Development of Body Oxygen Stores in Harbor Seals: Effects of Age, Mass, and Body Composition

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ABSTRACT

Harbor seal pups are highly precocial and can swim and dive at birth. Such behavioral maturity suggests that they may be born with mature body oxygen stores or that stores develop quickly during the nursing period. To test this hypothesis, we compared the blood and muscle oxygen stores of harbor seal pups, yearlings, and adults. We found that pups had smaller oxygen stores than adults (neonates 57%, weaned pups 75%, and yearlings 90% those of adults), largely because neonatal myoglobin concentrations were low (1.6 ± 0.2 g% vs. 3.8 ± 0.3 g% for adults) and changed little during the nursing period. In contrast, blood oxygen stores were relatively mature, with nursing pups having hematocrit (55% ± 0.2%), hemoglobin (21.7 ± 0.4 g%), and blood volume (12.3 ± 0.5 mL/kg) only slightly lower than the corresponding values for adults (57% ± 0.2%, 23.8 ± 0.3 g %, and 15.0 ± 0.5 mL/kg). Because neonatal pups had relatively high metabolic rates (11.0 mL O_2/kg min), their calculated aerobic dive limit was less than 50% that of adults. These results suggest that harbor seals' early aquatic activity is primarily supported by rapid development of blood, with immature muscle oxygen stores and elevated use rates limiting aerobic diving ability.

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Introduction

A fundamental question in physiological ecology is when physiological capacity constrains behavior and life-history patterns. Classic research in this field addresses questions concerning how species are adapted to their environment and how the observed adaptations have evolved. Comparative studies have indicated how close relatives have evolved to meet localized conditions and how different levels of adaptation result in different behavioral options (Prosser 1989; Feder and Block 1991; Mangum and Hochachka 1998). In the marine ecosystem, the ability of air-breathing vertebrates to make long and deep dives is an excellent example of the degree to which animals can adapt in the face of ecological constraints, and the study of the physiological modifications that advance diving ability has provided insights into fundamental questions about organ physiology, metabolic regulation, and life-history traits (Kooyman 1989; Castellini 1991; Butler and Jones 1997; Costa and Sinervo 2004).

During the past several decades, seminal laboratory research on the physiological adaptations of marine mammals has revealed that the ability of seals to make long and deep dives is contingent on having large reserves of oxygen and the ability to markedly reduce whole-body and organ metabolic rates (Kooyman 1989; Castellini 1991; Schreer and Kovacs 1997). Subsequent field studies have demonstrated that the diving behavior of adults is constrained by the size of their oxygen reserves and by their diving metabolic rate (DMR). The ratio of these two values, the aerobic dive limit (ADL), is the maximum dive duration before the balance of metabolism becomes anaerobic (Kooyman et al. 1980, 1983). In the absence of postdive lactate measurements, ADL is calculated as the ratio of total body oxygen stores to DMR. The calculated ADL can be used to effectively describe the routine diving behavior of adults (Kooyman 1989; Boyd and Croxall 1996; Butler and Jones 1997; Schreer and Kovacs 1997), and the physiological parameters that influence ADL can be directly correlated with species-specific life-history traits (Gentry and Kooyman 1986; Costa 1993; Costa and Gales 2003). Within this context, marine mammals are a model system for the study of physiological ecology; not only do physiological differences among species exist, but also these adaptations can and do limit the range of possible behaviors.

Until recently, our understanding of the physiological adaptations possessed by diving mammals was based largely on work on adults, and we lacked knowledge about the ontogeny

of diving physiology or its effect on behavior. Whereas studies on neonatal pinniped metabolic rates had indicated that pups, like most terrestrial mammalian young, had disproportionately high metabolic rates and had limited control of heart rate and other metabolic processes (Elsner et al. 1977; Rea and Costa 1992; Blackwell and Le Boeuf 1993; Castellini et al. 1994; Falabella et al. 1999), the implications of this physiological limitation on the diving ability of pups were not considered until more recently (Thorson and Le Boeuf 1994; Burns and Castellini 1996; Horning and Trillmich 1997). Over the past decade, however, studies of juvenile diving behavior and physiology have indicated that all pups are fairly inept divers at birth and that the rate at which pups mature physiologically and behaviorally is closely tied to the length of the dependent period, here defined as the time from birth to independent foraging (Thorson and Le Boeuf 1994; Horning and Trillmich 1997; Merrick and Loughlin 1997). By extension, these findings suggest that both the rate and extent of neonatal physiological development is under selection; pups must become effective marine predators within a few weeks of nutritional independence (at weaning or the end of the postweaning fast) or face death through starvation.

Of all the pinnipeds, harbor seals offer a unique opportunity to study the relationship between rates of physiological change and behavioral strategies. Harbor seal pups are some of the most precocial of all phocids. Pups can swim and dive at birth (Jorgensen et al. 2001; Greaves et al. 2005) and are one of only two species that are born with a subcutaneous blubber layer and adult pelage (Bigg 1981; Oftedal et al. 1991). In combination with the relatively short lactation period (~21–28 d; Lawson and Renouf 1987; Muelbert and Bowen 1993), such behavioral maturity suggests that harbor seal pups may be born with mature body oxygen stores or that stores develop quickly during the nursing period. Alternatively, if harbor seal pups are born with immature oxygen stores or these stores do not develop rapidly during the nursing period, it might suggest that there are limits to the rate at which these processes can mature. Since the identification of such fundamental physiological limits often provides insights into the interaction between physiology, behavior, and life-history patterns (Prosser 1989; Costa 1993; Mangum and Hochachka 1998), this study is focused on understanding the pattern of development of blood and tissue oxygen stores in young harbor seals as it relates to the ontogeny of diving capacity.

