

# Development of myoglobin concentration and acid buffering capacity in harp (*Pagophilus groenlandicus*) and hooded (*Cystophora cristata*) seals from birth to maturity

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

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**Abstract** Pinnipeds rely on muscle oxygen stores to help support aerobic diving, therefore muscle maturation may influence the behavioral ecology of young pinnipeds. To investigate the pattern of muscle development, myoglobin concentration ([Mb]) and acid buffering ability ( $\beta$ ) was measured in ten muscles from 23 harp and 40 hooded seals of various ages. Adult [Mb] ranged from 28–97 to 35–104 mg g tissue<sup>-1</sup> in harp and hooded seals, respectively, with values increasing from the cervical, non-swimming muscles to the main swimming muscles of the lumbar region. Neonatal and weaned pup muscles exhibited lower ( $\sim 30\%$  adult values) and less variable [Mb] across the body than adults. In contrast, adult  $\beta$  showed little regional variation (60–90 slykes), while high pup values ( $\sim 75\%$  adult values) indicate significant in utero development. These findings suggest that intra-uterine conditions are sufficiently hypoxic to stimulate prenatal  $\beta$  development, but that [Mb] development requires additional postnatal signal such as exercise, and/or growth factors. However, because of limited development in both  $\beta$  and [Mb] during the nursing period, pups are weaned with muscles with lower aerobic and anaerobic capacities than those of adults.

**Keywords** Myoglobin · Acid buffering · Muscle development · Harp seal · Hooded seal

## Abbreviations

$\beta$	Acid buffering capacity, slykes
$\beta_{\text{TBA}}$	Total body acid buffering capacity
$\beta_{\text{LD}}$	Acid buffering capacity for the <i>longissimus dorsi</i>
$\beta_{\text{PEC}}$	Acid buffering capacity for the <i>pectoralis</i>
LD	<i>Longissimus dorsi</i>
Mb	Muscle myoglobin, mg g wet tissue <sup>-1</sup>
[Mb] <sub>TBA</sub>	Total body myoglobin concentration
[Mb] <sub>LD</sub>	Myoglobin concentration for the <i>longissimus dorsi</i>
[Mb] <sub>PEC</sub>	Myoglobin concentration for the <i>pectoralis</i>
Pec	<i>Pectoralis</i>
PWF	Postweaning fast

## Introduction

Marine mammals exhibit a host of physiological adaptations to successfully forage in an oxygen limited environment. For diving animals, foraging duration depends primarily on two factors: the amount of oxygen (O<sub>2</sub>) that can be brought to depth and the rate at which this O<sub>2</sub> is utilized (Scholander 1940). Marine mammals optimize the amount of O<sub>2</sub> carried to depth by having high blood volumes, high hemoglobin and high myoglobin concentration ([Mb]) (Scholander 1940; Butler and Jones 1997; Ramirez et al. 2007). To reduce O<sub>2</sub> consumption while foraging, adult marine mammals exhibit a dive response that includes selective vasoconstriction, a compensatory bradycardia, and

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K. C. Lestyk (✉) · J. M. Burns  
Department of Biological Sciences, University of Alaska  
Anchorage, Anchorage, AK 99508, USA  
e-mail: askl122@uaa.alaska.edu

L. P. Folkow · A. S. Blix  
Department of Arctic Biology, Institute of Medical Biology,  
University of Tromsø, 9037 Tromsø, Norway

M. O. Hammill  
Maurice Lamontagne Institute, Fisheries and Oceans Canada,  
Mont-Joli, QC, Canada

tissue-specific reductions in metabolic rates (Butler and Jones 1997; Davis et al. 2004; Ramirez et al. 2007). While these adaptations are well developed in adults, young animals have less developed cardiovascular control, limited ability to vasoconstrict and reduce metabolic rate, and lower mass-specific O<sub>2</sub> stores (Kooyman et al. 1981; Greaves et al. 2005; Burns et al. 2007). Lower O<sub>2</sub> stores and higher O<sub>2</sub> use rates in pups result in dives that are shorter and shallower than those of adults (Thorson and Le Boeuf 1994; Jorgensen et al. 2001). Therefore, rapid development of O<sub>2</sub> stores is crucial for pups to successfully forage independently. Early work in several pinniped species has shown that blood O<sub>2</sub> stores develop quickly, while muscle development proceeds at a much slower pace (Noren et al. 2005; Burns et al. 2007). As a result, muscle maturation likely influences the behavioral ecology of young pinnipeds.

Marine mammal exercise (diving activity) occurs under O<sub>2</sub> limited conditions, but their skeletal muscles still rely primarily on lipid-based aerobic metabolism during the vast majority of their dives (Kooyman et al. 1981; Reed et al. 1994; Kanatous et al. 1999). This is possible due to high [Mb] in muscles that serves as an endogenous O<sub>2</sub> supply and aids in O<sub>2</sub> transport (Wittenberg and Wittenberg 2003). In addition to having [Mb] that are 10–30 times higher than endurance-adapted terrestrial athletes (Kooyman 1989), marine mammal muscles also possess high proportions of oxidative muscle fibers and high levels of oxidative enzymes (George and Ronald 1975; Reed et al. 1994; Kanatous et al. 1999). However, when O<sub>2</sub> stores are depleted, these same muscles must rely on anaerobic metabolism which creates acid byproducts leading to metabolic acidosis (Peronnet and Aguilaniu 2006). Multiple studies in terrestrial and marine mammals have linked acidosis to decreases in contractile ability and increased muscle fatigue (Bangsbo et al. 1996; Brooks et al. 2005; Kristensen et al. 2005), and determined that muscles contain a variety of bicarbonate and non-bicarbonate buffering compounds designed to maintain acid–base balance, and therefore function (Burton 1978). Given their underwater diving activity, and the potential need for anaerobic metabolism, it is not surprising that marine mammals possess high levels of non-bicarbonate buffers (Castellini and Somero 1981). Because species that routinely dive deeper and/or longer have higher [Mb] and aerobic enzyme activities (Kanatous et al. 1999; Noren et al. 2001), these parameters can be used as an index of aerobic diving ability. Similarly, acid buffering capacity ( $\beta$ ) can be used as an indicator for how long an animal can rely on anaerobic metabolism once O<sub>2</sub> stores are depleted (Castellini and Somero 1981; Noren 2004). Thus, measuring both [Mb] and  $\beta$  allows for inferences to be made about diving and foraging ability.

