (>6 yr)	191.5 ± 6.0	41.5 ± 4.5 (66 ± 4%)	15.9 ± 1.3[f,g] (27 ± 4%)	61.4 ± 4.6[f,g]	11.9 ± .9[a,f,g]

Note. Values are mean ± SE. Calculations were based on 10 seals in each age class, except for newborn muscle O_2, total O_2, and CADL ($n = 9$), and for 0-d postweaning (DPW) muscle O_2, total O_2, and CADL ($n = 7$). DPP = days postpartum. Lung O_2 was assumed to represent a constant proportion of body mass (at 4.1 mL kg^{-1}; see text) and was added to blood and muscle O_2 stores to compute total O_2 and percentage contributions (shown in parentheses). Significant differences across postweaning (PW) longitudinal sample points (repeated-measures ANOVA on PW longitudinal samples: 0, 12, and 24 DPW) were found for mass-specific oxygen stores in the blood ($F_{2,29} = 20.998$, $P < 0.001$) and in total ($F_{2,26} = 19.816$, $P < 0.001$) and for CADL ($F_{2,26} = 11.292$, $P = 0.001$). Although the mean value for mass-specific oxygen stores in the muscle appeared to increase throughout the PW period, the differences across sample points were not significant ($F_{2,26} = 0.763$, $P = 0.483$). Significant differences across population age classes (ANOVA on cross-sectional population samples: newborn, 24-DPW pup, yearling, and adult) were found for mass-specific oxygen stores in the blood ($F_{3,39} = 5.594$, $P = 0.003$), muscle ($F_{3,38} = 28.301$, $P < 0.001$), and in total ($F_{3,38} = 14.525$, $P < 0.001$) and for CADL ($F_{3,38} = 63.539$, $P < 0.001$).

[a] Values different than yearling (Tukey all pairwise comparison across cross-sectional population samples at $P < 0.05$).

[b] Values different than adult (Tukey all pairwise comparison across cross-sectional population samples at $P < 0.05$).

[c] Values different than 12 DPW (Tukey all pairwise comparison across postweaning longitudinal samples at $P < 0.05$).

[d] Values different than 24 DPW (Tukey all pairwise comparison across postweaning longitudinal samples at $P < 0.05$).

[e] Values different than 0 DPW (Tukey all pairwise comparison across postweaning longitudinal samples at $P < 0.05$).

[f] Values different than newborn (Tukey all pairwise comparison across cross-sectional population samples at $P < 0.05$).

[g] Values different than 24 DPW (Tukey all pairwise comparison across cross-sectional population samples at $P < 0.05$).

more, we provide evidence that the terrestrial PWF of phocid seals represents an important physiological development period. Despite differences in nursing and PWF durations, immature marine mammals appear to have converged onto developing a similar proportion of adult oxygen stores at the onset of independence at sea. This indicates that the rate of oxygen store development matches the rate at which independence is attained, and it suggests that there may be a critical level of development for successful diving and, by inference, foraging. Regardless, limited mass-specific oxygen reserves in combination with small body size constrain the diving capacity of juvenile marine mammals. Differences in diving capabilities throughout maturation may result in partitioning of prey resources, perhaps minimizing competition between age classes. To the extent that diving capacity limits access to prey, the ontogenetic changes described in this study may explain observed losses in juvenile pinnipeds during periods of limited prey availability (DeLong et al. 1991).

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