Material and Methods

Animal Capture and Handling

Harbor seals were captured throughout the year in Monterey Bay, California, from September 1997 through June 2000 ($n = 278$), and in Prince William Sound, Alaska, from June 24 to July 1 in 1998 and 1999 ($n = 117$). The two sites were chosen to complement each other, with a large number of animals over a limited age range captured in Alaska and a smaller number of animals over a broader range of ages captured in California. Capture logistics also limited the types of data that could be obtained from each group of animals. In both states, seals were live-captured near haul-outs by net entanglement as previously described (Jeffries 1986; Frost et al. 1995). Because individual animals were not targeted, we assume that animals captured were representative of the population. After removal from the capture net, seals were placed in individual nets and placed in a quiet location until handling. All seals were weighed (± 0.1 kg) with a hanging electronic load cell (O'Haus), measured (standard length, axial girth), sexed, and tagged (Dalton Allflex tag) for later identification. Animals either were manually restrained or were chemically immobilized by intravenous injection of diazepam (0.2–0.3 mg/kg; Abbott Laboratories, Chicago, IL). Blood samples were collected from the extradural vein into Vacutainer collection tubes and placed on ice (<6 h) or in −80°C storage until analyzed.

The ages of adult and yearling harbor seals were determined based on their size (standard length and mass) and pelage condition. Pups were aged based on appearance, body mass, time of year, and whether they had milk in their stomachs. During the pupping season (April–May in California) pups were classified as neonates (estimated <7 d old) if they weighed less than 15 kg and appeared thin for their body length (Dubé et al. 2003). Pups between 15 and 22 kg, those that had milk in their stomachs, and those that were seen with an attending female before or after capture were classified as nursing pups. Large (>22 kg) pups that did not have milk in their stomachs and were not seen with attending females were classified as weaned. In Alaska, all captures were performed during a 1-wk period at the end of June (June 24–July 1) when most pups were near weaning or weaned (Frost et al., forthcoming). However, those pups that had milk in their stomachs or were less than 22 kg were classified as nursing ($n = 6$). No pups larger than 25 kg had any milk in their stomachs.

A subset of animals handled in California ($n = 63$) and Alaska ($n = 58$) had their body compositions determined by deuterium-oxide dilution. Following collection of a presample, animals were intubated with deuterium oxide (Cambridge Isotopes, 99.9%, 0.5 g/kg), and two postequilibration blood samples were collected into red-top Vacutainers at 2 and 2.5 h after injection (Bowen and Iverson 1998). Serum samples from centrifuged vials were frozen at −20°C. Pure water was recovered from serum samples by vacuum distillation, and samples were analyzed by infrared spectrophotometry at Dalhousie University, Canada (Oftedal and Iverson 1987). Total body water (TBW) was determined from hydrogen-dilution space using equation (5) from Bowen and Iverson (1998). Total body fat and lean body mass percentages were determined from TBW using equations developed for gray seals (Reilly and Fedak 1990).

Hematology

An initial blood sample was collected into green-top (lyophilized lithium heparin) and purple-top (liquid EDTA) Vacutainers. Within 6 h of sample collection, hematocrit (HCT; percentage of red blood cells by volume) was determined from the green-top Vacutainers by centrifugation, and hemoglobin (Hb; g/100 mL blood) was determined by the cyanomethemoglobin method (Sigma Kit 625A). Mean corpuscular hemoglobin content (MCHC) was then determined as (100 × Hb)/HCT. A second purple-top Vacutainer of blood was collected from a subset of animals handled in California, and these samples were kept refrigerated until hematological analysis by a veterinary laboratory (1998, Meris Laboratories; 1999–2000, IDEXX Veterinary Services). Complete red blood cell (RBC) counts (1,000/μL blood) were conducted within 48 h of sample collection using Coulter Counter methods. To control for artifacts introduced into mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) measures by the optical counting method of Coulter-type laboratory equipment (Castellini et al. 1996; Fadely 1997; Bossart et al. 2001), MCV and MCH were calculated from combinations of field-determined HCT and Hb and the laboratory-determined RBC counts following Kerr (1989). Because RBC counts were not determined for samples collected in Alaska, no information on MCH or MCV is available for these animals. In addition, because hematological parameters are known to vary with respect to season (Fadely 1997), comparisons among age classes were made using only those samples collected during the pupping and breeding season (in California, April 1–July 1; in Alaska, June 20–July 4).

Blood and Muscle Development

Plasma volume (PV) was determined using the Evans blue methodology (International Committee for Standardization in Haematology 1973). Briefly, a preweighed dose of Evans blue dye (~0.5 mg/kg) was injected into the extradural vein. Three to six serial blood samples were then collected during the subsequent 30-min period. All samples were collected directly into green-top Vacutainers, well mixed, and placed on ice until processing (3–6 h later). HCT values were determined for each sample, and then tubes were centrifuged and the plasma separated. Separated plasma was stored frozen for later analysis. Before spectroscopic analysis, samples were thawed, mixed, and recentrifuged to remove any artifacts of freezing. The optical density of the plasma samples resulting from Evans blue dye was determined at 624 and 740 nm following El-Sayed et al. (1995) with modifications by Foldager and Blomqvist (1991). Standard curves were determined using harbor seal plasma. All concentration measures were log transformed and the concentration at injection determined by linear extrapolation. PV was calculated only if all the injectate was delivered intravenously and there was a linear change in concentration of dye. Blood volume (BV) was determined by dividing the estimated PV by $1 - \mathrm{HCT}_{max}$. Both BV and PV are reported on a mass-specific and lean-mass-specific basis.