Previous work on marine mammal muscle has focused primarily on adaptations possessed by adult animals and has looked at relatively few muscles within individual animals (Hochachka and Foreman 1993; Kanatous et al. 1999; Noren et al. 2001; Kanatous et al. 2008). However, the physiological properties of skeletal muscle differ based on muscle function and age (Muir 2000; Baldwin and Haddad 2001). Generally, muscles used primarily for locomotion are engineered for prolonged, aerobic exercise with large fiber diameters, high [Mb] and oxidative enzyme activities while muscles not used for sustained locomotion have smaller fiber diameters, lower [Mb] and oxidative enzyme activities (Close 1972; Pette and Staron 1990; Kanatous et al. 2002). Muscles also undergo large changes with age as most newborn mammals have largely undifferentiated muscles with lower mechanical ability, lower [Mb], and altered enzyme activities as compared to adults (Close 1972; Kanatous et al. 1999; Picard et al. 2002). In addition, the rate at which muscle matures depends on both use patterns and fiber types, with the largest differences seen in the major oxidative locomotory muscles (Ponganis et al. 1997; Picard et al. 2002; Jamon 2006). While developmental and functional patterns of muscle development have been well documented in many terrestrial species (Garry et al. 1996; Starck and Ricklefs 1998), there is little information available on the muscle development in marine mammals, despite the very different constraints faced by muscles which must perform when O<sub>2</sub> supply is limited.

Therefore, the goal of this project was to investigate developmental variation in [Mb] and  $\beta$  in several muscles across the body and between multiple age classes in harp and hooded seals to determine the muscle profile from birth to maturity. Harp and hooded seals differ in physiological maturity at birth and in foraging behaviors as adults, thus offering potential insight into the relative impact of growth rate and activity pattern on muscle development. Hooded seals are very precocial at birth, having the shortest nursing period of any mammal (~4 days), and are born more physiologically mature with dense pelage and a subcutaneous blubber layer (Blix and Steen 1979; Bowen et al. 1987). Adult hooded seals forage in deep, offshore waters along the continental shelf break, with recorded dives in excess of 1,000 meters and average dives between 100–600 m and 5–25 min (Folkow and Blix 1999). In contrast, the more altricial harp seal pups are born with a lanugo, lack significant blubber stores and nurse for ~12 days (Kovacs and Lavigne 1986; Worthy and Lavigne 1987). Unlike hooded seals, adult harp seals normally forage over shelf areas, with dives generally shallower than 400 m and shorter than 10 min, although dives up to 700 m have been documented (Folkow et al. 2004; Nordoy et al. 2008). In spite of these differences, pups of both species endure a

postweaning fast (PWF) that lasts 4–6 weeks during which time a significant amount of mass (~30%) is lost (Kovacs and Lavigne 1986; Bowen et al. 1987). While the purpose of the PWF is unknown, it may provide time needed for muscles to mature before diving and foraging begin. Thus, examining the timing and rate of muscle development in harp and hooded seals may shed light on the mechanisms that lead to an increased ability to forage in an oxygen limited environment.

**Methods**

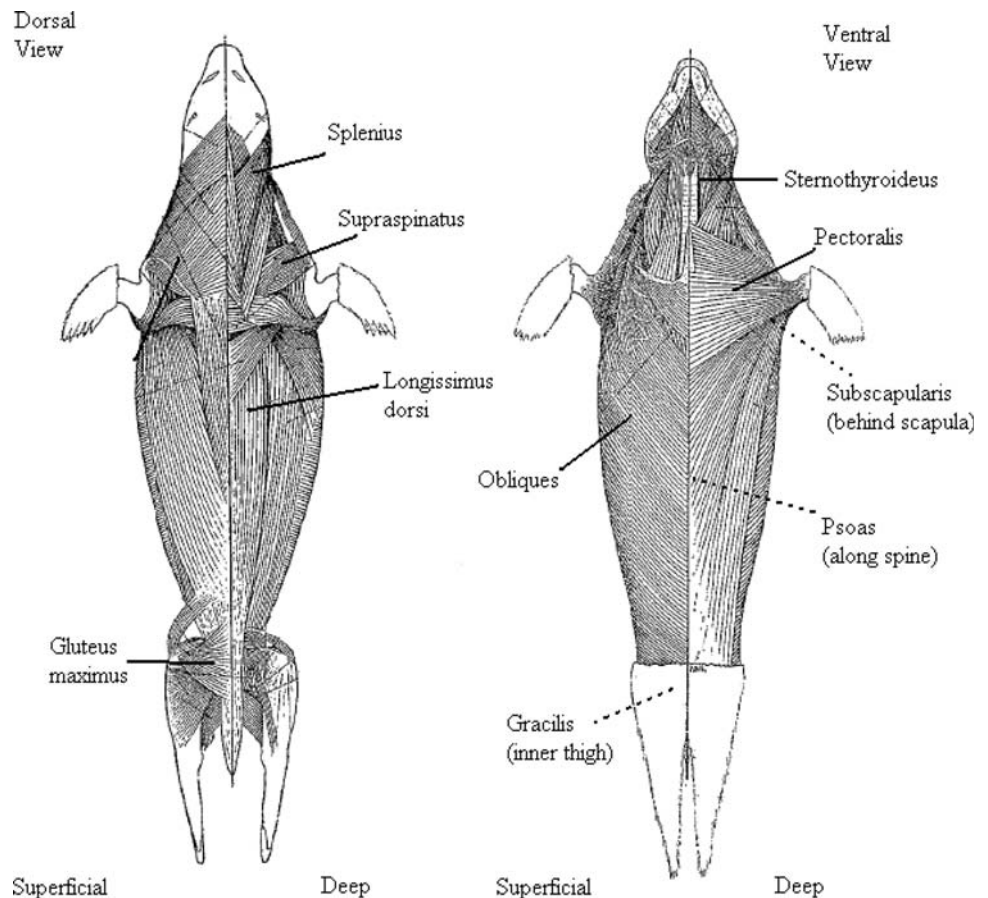
**Animal handling**

Fourteen hooded seals of the Greenland Sea stock were captured between 23 March and 5 April 1999 and 11 hooded seals and 8 harp seals were captured between 21 March and 1 April 2000 in the Greenland Sea (~72°N, 15°W). Fifteen harp and 15 hooded seals of the Canadian stock were also captured between 3 March and 16 March 2005 in the Gulf of St Lawrence, Canada (~47°23'N, 61°52'W). Harp seal pups were categorized as neonates

(yellowcoats and thin whitecoats), nursing (fat whitecoats), or weaned (ragged jackets and beaters) following Stewart and Lavigne (1980). Hooded seal pups were categorized as neonates (1–2 days old), weaned pups, or yearlings following Bowen et al. (1987). Seals were weighed (±0.5 kg) and blood samples were taken. Animals were sacrificed using methods approved for scientific harvest in Norway and Canada and under governmental permits authorizing collection.

Ten muscles were collected from across the body. The muscles selected represented two muscles that perform primarily postural functions (*splenius* and *sternothyroideus*), four involved primarily in terrestrial locomotory activities (*subscapularis*, *supraspinatus*, *pectoralis*, *obliques*) and four primarily used during aquatic locomotion (*longissimus dorsi*, *psaos*, *gracilis*, and *gluteus maximus*) (Howell 1929) (Fig. 1). Muscle samples (~5 g) were placed on ice, stored at -20°C for up to 2 weeks before being stored at -80°C prior to analysis. Muscle mass was determined on animals collected from the Greenland Sea stock by complete dissection. During dissection the body was divided into 5 regions for muscle mass measurements, and all muscles in each region were weighed

**Fig. 1** Skeletal muscle anatomy of seals (from Howell 1929) showing the 10 muscles sampled as part of this study. Dashed lines indicate the approximate location of muscles not revealed by these schematics



together. These regions were the head and neck (including among others, *splenius* and *sternothyroideus*), shoulder (including among others, *subscapularis* and *supraspinatus*), thorax (including among others, *pectoralis* and *obliques*), swimming (including among others, *psoas* and *longissimus dorsi*), and the hindlimb (including among others, *gracilis* and *gluteus maximus*). The relationship between total and regional muscle mass and total body mass determined for seals collected from the Greenland Sea stock was used to estimate muscle mass for the seals collected in Canada.

#### Myoglobin concentration and acid buffering capacity

Myoglobin concentration ([Mb] mg g wet tissue<sup>-1</sup>) was determined for all muscles following the methods in (Reynafarje 1963). Muscle samples were trimmed, weighed, and sonicated (Fisher Scientific, Sonic Dismembrator model 500) in ice-cold 0.04 M phosphate buffer (19.25 ml g<sup>-1</sup> tissue, pH 6.6). The homogenate was then centrifuged, and the resulting supernatant reduced with CO gas and excess sodium hydrosulfite. The absorbance was obtained at wavelengths 568 and 535 nm (Beckman Spectrophotometer DU530), and Mb calculated following equation 4 in Reynafarje (1963). All samples were run in triplicate and tissue samples with previously measured [Mb] from northern elephant seals (*Mirounga angustirostris*), harbor seals (*Phoca vitulina*), and lyophilized horse myoglobin (Sigma-Aldrich, Allentown, PA) were simultaneously assayed as controls (Thorson and Le Boeuf 1994; Clark et al. 2007).

Non-bicarbonate buffering capacity ( $\beta$ ), the  $\mu$ moles of base needed to change the pH of homogenate one pH unit per gram of muscle tissue, was determined in 23 harp and 26 hooded seals following Bate Smith (1938) and Castellini and Somero (1981). Frozen muscle samples ( $\sim$ 75 mg) were sonicated (Fisher Scientific, Sonic dismembrator Model 500) in 3 ml of ice-cold normal saline (0.9% NaCl). The homogenates were brought to 37°C and stirred constantly while being titrated with NaOH (0.1 M) from approximately pH 6 to pH 7. All samples were run in triplicate and harbor seal muscle tissue with a previously measured  $\beta$  was simultaneously titrated as a control (Castellini and Somero 1981).

#### Statistical analysis

To determine if there were differences in live mass and total percent muscle mass due to age or geographic region, two-way ANOVAs with Bonferroni post hoc tests (SPSS v. 14.0, Chicago, IL) were used within each species. Because the analysis for live mass indicated a significant

difference between adult females (but not pups) from the Canadian and Greenland stocks, adult females were treated separately throughout all subsequent analyses. One-way ANOVAs were performed to determine differences in the proportion of muscle mass within each body region between age classes for each species separately. Prior to analysis, data were tested for normality and all proportional data were arcsin transformed. Significance was set at  $P < 0.05$  and all values are reported as mean  $\pm$  SEM.

Three-way ANOVAs were used to determine differences in [Mb] or  $\beta$  between age classes, muscles and species. Due to significant three-way interactions in these models, species were separated and two-way ANOVAs with Bonferroni post hoc tests were used to determine differences in [Mb] or  $\beta$  within each species due to age class and muscle. Based on ANOVA results that demonstrated no difference in [Mb] or  $\beta$  between the two muscles sampled from within each body region (head, shoulder, abdominal, swimming, and hip/hind regions), the average [Mb] (and  $\beta$ ) for each body region was multiplied by the regional muscle mass proportion. These scaled values were summed for each animal, and the resulting total body average myoglobin ([Mb]<sub>TBA</sub>) and total body average acid buffering capacity ( $\beta$ <sub>TBA</sub>) were compared between age classes and species with two-way ANOVAs and Bonferroni post hoc tests. Comparisons between species were limited to age classes that were represented by both species.

To assess the accuracy of the common practice of using single muscle biopsy samples to infer [Mb]<sub>TBA</sub>, the percent difference between [Mb]<sub>TBA</sub> and the [Mb] of the *longissimus dorsi* ([Mb]<sub>LD</sub>) and *pectoralis* ([Mb]<sub>PEC</sub>) was calculated. These two muscles are often taken as representative of the Mb load in swimming and non-swimming muscles, respectively (Castellini and Somero 1981; Lydersen et al. 1992; Kanatous et al. 1999; Kanatous et al. 2002; Burns et al. 2005; Clark et al. 2007). Paired *t* tests were used to compare [Mb]<sub>TBA</sub> to [Mb]<sub>LD</sub>, [Mb]<sub>PEC</sub> and the Mb value when the [Mb]<sub>LD</sub> and [Mb]<sub>PEC</sub> were averaged.

Since [Mb] can act as a non-bicarbonate buffer (Burton 1978), the extent to which age and/or muscle related differences in  $\beta$  were driven primarily by differences in [Mb] was assessed. To do this, repeated measures ANCOVAs were used within each species to determine if differences exist between juveniles (neonates, nursing, weaned, yearlings) and adults in  $\beta$  with [Mb] as the covariate. Then regression lines for the  $\beta$  to Mb relationship were compared to determine how the relationship differed between age classes. Finally, linear regression was used to determine the relationship between previously reported average dive duration and [Mb] in the major locomotory muscles of a variety of different diving animals.



**Results**

Live mass and muscle mass data

There were significant increases in live mass with age in harp ( $F_{4,18} = 143.2, P < 0.001$ ) and hooded seals ( $F_{4,35} = 316.3, P < 0.001$ ), and adult female harp and hooded seals of the Canadian stock were heavier than those from the Greenland Sea stock (Table 1). Based on measurements of the animals from the Greenland Sea stock only, pups had significantly less muscle mass on a percent basis than adult females (harp:  $F_{1,5} = 43.8, P < 0.01$ ; hooded:  $F_{3,21} = 13.2, P < 0.001$ ), and a different distribution of that mass across the body (harp:  $F_{4,30} = 212.6, P < 0.001$ ; hooded:  $F_{4,120} = 419.5, P < 0.001$ ) (Table 2). Neonatal pups had proportionately more muscle in the abdominal region, and less in the swimming region compared to adult animals (Table 2).

Myoglobin

There was significant variation in [Mb] due to age, muscle, and their interaction (harp: age  $F_{4,180} = 838.4, P < 0.001$ ; muscle  $F_{9,180} = 138.4, P < 0.001$  and interaction  $F_{36,180} = 15.38, P < 0.001$ ; hooded: age  $F_{4,207} = 1,086.1, P < 0.001$ ; muscle  $F_{9,207} = 174.5, P < 0.001$ ; interaction  $F_{36,207} = 20.38, P < 0.001$ ). In all cases, pup muscles had lower [Mb] values than adult muscles, with the largest differences in the major swimming muscles (Fig. 2). For example, the [Mb] of the *sternothyriodeus* (used for postural activities) in weaned harp and hooded seal pups was  $67.4 \pm 9.5\%$  and  $54.3 \pm 2.1\%$  that of adults, while the [Mb] in the *longissimus dorsi* (LD, used for major

swimming activities) was only  $35.5 \pm 2.8\%$  and  $30.2 \pm 1.6\%$  of adult values, respectively.