Muscle samples (~0.2 g) were collected by biopsy from the *longissimus dorsi* of sedated harbor seals using either a 6-mm dermal-biopsy punch (Miltex) or a 6-mm Bergrström-Stille muscle-biopsy cannula. Samples were placed into a liquid nitrogen dewar or onto ice in the field and were then frozen at −80°C until analysis. Myoglobin content was determined following Reynafarje (1963) as modified by Castellini and Somero (1981). Buffer blanks and elephant seal muscle tissue of known myoglobin concentration were used as assay controls.

Total Body Oxygen Stores

Total body oxygen stores were determined by adding the stores in lung, muscle, and blood (Lenfant et al. 1970; Kooyman et al. 1983). Muscle oxygen stores were estimated by multiplying the measured myoglobin content by the oxygen-binding capacity of myoglobin (1.34 mL O_2/g) and the total muscle mass. Muscle mass was assumed to be 19.2% for pups and 28.8% for adults, and lung oxygen stores were estimated as 5.3 mL O_2/kg for pups and 12.2 mL O_2/kg for adults. These values are based on our measurements of lung volume and muscle mass for 11 pup and nine adult hooded seals (*Cystophora cristata*; Burns et al. 2000). The adult value of 28.8% muscle is similar to that commonly used in measurements of total body oxygen stores (30%; Kooyman et al. 1983). The value used for pups, 19.2%, is similar to our single direct measurement for a weaned harbor seal pup (19.1%) and for four young harp seal pups (18.4%). Blood oxygen stores were determined using the individually measured HCT_{field}, Hb_{field}, and BV. We assumed that one-third of the BV was arterial and that 75% of the oxygen in the arterial system was available, with an additional 5% of the oxygen available through the venous system (Ponganis et al. 1993). Total body oxygen stores were calculated for all animals for which there were muscle and blood oxygen-store measurements. In addition, average total body oxygen stores were calculated for each age class based on the average BV, HCT, Hb, and muscle myoglobin content for that age class.

Metabolic Rate and Aerobic Dive Capacity

The metabolic rate of neonatal harbor seal pups housed at the Marine Mammal Center (Sausalito, CA) was determined in air using indirect flow-through calorimetry. These pups were brought into the center after being abandoned by their mothers for unknown reasons. Only pups that were judged to be healthy by the on-site veterinary staff were selected for use, and all trials occurred within 1 wk of entry to the center. The metabolic chamber consisted of an opaque plastic tub with a lid sealed with a foam-rubber gasket. The lid had a central Plexiglas window through which the seal was observed at all times. Ambient

Table 1: Mean mass and body fat percentage of harbor seals captured in California and Alaska from 1997 to 2000

	Mass (kg)		Body Fat Percentage	
Age Class	California	Alaska	California	Alaska
Neonates	$10.3 \pm .4^{a}$ (6, 9)	…	15.4 ± 2.7^{a} (2, 3)	…
Nursing pups	$17.7 \pm .6^{b*}$ (18, 14)	24.9 ± 1.5^{a} (5, 1)	31.3 ± 2.1^{bc} (9, 4)	35.3 ± 1.7^{a} (4, 0)
Weaned pups	$24.1 \pm .4^{c*}$ (26, 11)	$28.9 \pm .5^{a}$ (14, 15)	36.6 ± 2.0^{bc} (4, 2)	40.0 ± 1.3^{a} (12, 13)
Yearlings	32.0 ± 1.5^{d} (9, 13)	$33.1 \pm .5^{b}$ (17, 13)	$20.8 \pm 2.5^{ab*}$ (3, 2)	$25.9 \pm .9^{b}$ (13, 8)
Adults	$69.6 \pm 5.7^{e*}$ (95, 77)	52.1 ± 1.6^{c} (25, 27)	29.4 ± 2.6^{abc} (2, 0)	$24.6 \pm .9^{b}$ (5, 8)

Note. Data are ±SE, with sample sizes (females, males) given in parentheses. Mass and composition did not vary significantly with sex. Similar alphabetical superscripts indicate similar mass or body composition across age classes within each region.

* Significant difference between regions within each age class, as judged by Bonferroni post hoc comparisons.

air was drawn through the chambers using a modified vacuum cleaner as an air pump, and flow rates (approximately 40 L/min) were monitored with an in-line dry gas meter (DTM-325, American Meter). Flow rate, chamber temperature, relative humidity, and ambient pressure were recorded every 10 min. Water and carbon dioxide were removed from a subsample of excurrent air after passage through the flow meter using an alternating series of Baralyme and Drierite tubes. Oxygen concentration was determined by an Ametek S-3A oxygen analyzer that was directly linked to a personal computer. Oxygen consumption data were logged and metabolic rates calculated using equation (4b) from Withers (1977) and respirometry software (Datacan V, Sable Systems, Salt Lake City, UT). The respiratory quotient used was 0.76 (19.3 kJ/L O_2), which assumes a 50 : 50 lipid : protein fuel source (Schmidt-Nielsen 1990), as suggested by the diet of fish and milk replacement fed to pups at the center. The system was calibrated daily using nitrogen gas (Fedak et al. 1981).

Pups were placed within the chamber and allowed to acclimatize for 30 min before the 1-h recording period. Pups were observed at 10-min intervals and their activity scored as asleep, quiescent (awake but calm), active (awake, moving calmly), or agitated. In-air resting metabolic rates were determined for only those 10-min periods when pups were quiescent, and only the lowest oxygen consumption values are reported here. All metabolic rate measurements were determined in air within the animal's thermoneutral zone (Rosen and Renouf 1997). The ADL of harbor seals was calculated by dividing total body oxygen stores by resting metabolic rates. Because we measured metabolic rates only for neonatal pups, all other values were taken from the literature.