It follows that there was much less variation in [Mb] across the body in pups than in adults (Fig. 2). In hooded seal pups, the [Mb]<sub>LD</sub> content was only twice as high as that in the *sternothyriodeus* muscle ( $16.47 \pm 1.12$  vs.  $31.93 \pm 1.12$  mg g wet tissue<sup>-1</sup>), while in adults, there was more than a threefold difference ( $31.93 \pm 1.12$  vs.  $103.71 \pm 4.82$  mg g wet tissue<sup>-1</sup>). This increase in variation across the body with age was reflected by post hoc analyses, which revealed that in adults, muscles could be grouped into three distinct body regions within which [Mb] did not differ significantly: (1) the head and neck region (*splenius* and *sternothyriodeus*), (2) the shoulder and thoracic region (*subscapularis*, *supraspinatus*, *pectoralis*, *obliques* including the *gracilis*), and (3) the major swimming muscles (*psaos*, *longissimus dorsi* and *gluteus maximus*). In contrast, among both harp and hooded seal pups (neonates, nursing and weaned pups) only the head and neck muscles differed significantly from other muscles. No interspecific comparisons among individual muscles were performed.

When comparing the [Mb]<sub>TBA</sub> between harp and hooded seals, significant differences were found due to age ( $F_{3,48} = 412.43, P < 0.001$ ) and the species-by-age interactions ( $F_{3,48} = 5.91, P < 0.01$ ), but not by species alone ( $F_{1,48} = 3.08, P = 0.086$ ) (Fig. 3). Among harp seals, neonates had the lowest [Mb]<sub>TBA</sub>, nursing and weaned pups had similar [Mb]<sub>TBA</sub>, and adults had the highest values, with adult females from the Greenland Sea having significantly higher [Mb]<sub>TBA</sub> than those from Canada. Hooded seal neonates and weaned pups had similar [Mb]<sub>TBA</sub>, but these values were lower than in yearlings, which had similar values to Canadian adults, but both had lower [Mb]<sub>TBA</sub> than the Greenland Sea adults. Post hoc tests revealed that the more precocious hooded seal neonates had higher [Mb]<sub>TBA</sub> values than harp seal neonates, but there were no species differences in [Mb]<sub>TBA</sub> in weaned pups, or adults from Canada or the Greenland Sea.

For all animals, the [Mb]<sub>TBA</sub> differed significantly from those of the commonly sampled *longissimus dorsi* (LD) and *pectoralis* (PEC) muscles, indicating that using the Mb value for either of these muscles singly to estimate total Mb load will introduce bias. For example, the [Mb]<sub>LD</sub> was  $21.6 \pm 2.3\%$  higher than [Mb]<sub>TBA</sub> in harp neonates and  $18.3 \pm 2.5\%$  higher than [Mb]<sub>TBA</sub> in hooded seal neonates. For adults, the [Mb]<sub>LD</sub> was  $27.7 \pm 2.3\%$  and  $29.7 \pm 2.9\%$  higher than [Mb]<sub>TBA</sub> in harp and hooded seals, respectively. However because the [Mb]<sub>LD</sub> was typically higher and the [Mb]<sub>PEC</sub> lower than [Mb]<sub>TBA</sub>, averaging these two values provided an estimate that was closer to the overall value (within  $7.2 \pm 1.1$  and  $10.4 \pm 1.0\%$  of the [Mb]<sub>TBA</sub> for harp and hooded seals, respectively).

**Table 1** Live mass of harp and hooded seals by age class

	n (M, F)	Live mass (kg)
<b>Harp seal</b>		
Neonate pups	5 (1, 4)	10.3 ± 3.9 <sup>a</sup>
Nursing pups	4 (3, 1)	29.1 ± 4.3 <sup>b</sup>
Weaned pups	5 (2, 3)	36.6 ± 3.9 <sup>b</sup>
GS adults	4 (0, 4)	98.5 ± 4.3 <sup>c</sup>
Canadian adults	5 (0, 5)	120.1 ± 3.9 <sup>d</sup>
<b>Hooded seal</b>		
Neonate pups	7 (5, 2)	24.8 ± 5.2 <sup>a</sup>
Weaned pups	16 (7, 9)	40.2 ± 3.4 <sup>a</sup>
Yearlings	3 (1, 2)	50.0 ± 7.9 <sup>a</sup>
GS adults	9 (0, 9)	152.3 ± 4.6 <sup>b</sup>
Canadian adults	5 (0, 5)	249.2 ± 6.1 <sup>c</sup>

Superscript alphabets indicate significant differences between age classes within a species

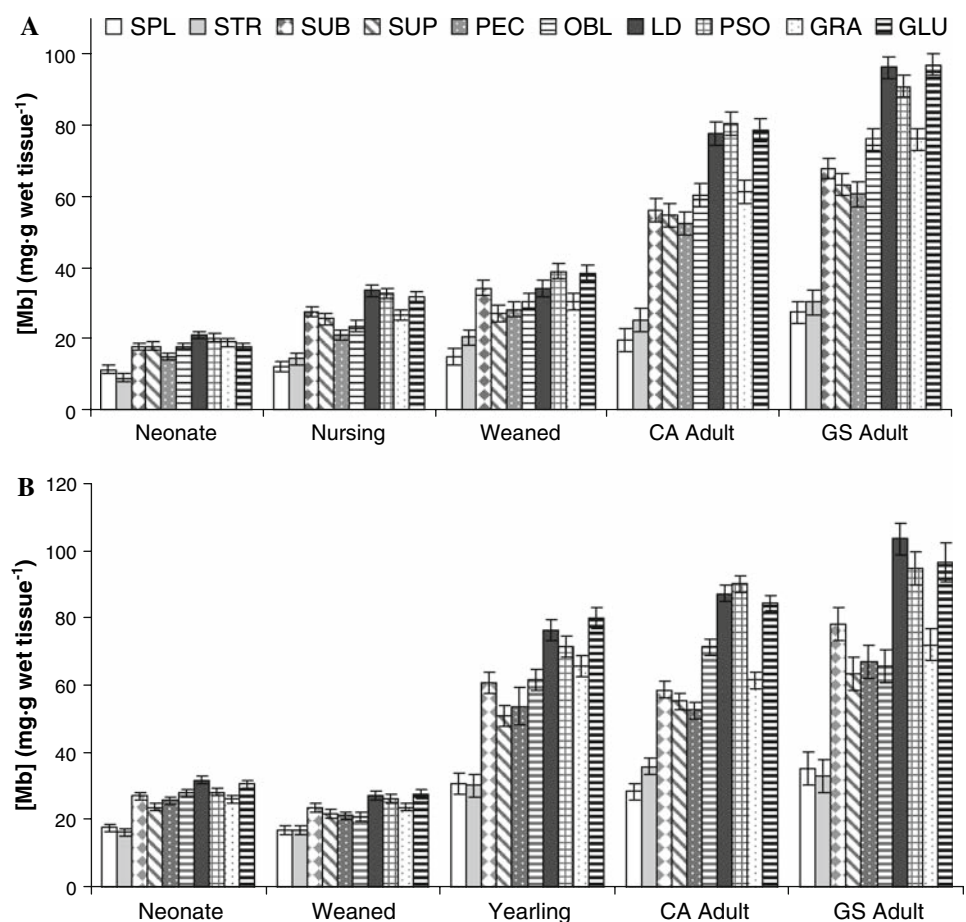
GS Greenland Sea

**Table 2** Percent muscle mass and proportion of that muscle mass located in each body region as determined through complete dissection from animals collected in the Greenland Sea

	<i>n</i> (M, F)	Muscle mass (%)	MM head (%)	MM shoulder (%)	MM abdominal (%)	MM swimming (%)	MM hind/hip (%)
<b>Harp seal</b>							
Nursing pups	4 (3, 1)	18.4 ± 0.8	13.1 ± 0.8	20.6 ± 0.8	40.5 ± 0.8 <sup>a</sup>	13.0 ± 0.8 <sup>a</sup>	12.8 ± 0.8
Adults	4 (0, 4)	25.6 ± 0.6	10.3 ± 0.9	21.4 ± 0.9	37.9 ± 0.9 <sup>b</sup>	17.9 ± 0.9 <sup>b</sup>	12.6 ± 0.9
<b>Hooded seal</b>							
Neonate pups	2 (2, 0)	19.6 ± 1.0 <sup>a</sup>	14.7 ± 0.9	14.6 ± 0.9 <sup>a</sup>	48.7 ± 0.9 <sup>a</sup>	9.5 ± 0.9 <sup>a</sup>	12.4 ± 0.9
Weaned pups	11 (5, 6)	20.1 ± 1.2 <sup>a</sup>	14.5 ± 0.8	22.0 ± 0.8 <sup>b</sup>	39.3 ± 0.8 <sup>b</sup>	13.3 ± 0.8 <sup>b</sup>	10.9 ± 0.8
Yearlings	3 (1, 2)	25.2 ± 1.0 <sup>ab</sup>	11.5 ± 1.0	19.6 ± 1.0 <sup>ab</sup>	43.1 ± 1.0 <sup>b</sup>	15.2 ± 1.0 <sup>bc</sup>	10.6 ± 1.0
Adults	9 (0, 9)	28.0 ± 0.6 <sup>b</sup>	10.6 ± 0.7	18.2 ± 0.7 <sup>a</sup>	42.5 ± 0.7 <sup>b</sup>	17.9 ± 0.7 <sup>c</sup>	10.8 ± 0.7

Superscript alphabets indicate significant differences between age classes within a species

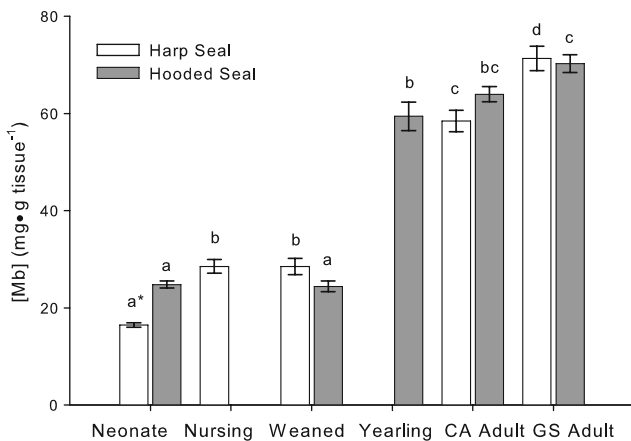
**Fig. 2** Mean ± SE myoglobin levels (mg g wet tissue<sup>-1</sup>) in muscles across the body in harp (a) and hooded (b) seals. Muscles are ordered from head to tail, left to right; SPL = *splenius*, STR = *sternothyroideus*, SUB = *subscapularis*, SUP = *supraspinatus*, PEC = *pectoralis*, OBL = *obliques*, LD = *longissimus dorsi*, PSO = *psaos*, GRA = *gracilis*, GLU = *gluteus maximus*. Neonate, nursing and weaned refer to pup age classes. CA Canada, GS Greenland Sea



### Acid buffering

There were age and muscle differences in  $\beta$  (harp: age  $F_{4,180} = 40.7$ ,  $P < 0.001$ ; muscle  $F_{9,180} = 16.3$ ,  $P < 0.001$ ; hooded: age  $F_{4,207} = 72.82$ ,  $P < 0.001$ ; muscle  $F_{9,207} = 17.65$ ,  $P < 0.001$ ; interactions  $F_{36,207} = 1.96$ ,  $P < 0.01$ ) (Fig. 4). As in [Mb], pups of both species had significantly lower  $\beta$  values than adults in all muscles, with

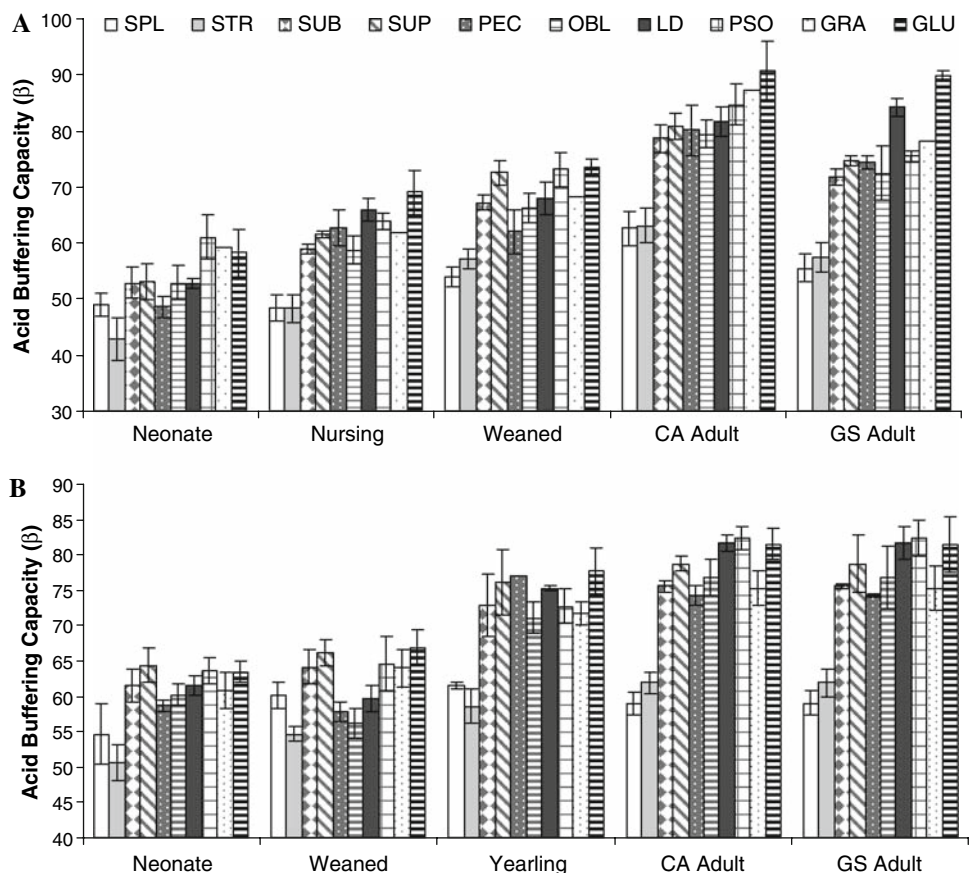
the largest differences present in the main swimming muscles. For instance, the  $\beta$  for the LD was  $31.43 \pm 1.48$  slykes (37.4%) lower in harp seal pups as compared to adult values, while the  $\beta$  for the *sternothyroideus* was only  $14.62 \pm 3.20$  slykes, or 25.5% lower. However,  $\beta$  values in pup muscles were more similar to those in adults (range 42–61 slykes) than were Mb values (range 9–21 mg g tissue<sup>-1</sup>). In addition, there was less variation