Statistical Analyses

The primary goal of this study was to characterize the impact of animal age on various hematological parameters and body oxygen stores. However, it was also necessary to test for differences resulting from collection year, sample location (California or Alaska), and sex. These comparisons were conducted using a general linear model (GLM in SPSS v. 12.0) to test for all effects simultaneously. When significant regional differences were identified in the initial test, the two regions were separated for all subsequent analyses. Before all analyses, data normality was assessed using probability plots, and data were log transformed as necessary to achieve normality. Significance was assumed at $P < 0.05$ in the GLM models, and Bonferroni post hoc comparisons were used to identify age groups that differed significantly. Data are presented as mean ± 1 SE unless otherwise noted.

Results

Animal Morphology

Over the course of this study, 395 harbor seals were captured, sexed, weighed, and sampled. There were significant differences in mass by region ($F_{1,388} = 5.332$, $P = 0.020$), so regions were separated for subsequent analysis. As expected, within each region, mass increased with age (California: $F_{4,268} = 354.24$, $P < 0.00$; Alaska: $F_{3,109} = 79.80$, $P < 0.00$) but did not vary significantly with sex or sampling year (Table 1). There were significant differences across age classes between the states, with Alaskan pups larger than those from California (nursing: $F_{1,37} = 20.038$, $P < 0.000$; weaned: $F_{1,65} = 58.333$, $P < 0.000$) and adults smaller ($F_{1,222} = 42.336$, $P < 0.00$). There were no differences in the average size of yearlings. In contrast, only yearlings differed in body composition by state, with yearlings from Alaska having larger fat stores than those from California ($F_{1,25} = 5.07$, $P = 0.034$). There were significant differences in body composition with age (California: $F_{4,30} = 10.00$, $P < 0.001$; Alaska: $F_{3,62} = 39.946$, $P < 0.001$), and in general, fat percentage increased during the nursing period and then declined to values seen in adults and yearlings (Table 1). There were no differences in body composition resulting from sex or collection year. Because not all parameters were measured on all animals, sample sizes are reported separately with each analysis.

Hematology

Neither sample collection year nor sex had a significant impact on any hematological parameter measured. There were significant regional differences in Hb ($F_{4,213} = 7.310$, $P < 0.001$) and MCHC ($F_{4,213} = 7.310$, $P < 0.001$) but not in HCT ($F_{4,213} = 7.310$, $P < 0.001$). Because these three parameters are related, all subsequent analyses were separated by region. In California, there were age-related differences in HCT ($F_{4,109} = 2.520$, $P = 0.046$) and MCHC ($F_{4,101} = 4.499$, $P = 0.002$) but not in Hb ($F_{4,101} = 2.129$, $P = 0.083$). In contrast, all three parameters varied with age in Alaska (HCT: $F_{3,110} = 6.585$, $P < 0.001$; Hb: $F_{3,111} = 6.522$, $P < 0.001$; MCHC: $F_{3,109} = 4.726$, $P = 0.004$). In general, HCT and Hb were high in newborns, lower during the nursing period, and increasing toward weaning and adulthood, while MCHC increased uniformly with age. The results from all post hoc comparisons are shown in Table 2. There were age-related changes in red-cell characteristics for animals captured in California. While RBC counts did not vary with age, sex, or collection year, MCV and MCH increased with age (MCV: $F_{4,47} = 2.944$, $P = 0.028$; MCH: $F_{4,47} = 8.307$, $P < 0.001$). Post hoc tests revealed that adult and yearling harbor seals had significantly higher MCV and MCH values than did pups of all ages (Table 2).

Blood and Muscle Development

PV and BV were determined for 130 animals, of which 81 also had body composition determined. To determine what factors most influenced PV and BV, we examined fluid volumes on both a mass-specific and lean-body-mass-specific basis with respect to age, sampling location, collection year, and sex. Because there were no differences by location, sex, or collection year, samples from both regions were combined. Age was the only variable that had a significant effect on mass-specific PV or BV (PV: $F_{4,124} = 9.296$, $P < 0.001$; BV: $F_{4,124} = 13.318$, $P < 0.001$). Both fluid volumes were elevated in the youngest pups (PV: 8.8 ± 1.0 mL/kg; BV: 20.3 ± 1.9 mL/kg), declined in weaned pups (PV: 5.3 ± 0.2 mL/kg; BV: 12.3 ± 0.3 mL/kg), and then increased to adult values in yearlings and adults (PV: 6.3 ± 0.2 mL/kg; BV: 15.1 ± 0.7 mL/kg; Fig. 1). A similar pattern of age-related change was evident when PV and BV were scaled to lean body mass (PV: $F_{4,72} = 3.933$, $P = 0.006$; BV: $F_{4,72} = 4.149$, $P = 0.004$), although the magnitude of the age-related differences was smaller for BV than for PV (Fig. 1).

In keeping with these age-related trends, mass-specific total blood oxygen stores increased with age ($F_{4,95} = 10.189$, $P < 0.001$) but not with collection year, location, or sex (Table 3). Because blood oxygen stores integrate BV and Hb, the trend was similar to that seen with respect to fluid volumes: while adults and yearlings had greater mass-specific oxygen stores than nursing and weaned pups, neonates had the greatest values of all (Table 3). In contrast, blood oxygen stores varied only slightly with age on a lean-body-mass-specific basis ($F_{1,67} = 3.405$, $P = 0.014$), with post hoc tests revealing that the only significant difference was between yearlings and adults (53.6 ± 1.6 mL/kg lean tissue) and early nursing pups (43.4 ± 3.1 mL/kg lean tissue).