**Fig. 3** Mean  $\pm$  SE total body myoglobin concentration ( $[Mb]_{TBA}$ ) for harp and hooded seals. Superscripted letters indicate significant differences between age classes within a species, while asterisk indicates significant differences within an age class between species. CA Canada, GS Greenland Sea

across the body in  $\beta$  than in Mb, with neonates showing no consistent differences in  $\beta$  among the ten muscles. Among adults, only the  $\beta$  in head muscles (*splenius* and *sternothyroideus*) was significantly lower than all remaining muscles and there were no differences between adults from Canada and the Greenland Sea.

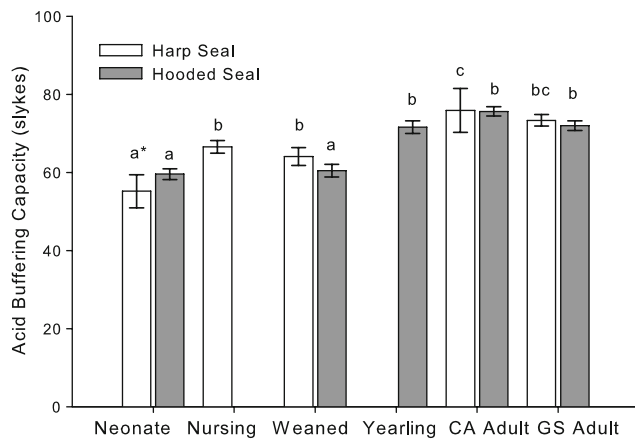
**Fig. 4** Distribution of mean  $\pm$  SE acid buffering capacity (slykes) across the body in harp (a) and hooded (b) seals. Muscles are ordered from head to tail, left to right; SPL = *splenius*, STR = *sternothyroideus*, SUB = *subscapularis*, SUP = *supraspinatus*, PEC = *pectoralis*, OBL = *obliques*, LD = *longissimus dorsi*, PSO = *psoas*, GRA = *gracilis*, GLU = *gluteus maximus*. Neonate, nursing and weaned refer to pup age classes. CA Canada, GS Greenland Sea



There were significant differences in  $\beta_{TBA}$  due to age class ( $F_{3,34} = 54.06, P < 0.001$ ) and age by species interactions ( $F_{3,34} = 4.90, P < 0.01$ ), but no significant species effect ( $F_{1,34} = 0.15, P = 0.71$ ; Fig. 5). Within harp seals, neonates had significantly lower  $\beta$  than all older animals. For hooded seals, neonates and weaned pups had significantly lower  $\beta$  than yearlings and adults, which were similar. When results were analyzed within each age class, harp seal neonates had lower  $\beta_{TBA}$  than hooded seal neonates, but there were no differences between species for any other age class. Because there was limited variation across the body in  $\beta$ , using the *longissimus dorsi* (or *pectoralis*)  $\beta$  value to estimate  $\beta_{TBA}$  does not produce as much bias as it did in  $[Mb]$  (harp  $\beta_{LD}$   $4.2 \pm 1.1\%$  higher than  $\beta_{TBA}$ ; hooded  $3.0 \pm 1.8\%$  higher), and averaging the  $\beta_{LD}$  and  $\beta_{PEC}$  accurately approximated  $\beta_{TBA}$  (difference of:  $0.28 \pm 1.0\%$  for harps,  $0.44 \pm 1.5\%$  for hooded seals).

Relationships between Mb,  $\beta$  and average dive duration

ANCOVA results indicated significant effects of both  $[Mb]$  and age class on  $\beta$  ability in both species (harp  $F_{1,185} = 214.35, P < 0.001$ ; hooded  $F_{1,187} = 278.24, P < 0.001$ ). While  $[Mb]$  and  $\beta$  were significantly positively correlated in all age classes, Mb had a much greater impact on  $\beta$  in



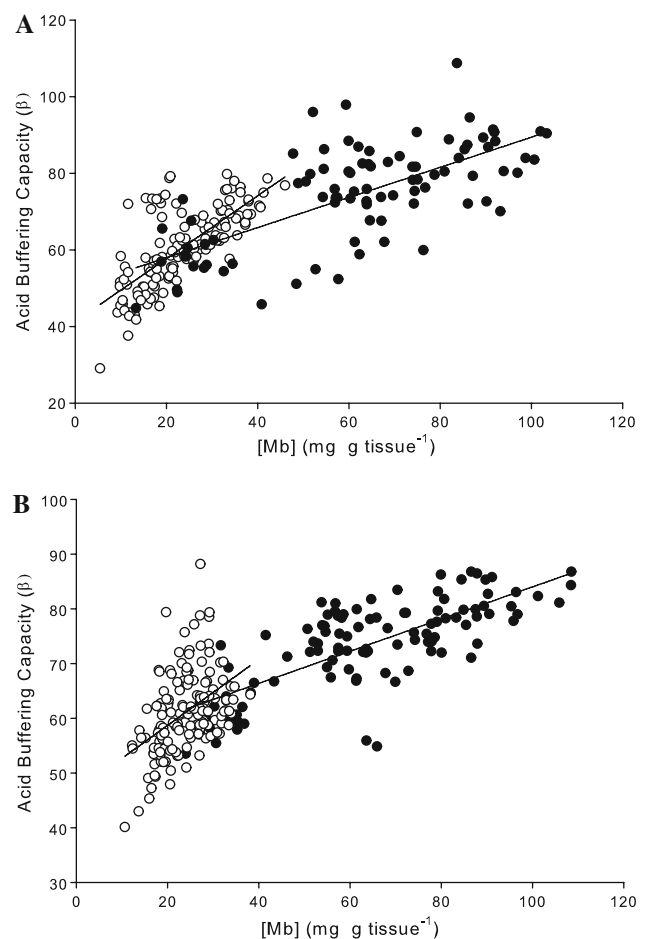
**Fig. 5** Mean  $\pm$  SE total body acid buffering capacity ( $\beta_{TBA}$ ) for harp and hooded seals. Superscripted letters indicate significant differences between age classes within a species, while asterisk indicates significant differences within an age class between species. CA Canada, GS Greenland Sea