There were no differences in myoglobin content of the *lon-*

Table 2: Hematology measures for harbor seals captured in California and Alaska during the pupping period

Age Class	*N*	HCT (%)	Hb (g/100 mL)	MCHC (g/100 mL)	RBC Count (10^6/mm^3)	MCV (fL)	MCH (pg)
Neonates:							
California	10 (8)	57.7 ± 1.5	22.6 ± .7	39.2 ± .5^{123}	5.53 ± .20	103.4 ± 3.1	40.5 ± 1.6^{ab}
Alaska	…	…	…	…	…	…	…
Nursing pups:							
California	20 (10)	54.9 ± .9	21.7 ± .4	39.4 ± .3^{12}*	5.25 ± .15	105.5 ± 3.3	41.4 ± 1.5^{abc}
Alaska	6	54.3 ± .6^{abc}	22.4 ± .4^{a}	41.3 ± .8^{abc}	…	…	…
Weaned pups:							
California	22 (8)	56.0 ± .7	22.1 ± .4*	39.5 ± .5^{12}*	5.23 ± .10	106.8 ± 3.0	41.2 ± .8^{abc}
Alaska	29	57.4 ± .5^{ab}	24.0 ± .3^{ab}	41.9 ± .3^{ab}	…	…	…
Yearlings:							
California	7 (6)	58.5 ± .9	24.1 ± .6	41.2 ± .4^{123}	5.18 ± .19	112.9 ± 3.7	46.7 ± 1.6^{bcd}
Alaska	29	58.1 ± .8^{ab}	25.0 ± .3^{b}	43.3 ± .6^{abc}	…	…	…
Adults:							
California	50 (20)	54.1 ± .7	22.6 ± .3*	41.6 ± .5^{13}*	4.97 ± .11	113.6 ± 1.8	47.0 ± .7^{cd}
Alaska	49	54.9 ± .5^{ac}	24.0 ± .2^{a}	43.5 ± .3^{ac}	…	…	…

Note. Sample sizes are for hematology measures; those in parentheses are for RBC counts. Similar alphabetical (Alaska) and numerical (California) superscripts indicate similar hematological parameters across age classes within each region. There were no significant differences with age in HCT, Hb, RBC, or MCV values from California groups.

* Significant difference between regions within each age class, as judged by Bonferroni post hoc comparisons.

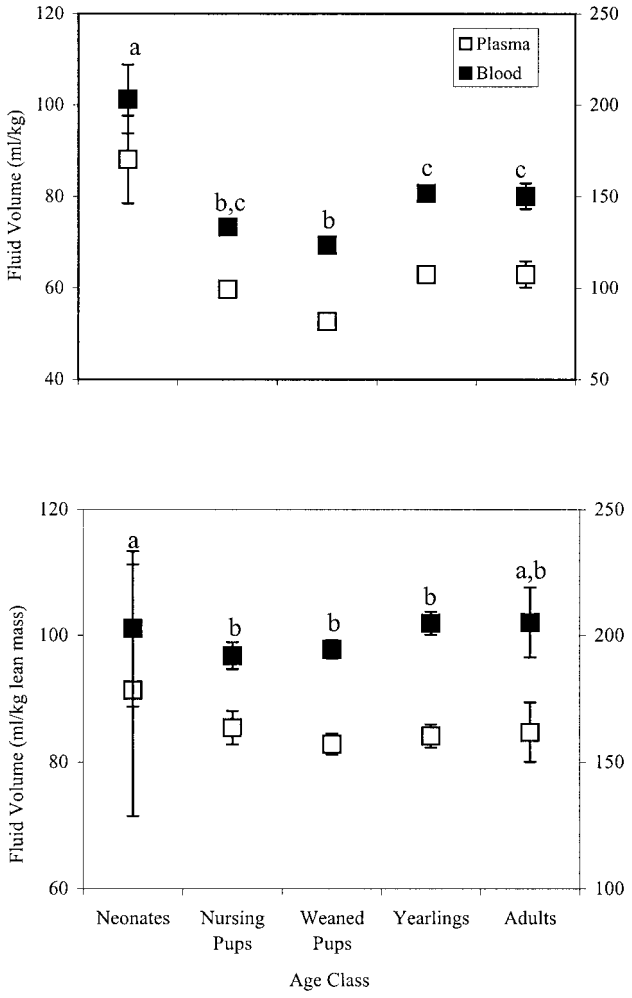

Figure 1. Changes in plasma and blood volumes (mean ± SE) with age on both a mass-specific and lean-body-mass-specific basis for harbor seals in each of five age classes: neonates, nursing pups, weaned pups, yearlings, and adults. Plasma volume is shown on the left axis, blood volume on the right. Age classes with similar alphabetical superscripts were not different from each other, as measured by Bonferroni post hoc comparisons. Because there were no significant differences resulting from location, samples from both regions were combined.

gissimus dorsi resulting from sex or handling location. However, there were significant differences resulting from age ($F_{4,31} = 5.418$, $P = 0.002$). Post hoc comparisons indicated that adults and yearlings had significantly greater myoglobin content than did pups of all ages (Fig. 2). Similarly, mass-specific muscle oxygen stores varied significantly only with age ($F_{3,33} = 21.669$, $P = 0.000$), and adult and yearling values were significantly greater than those for pups of all ages (Table 3).