pups (neonates, nursing and weaned pups, pooled based on ANCOVA post hoc results, harp  $R^2 = 0.524$ ,  $y = 0.821x + 41.3$ ; hooded  $R^2 = 0.212$ ,  $y = 0.650x + 45.7$ ) than in all older animals (yearlings, Canadian and Greenland Sea adults, harp  $R^2 = 0.467$ ,  $y = 0.393x + 50.1$ ; hooded  $R^2 = 0.687$ ,  $y = 0.327x + 53.3$ ; Fig. 6). Average dive duration was significantly correlated to [Mb] in the major locomotory muscles of diving mammals and birds ([Mb] =  $2.89 \times$  avg. dive duration + 26.9,  $R^2 = 0.64$ ; Fig. 7).

## Discussion

Results from this study suggest that pup muscles are not fully developed at birth and that, even once weaned, pup muscles are unable to sustain aerobic or anaerobic energy production during underwater activity to the same extent as in adults. In addition, the rate and timing of [Mb] and  $\beta$  development differed based on muscle function, but not between harp and hooded seal pups suggesting that constraints on muscle development are similar across the phocid lineage, regardless of differing life history patterns. More specifically, harp and hooded seal pups have both lower muscle mass than adults and allocate less mass to the major swimming muscles. The lower [Mb] and  $\beta$  in pup muscles, and the much reduced variation in these parameters in younger animals indicate that significant maturation of the major locomotory muscles must occur postnatally, in response to swimming and diving activity and/or growth factors.

As seen in other species (Ontell and Dunn 1978; Carrier 1983; Starck and Ricklefs 1998), harp and hooded seal pups had significantly less total muscle mass than adults,

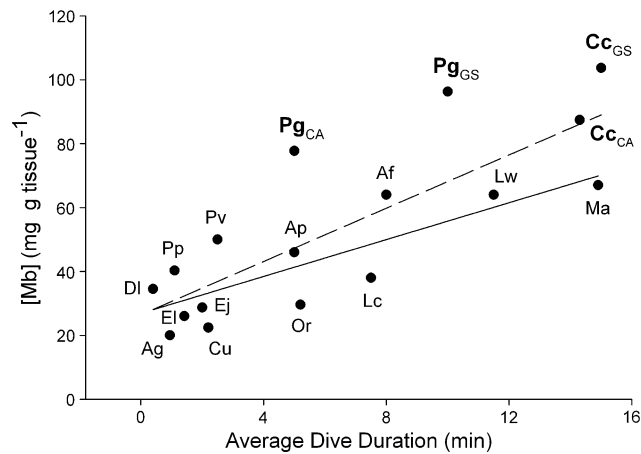


**Fig. 6** Relationship between [Mb] and  $\beta$  for harp (a) and hooded (b) seals. Dark circles represent adults while open circles represent pup age classes (neonates, nursing and weaned pups). Trend lines are best-fit regression lines to depict differences between pups and adults

and the muscle mass was proportioned differently across the body. Both of these differences likely reflect the limited activity pups demonstrate while on-ice during the nursing and early PWF period (Worthy and Lavigne 1987). During this period, a greater fraction of total muscle mass is found in those areas used for terrestrial locomotion (i.e. shoulder and abdominal muscles) than in those used for aquatic locomotion. Similarly, galliform birds develop leg muscles before flight muscles (Starck and Ricklefs 1998) and marsupials develop cranio-facial muscles (to accommodate teat attachment) well before limb musculature (Grand and Barboza 2001). However, as in most terrestrial and marine mammals, muscles account for  $\sim 1/3$  of adult mass, with most of the mass concentrated in the midsection and tail regions, as expected based on the swimming style of phocids (Bryden 1972; Grand 1977; Fish and Ronald 1988).

In addition to the lower mass, muscles critical to underwater activities were not fully mature in pups. While the lower [Mb] and  $\beta$  values in juveniles were expected





**Fig. 7** Average dive duration is strongly correlated with [Mb] in the major locomotory muscles of diving animals. Note that harp and hooded seals from both geographic regions have higher [Mb] than predicted based on previously reported data (solid line;  $[Mb] = 2.89 \times \text{avg. dive duration} + 26.90$ ,  $R^2 = 0.64$ ). When harp and hooded seals are included (dotted line;  $[Mb] = 4.17 \times \text{avg. dive duration} + 26.44$ ,  $R^2 = 0.66$ ) harp seals from both regions (Canada:  $Pg_{CA}$ ; Greenland Sea:  $Pg_{GS}$ ) and hooded seals from the Greenland sea ( $Cc_{GS}$ ) still have significantly higher [Mb] than predicted, while Canadian hooded seals ( $Cc_{CA}$ ) fall on the predicted line. *Af* emperor penguin, *Ag* Antarctic fur seal, *Ap* king penguin, *Cu* Northern fur seal, *DI* Pacific white-sided dolphin, *Ej* Steller sea lion, *El*: sea otter, *Lc* crabeater seal, *Lw* Weddell seal, *Ma* Northern elephant seal, *Or* walrus, *Pp* harbor porpoise, and *Pv* harbor seal (data from Lenfant et al. 1970; Kooyman et al. 1992; Reed et al. 1994; Butler and Jones 1997; Merrick and Loughlin 1997; Ponganis et al. 1997; Folkow and Blix 1999; Kanatous et al. 1999; Noren and Williams 2000; Bodkin et al. 2004; Folkow et al. 2004; Bajzak et al. 2009; M. O. Hammill, unpublished data)

based on previous studies (Ponganis et al. 1999; Noren et al. 2001; Burns et al. 2005), the regional differences reported here indicate that the largest differences among age classes exist in the major swimming muscles. Adult muscles could be grouped into three functional categories based on similarity in [Mb]. In order of increasing [Mb], these categories were (1) the postural muscles of the head and neck region (including *splenius* and *sternothyroideus*), (2) the muscles of the thoracic region used primarily in terrestrial locomotion muscles (including *supraspinatus*, *subscapularis*, *pectoralis*, *obliques* and *gracilis*), and (3) the major swimming muscles of the lumbar region (including LD, *psoas* and *gluteus maximus*). Surprisingly, while the *gracilis* is physically located in the hind region, its [Mb] was significantly lower than that observed in other posterior swimming muscles, suggesting that its role in aquatic locomotion is limited. These findings indicate that [Mb] does not simply progress from low anterior values to high posterior values (Venters et al. 1999), but instead reflects muscle function and use.