Total Oxygen Stores

Total oxygen stores varied with age on both a mass-specific ($F_{4,24} = 12.330$, $P < 0.001$) and lean-body-mass-specific basis ($F_{4,21} = 5.736$, $P = 0.003$) but not as a result of region, collection year, or sex. On both a mass- and a lean-body-mass-specific basis, seals fell into two groups, with pups of all ages having smaller stores than yearlings and adults (Table 3). However, the pattern of variation with age did differ; on a mass-specific basis oxygen stores declined during lactation, while on a lean-body-mass-specific basis they increased slightly. The mean total body oxygen stores determined for individual seals when both blood and muscle stores were measured was similar to that estimated by summing the average blood, muscle, and lung stores (Table 3).

The significant age-related increase in total oxygen stores was accompanied by a shift in the primary oxygen storage site. Young pups stored a significantly greater proportion of their oxygen in the blood than did older age classes ($F_{4,23} = 21.773$, $P < 0.001$), and this proportion became more "adult-like" as muscles developed (Fig. 3). For example, neonatal pups stored 81% of their oxygen in blood and only 8% in muscle, while adults stored 58% in blood and 24% in muscle.

Metabolic Trials and Aerobic Dive Capacity

In March and April 1998, metabolic trials were conducted once on each of 14 neonatal pups (8.1 ± 0.3 kg, 10 ± 1 d old). The average resting metabolic rate in air was 11.0 ± 0.4 mL O_2/kg min at an ambient temperature of 17.5° ± 0.2°C. There was no difference between metabolic rates of male and female pups ($t_{12} = 0.72$, $P = 0.49$). The calculated aerobic dive capacity (cADL) of harbor seals of different ages was determined by dividing total body oxygen stores by resting metabolic rates. There was a clear increase in cADL near weaning and further differences among age classes resulting from age and mass (Table 3).

Discussion

There were four main findings from this research. First, harbor seal pups, despite their highly precocial behavior, are not physiologically mature at birth or at weaning. Pups of all ages had smaller mass-specific total oxygen stores than yearlings or adults, and these differences were apparent even when differences in body composition were considered. Second, this research clearly demonstrated that hematology and blood oxygen stores were more mature at birth than muscle oxygen stores. In fact, results indicate that on a lean-body-mass-specific basis, pups are born with blood oxygen stores that are similar to those of adults. Third, we found that the oxygen storage capacity of yearlings and adults is similar despite the large difference in body size between these age classes, which suggests that postweaning physiological development of body oxygen stores is fairly rapid and is largely completed within the first year of life. Finally, this study revealed that adult harbor seals have fairly large total body oxygen stores (68 mL O_2/kg total

Table 3: Body oxygen stores, resting metabolic rates, and calculated aerobic dive capacity in harbor seals

Age Class	*N* (Blood, Muscle)	Blood O_2 Stores (mL O_2/kg)	Blood O_2 Stores (mL O_2/kg Lean Tissue)	Muscle O_2 Stores (mL O_2/kg)	Muscle O_2 Stores (mL O_2/kg Lean Tissue)	Total O_2 Stores (mL O_2/kg)	Total O_2 Stores (mL O_2/kg Lean Tissue)	Mean Total O_2 Stores (mL O_2/kg from Averages)	Resting Metabolic Rate in Air (mL O_2/kg min)	Calculated Aerobic Dive Limit (min)
Neonates	8, 4	45.6 ± 5.2[a]	44.6 ± 4.0[ab]	4.0 ± .6[a]	3.9 ± 2.5[a]	51.8 ± 7.1[a]	52.1 ± 7.7[a]	57.4	11.0 ± .4	3.0
Nursing pups	18, 7	31.2 ± 1.3[b]	43.4 ± 3.1[ab]	5.9 ± .5[a]	8.8 ± 2.0[a]	39.4 ± 2.1[a]	56.6 ± 6.7[a]	41.7	13.3*	3.1
Weaned pups	33, 7	30.4 ± 1.0[b]	48.7 ± 1.5[ab]	7.0 ± .7[a]	12.1 ± 1.8[a]	40.0 ± 1.4[a]	67.4 ± 5.5[a]	42.6	8.6**	4.9
Yearlings	28, 6	39.1 ± 1.1[a]	53.6 ± 1.7[a]	16.3 ± 1.6[b]	22.5 ± 1.8[b]	68.4 ± 3.1[b]	93.9 ± 5.5[b]	68.0	8.3**	7.3
Adults	18, 14	38.4 ± 2.4[a]	53.5 ± 2.7[ab]	14.7 ± 1.1[b]	21.4 ± 1.6[b]	68.7 ± 4.1[b]	90.3 ± 4.7[b]	64.4	6.2**	10.2

Note. Total body oxygen stores ± SE are from individuals for which all components were measured, while the mean total oxygen stores were determined from the average blood volume, hemoglobin, and myoglobin values for that age class. Dive capacity was calculated by taking the ratio of body mass to total oxygen stores. Values are shown on a mass-specific and lean-body-mass-specific basis. Age classes that were similar to each other in post hoc comparisons are indicated with similar superscripts. Lung oxygen was assumed to be 5.3 mL/kg for pups and 12.2 mL/kg for adults.

* Miller and Irving 1975.