Pup muscles contained much less Mb than adult muscles, and there was much less variation in [Mb] across the

body. As a result, their muscles could be categorized into only two functional groups: the relatively mature muscles of the head and neck, and the much less mature muscles in the remainder of the body. Given that the primary activity of young pups is nursing (Sivertsen 1941; Oftedal et al. 1996), early development of postural neck muscles is crucial and this is reflected in neck [Mb] 40% of adult values. In contrast, swimming activity does not begin until 5–6 weeks of age (Worthy and Lavigne 1987; Lydersen et al. 1997), allowing much slower development of muscles involved in underwater locomotion ([Mb] ~25% those of adults at birth and at weaning). Moreover, the limited development of Mb during the PWF suggests that additional nutrients, exercise, or hypoxia is required for Mb upregulation and final muscle maturation (Holloszy and Booth 1976; Terrados et al. 1990; Garry et al. 1996).

In contrast to the significant differences in [Mb] due to age and muscle function, and the apparent need for additional external signals to stimulate [Mb] upregulation following the PWF, muscle  $\beta$  ability was more mature and showed far less regional variation. For example, acid buffering ability in harp and hooded seal pups was approximately 2/3 that of adults, and regional differences were either absent (hooded seals) or limited to differences between the postural muscles of the head and neck and the rest of the body (harp seals). This suggests that swimming and diving activity is not necessary for the majority of  $\beta$  development, as is the case for Mb development. Instead, the relative maturity of muscle  $\beta$  in neonatal pups likely reflects the need for prenatal development of significant buffering ability to withstand the low intrauterine  $O_2$  tensions that occur during periods when pregnant females are actively foraging (Liggins et al. 1980). Furthermore, early development of  $\beta$  in utero may be an adaptation to protect young pups against metabolic acidosis that would likely occur during early post-natal diving activity due to their low tissue  $O_2$  stores. Finally, the temporal mismatch between Mb and  $\beta$  development provides further evidence that the positive correlation between Mb and  $\beta$  should not be considered causal (Castellini and Somero 1981; Noren 2004). Indeed, the age-related change in the contribution of Mb to total buffering likely indicates that other components such as muscle proteins contribute significantly to the buffering capacity of pups.

In combination, the lower muscle mass, [Mb], and  $\beta$  ability of juveniles likely reduce the ability of their muscles to sustain both aerobic and anaerobic metabolism during diving. Low tissue  $O_2$  stores and high mass-specific metabolic rates result in a reduced aerobic dive limit, and likely impact juvenile foraging efficiency and prey choice (Bowen et al. 1999; Weise and Costa 2007). However, while both the lower [Mb] and  $\beta$  in pup muscles may have negative impacts on foraging behavior, it is important to

note that these values are low only when compared to those of adult marine mammals. Indeed, pup values are comparable to [Mb] and  $\beta$  found in the major locomotory muscles of adult terrestrial, endurance athletes confirming the high level of adaptation in marine mammals at birth (Castellini and Somero 1981; Evans et al. 1994). Moreover, even though the young hooded seal falls short of the spectacular diving performance of adults, we have recently shown that 1–3-month-old pups are able to dive to depths of 250–400 m and stay submerged for 20–30 min (L. P. Folkow, E. S. Nordoy and A. S. Blix, unpublished results).

Surprisingly, while hooded seals are more precocious at birth than harp seals (Blix and Steen 1979) and adults preferentially forage in deeper, more offshore waters than harp seals (Folkow and Blix 1999; Folkow et al. 2004; Nordoy et al. 2008), interspecific differences in [Mb] and  $\beta$  were limited to small differences in the [Mb] of neonatal pups; there were no interspecific differences in  $\beta$  in any age class. The lower [Mb] in neonatal harp seals fits with their more altricial status at birth, while the similarity in [Mb] at weaning indicates that there was more pre-weaning development in harp seal muscles than in hooded seals. In contrast, at each age, harp and hooded seals had similar  $\beta$  ability suggesting that the extensive development of  $\beta$  ability in utero is sufficient to compensate for any differences in developmental rate. Such findings also fit with the observation that performance-driven differences in Mb (both among muscles within individuals, and across different species) are much larger than those in  $\beta$  (MacArthur et al. 2001; Noren 2004).

That Mb levels can vary more extensively than  $\beta$ , is further suggested by the remarkable interspecific similarity in [Mb] between harp and hooded seal adults from Canada (or the Greenland Sea), and the geographical differences within each species, patterns not evident in  $\beta$ . The higher Mb levels in adult female harp and hooded seals from the Greenland Sea as compared to their Canadian conspecifics could be due to genetic differences between the two stocks. Such phenotypic plasticity could also be due to behavioral differences, as higher Mb levels are seen in animals from the longer-diving Greenland Sea stocks (Folkow and Blix 1999; Folkow et al. 2004, M. O. Hammill, unpublished data), just as occurs in California sea lions (*Zalophus californianus*, Weise et al. 2007). Differences in activity levels might also account for the unexpectedly high [Mb] found in adult harp seals from both Canada and the Greenland Sea. While Canadian hooded seal adults have [Mb] similar to those predicted, harp seals from both geographic areas and Greenland Sea hooded seals have values that are significantly higher than predicted based on the relationship between average dive duration and [Mb] in the major locomotory muscles of diving mammals and birds (Fig. 7). However, because the amount of O<sub>2</sub> needed to sustain

aerobic metabolism while underwater is determined both by the length of the dive and by the rate at which O<sub>2</sub> is used, the average diving activity level is at least as important as dive duration (Williams et al. 2000). Harp seals are small relative to other species that dive to similar depth and duration, and they appear to be more active while diving, which may result in their having a much higher diving metabolic rate than the larger hooded seals. As a result, harp seals, like small cetaceans (Noren 2004), may need higher [Mb] and  $\beta$  than expected based on dive depth alone. Alternatively, the similarity in [Mb] in adult harp and hooded seals may represent a physiological upper limit to the amount of Mb that can occur in muscles, without compromising muscle contractile function, as has been suggested relative to the high mitochondrial densities in hummingbird muscles (Suarez 1998).

In conclusion, our results confirm that adult harp and hooded seal skeletal muscles are poised for aerobic metabolism based on high O<sub>2</sub> content within all muscles, particularly those that support swimming activity, and that pup muscles are at a disadvantage based on significantly lower O<sub>2</sub> stores at weaning compared to adults. Additionally, the similarity in developmental patterns between harp and hooded seals suggests that muscle function, rather than life history strategies, exerts a stronger influence on muscle development. Pup muscles of both species employ strategies to cope with anaerobic metabolism before exercise-induced O<sub>2</sub> stores are developed. While high [Mb] contributes to substantial acid buffering capacity other components, such as muscle protein, must also be important based on the [Mb]/ $\beta$  relationship. Finally, inter- and intraspecific differences in both Mb and  $\beta$  levels likely reflect pre- and post-natal activity patterns, and the fact that adaptations to tolerate tissue hypoxia develop before those necessary to support underwater exercise. Because [Mb] is a main characteristic of muscle fiber types (Close 1972), the large changes in [Mb] documented here likely co-occur with similarly large changes in the metabolic functioning and structure of these tissues which may further constrain juvenile diving behavior.

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