** Ashwell-Erickson and Elsner 1981.

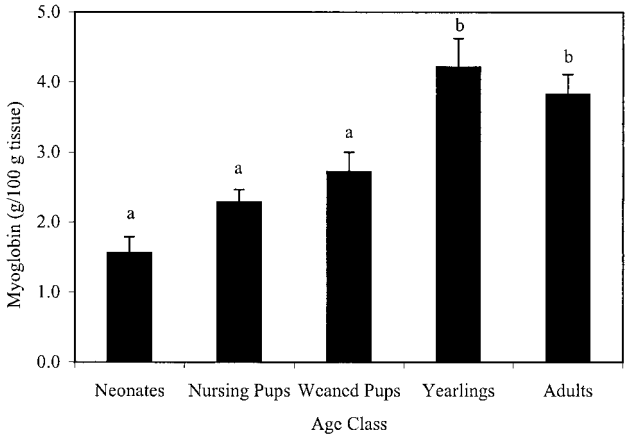

Figure 2. Mean ± SE myoglobin content in the *longissimus dorsi* muscle for harbor seals of different ages. Because there were no significant differences resulting from region, Alaskan and Californian data were combined. Age classes with similar alphabetical superscripts were not different from each other, as measured by Bonferroni post hoc comparisons.

mass, 90 mL O_2/kg lean mass) for a species that is typically thought of as a shallow-water forager (Lesage et al. 1999; Bekkby and Bjorge 2000; Frost et al. 2001; Jorgensen et al. 2001).

Development of Oxygen Stores

As harbor seals aged, their blood oxygen storage capacity increased primarily as a result of changes in the characteristics of the red cells rather than an expansion in mass-specific BV. In general, as animals got older, the size of the red cells increased, as did the amount of hemoglobin stored within cells (MCHC, MCV, MCH). In combination with a relatively unchanged HCT, this alteration in Hb indicates that older animals can store more oxygen per milliliter of blood. While absolute values for HCT, Hb, and MCHC varied between study locations, the ontogenetic trends were similar in scope and timing, and the observed differences were most likely results of regional differences in baseline values (Fadely 1997; Trumble and Castellini 2002). HCT, Hb, and MCHC values were likely high in neonates because fetal blood must have a high oxygen affinity to efficiently extract oxygen across the placenta. Similarly, observed age-related changes in cell size and MCH are consistent with the pattern of blood development previously observed in juvenile mammals from both terrestrial and marine habitats (Spensley et al. 1987; Sepulveda et al. 1999; Noren et al. 2002). Therefore, these changes do not reflect adaptations for aquatic existence or alterations in the basic pathways by which blood develops (Hochachka and Mottishaw 1999).

Although the oxygen-carrying capacity in the blood (mL O_2/mL blood) increased with age, PV and BV (adjusted for body size) did not. In fact, when examined on a mass-specific basis, PV and BV declined as pups grew, suggesting that blood stores could not keep pace with tissue development (Jorgensen et al. 2001). However, when analyzed on a lean-body-mass-specific basis, the only age-related differences were between neonates and all other age classes, indicating that BV is scaled to lean tissue mass rather than total mass in all but the youngest pups. The high fluid volumes in neonates were likely results of their low body fat percentage and high hydration state (Castellini et al. 1990). Beyond this neonatal period, PV and BV increased in proportion to the amount of lean tissue deposited, indicating that BV development is not constrained by the pups' rapid growth (as was suggested by Jorgensen et al. [2001]). In combination, these findings indicate that age-related increases in the total amount of oxygen carried in the blood result from increases in the hemoglobin content of the RBCs and not from differences in relative BV.

However, the rate at which hematological changes take place is clearly accelerated in harbor seals. Compared to northern elephant seals (*Mirounga angustirostris*; Thorson 1993), harbor seals are born with more mature blood O_2 stores (118% of adult values vs. 37% for northern elephant seals). Even though

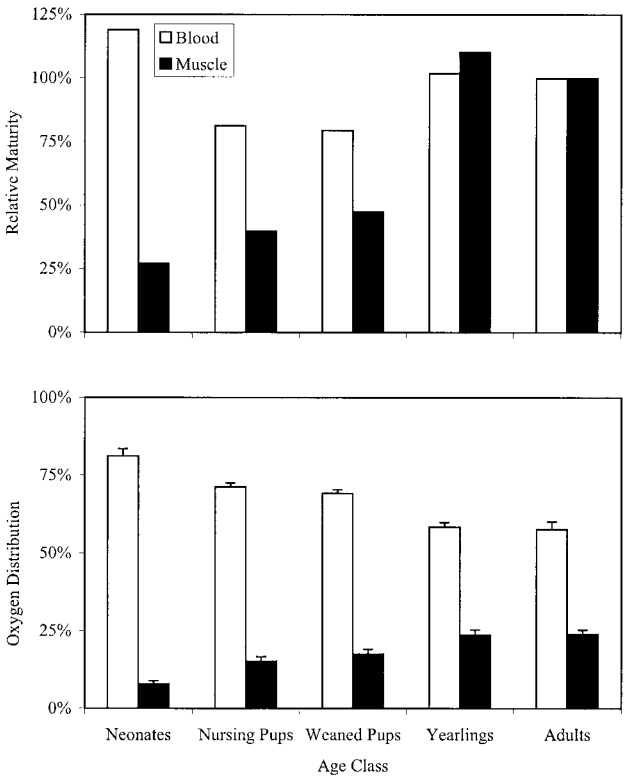

Figure 3. Relative maturity of blood and muscle oxygen stores for juvenile harbor seals as compared to adult values (*top*), and proportion of total oxygen stored in muscle and blood for harbor seals of all age classes (*bottom*).

oxygen storage capacity declines immediately after birth because of a decline in HCT and Hb, by the time pups are weaned, hematological values have largely recovered (BV is 82% that of adults on a mass-specific basis and 94% that of adults on a lean-body-mass-specific basis). As a result, weaned harbor seal pups can store almost as much oxygen in their blood as can adults, whereas species such as the northern elephant seal, which has a prolonged postweaning fast, have blood O_2 stores at weaning that are much smaller than those of adults (Thorson and Le Boeuf 1994). The greater oxygen storage capacity at birth and weaning in harbor seals is likely related to the short lactation period and earlier onset of independent foraging.

In contrast to the rapid development of blood oxygen stores, the slower development of muscle oxygen stores suggests that a more fundamental physiological constraint exists. Despite their highly precocial nature, neonatal harbor seal pups had muscle myoglobin concentrations that were only 37% those of adults. Although harbor seal muscle myoglobin load did increase slightly during the lactation period, the relative maturity of muscle oxygen stores at weaning (64%) was much lower than that in the blood. This finding is not unusual, since in all marine species studied to date, muscle myoglobin concentration does not reach adult values until after independent foraging has begun (Thorson and Le Boeuf 1994; Burns and Castellini 1996; Ponganis et al. 1999; Burns et al. 2000). Because some of these species demonstrate extensive preweaning diving, these results suggest that diving alone is not sufficient for complete muscle maturation and that some other factor triggers the final maturation.

Given that pinnipeds, like other mammals, have milk that is relatively low in protein and iron (Van Horn and Baker 1971; Halvorsen and Halvorsen 1973; Webb et al. 1984; Moreno-Rojas et al. 1993), it may be that the availability of dietary iron, necessary for the production of the heme molecule, limits the production of new red cells and muscle myoglobin (Burns et al. 2004). Since a larger proportion of body oxygen is stored in the blood, and blood oxygen is the primary source of oxygen for the heart, lung, and brain, if both stores cannot be developed simultaneously, it may be that hemoglobin is produced first so that the metabolic demands of these tissues can be met. In this scenario, muscle maturation may be delayed until after growth slows and/or diet changes. However, the molecular triggers for myoglobin and hemoglobin production are poorly understood, and we do not yet know whether ontogenetic or environmental factors (such as hypoxia) are the primary determinants of heme production in these tissues. Still, since muscle heme remains low during development, working muscles are likely to experience different hypoxic stresses in pups and adults. To compensate, muscles of young pups may differ in biochemical and histochemical parameters; juvenile marine mammals are known to have muscles with different ultrastructure (Kohin 1998), fiber types (Polasek and Davis 2001; Kanatous et al. 2002), enzyme concentrations (Richmond 2004), and acid-buffering capacity (Castellini and Somero 1981; Noren 1997) than those of adults. As animals mature, the shifts in all these parameters enhance the ability of muscles to remain aerobic during long dives.

Use Rates and Aerobic Capacity

Oxygen storage is not the only determinant of diving capacity; oxygen consumption rate is also critical. Here too, harbor seals develop rapidly; the elevated metabolic rates measured in neonatal harbor seals decline during the lactation period such that by the time pups are weaned, their rates of oxygen utilization are similar to those of yearlings (Ashwell-Erickson and Elsner 1981). In addition, weaned pups are able to regulate diving heart rate at levels significantly below eupneic rates (Greaves et al. 2005). When measured resting metabolic rates were used to calculate the aerobic dive capacity, our results were similar to those previously calculated for nursing and weaned pups (Ashwell-Erickson and Elsner 1981; Bowen et al. 1999; Jorgensen et al. 2001) and demonstrate that harbor seal pups, like other pinnipeds (Thorson and Le Boeuf 1994; Burns 1999; Clark 2004; Richmond 2004), have a lower aerobic dive capacity than older animals of similar size because of their age. The reduced aerobic capacity of weaned pups is caused primarily by immature oxygen stores and not elevated metabolic rates. We therefore suggest that the precocial nature of harbor seals and their preweaning diving activity does not fully prepare them to be aquatic predators. We further suggest that the rate-limiting step in achieving physiological maturity is not the regulation of oxygen consumption but the development of adequate oxygen stores.

This lower dive capacity has the potential to impact juvenile harbor seal foraging behavior. Pups are unable to dive as deep or for as long as older individuals because of their smaller size and reduced physiological capacity. However, because harbor seals forage in shallower coastal environments, and the majority of their dives are well within aerobic dive limits (Lesage et al. 1999; Jorgensen et al. 2001; Frost et al., forthcoming), young harbor seals may not be as affected by their reduced physiological capacity as are juveniles of deep-diving species (Burns 1999). Still, young harbor seal pups spend more time in the water and make more dives than adults despite their smaller size and lower absolute food requirements (Lesage et al. 1999; Jorgensen et al. 2001). In addition, inexperienced foragers that are working closer to their ADL may have less behavioral flexibility than older animals, which in turn may make them more vulnerable to shifts in prey abundance and distribution (Burns 1999; Costa et al. 2001).

In summary, this research demonstrates that harbor seal pups develop the physiological tools necessary for breathhold diving rapidly during the short lactation period. Blood oxygen stores mature first, primarily through shifts in characteristics of RBCs and not through postnatal expansion of BV (mL/kg). Indeed,

PV is constant across all age groups when measured on a lean-body-mass-specific basis, suggesting that fluid volume responds primarily to changes in the amount of metabolically active tissue rather than total mass. In contrast, muscle development lags behind that of blood, with large postweaning increases in mass and myoglobin content necessary to achieve adult oxygen stores. In combination, these findings suggest that it is critical to understand the factors that regulate muscle development in pinnipeds if we are to understand the underlying constraints on the rate at which body oxygen stores develop. Metabolic rates, while elevated relative to mature animals, dropped quickly, and this, in combination with mature cardiovascular control, allowed newly weaned harbor seals to dive for upward of 5 min without the need for anaerobic metabolism. Given the relatively short and shallow nature of most dives, it does not appear that oxygen availability and use rates are the sole constraints on the diving behavior of newly weaned harbor seals.

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