

RESEARCH ARTICLE

Rapid maturation of the muscle biochemistry that supports diving in Pacific walrus (*Odobenus rosmarus divergens*)

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ABSTRACT

Physiological constraints dictate animals' ability to exploit habitats. For marine mammals, it is important to quantify physiological limits that influence diving and their ability to alter foraging behaviors. We characterized age-specific dive limits of walrus by measuring anaerobic (acid-buffering capacity) and aerobic (myoglobin content) capacities of the muscles that power hind (longissimus dorsi) and fore (supraspinatus) flipper propulsion. Mean buffering capacities were similar across muscles and age classes (a fetus, five neonatal calves, a 3 month old and 20 adults), ranging from 41.31 to 54.14 slykes and 42.00 to 46.93 slykes in the longissimus and supraspinatus, respectively. Mean myoglobin in the fetus and neonatal calves fell within a narrow range (longissimus: 0.92–1.68 g 100 g⁻¹ wet muscle mass; supraspinatus: 0.88–1.64 g 100 g⁻¹ wet muscle mass). By 3 months post-partum, myoglobin in the longissimus increased by 79%, but levels in the supraspinatus remained unaltered. From 3 months post-partum to adulthood, myoglobin increased by an additional 26% in the longissimus and increased by 126% in the supraspinatus; myoglobin remained greater in the longissimus compared with the supraspinatus. Walrus are unique among marine mammals because they are born with a mature muscle acid-buffering capacity and attain mature myoglobin content early in life. Despite rapid physiological development, small body size limits the diving capacity of immature walrus and extreme sexual dimorphism reduces the diving capacity of adult females compared with adult males. Thus, free-ranging immature walrus likely exhibit the shortest foraging dives while adult males are capable of the longest foraging dives.

KEY WORDS: Odobenid, Myoglobin, Acid buffering, Ontogeny, Aerobic dive limit, Arctic

INTRODUCTION

With continued human perturbation to the environment, it is becoming increasingly important to predict the ability of animals to adapt to changes in their environment. For example, the rate of change within Arctic ecosystems, including changes in sea ice cover, sea level, water temperature and ocean currents (Rothrock et al., 1999; Parkinson and Cavalieri, 2002; Comiso and Parkinson, 2004; Walsh, 2008), is exceeding trends recorded over the past several millennia (Root et al., 2003; Overpeck et al., 2005; Walsh, 2008). As long-lived species with slow reproductive rates, Arctic marine mammals are poorly equipped to respond to sudden alterations in climate (Moore

and Huntington, 2008). Small population sizes following centuries of commercial harvest and a reliance on specific sea ice conditions further heighten the sensitivity of these species to environmental perturbations (O'Corry-Crowe, 2008).

Pacific walrus [*Odobenus rosmarus divergens* (Illiger 1811); Fig. 1] may be vulnerable to the effects of global climate change (Laidre et al., 2008). They are highly specialized shallow benthic foragers and consume a wide range of invertebrates, primarily feeding on clams, snails and marine worms (Fay, 1982; Sheffield and Grebmeier, 2009). Recently, walrus have altered their foraging behaviors and distribution patterns in the Chukchi Sea (Jay et al., 2012) in response to receding summer sea ice cover over the continental shelf. For example, adult females and their young have increased their use of terrestrial haul-outs when the sea ice is over deep waters (Kavry et al., 2008; Jay et al., 2012). The number of walrus converging on coastal haul-outs is expected to increase as summer sea ice continues to decline (Jay et al., 2011), but the ability of localized food supplies in these coastal regions to support large numbers of walrus over the long term is unknown (Ovsyanikov et al., 2008). Thus, these changes in behavior could affect the ability of walrus to meet daily energetic requirements (Noren et al., 2012).

Quantifying the physiological capacities of animals improves the ability of scientists and managers to determine the range of environmental conditions under which an animal can persist without declines in fitness (Wikelski and Cooke, 2006). For marine mammals, the importance of physiological constraints is evident during foraging, as their diving behaviors, and hence habitat utilization patterns, are defined by their breath-hold limits (Costa et al., 2001). To maximize aerobic submergence times, adult diving mammals have larger mass-specific body oxygen stores compared with terrestrial mammals as a result of elevated blood volume, high hemoglobin levels and greater muscle myoglobin content (Lenfant et al., 1970; Snyder, 1983; Castellini and Somero, 1981; Kooyman, 1989; for review, see Butler and Jones, 1997). Compared with adults, neonatal and juvenile marine mammals have low oxygen storage capacities in the blood and muscle (Bryden and Lim, 1969; Ronald et al., 1969; Geraci, 1971; Lane et al., 1972; Kodama et al., 1977; Thorson, 1993; Lewis et al., 2001; Noren et al., 2001, 2002, 2005, 2014; Burns et al., 2005, 2007; Richmond et al., 2005, 2006; Fowler et al., 2007; Weise and Costa, 2007; Verrier et al., 2011) while the cost of growth and small body size result in comparatively high mass-specific oxygen utilization rates (Kleiber, 1975). Ultimately, the ratio of oxygen stores to the rate at which these stores are depleted defines aerobic dive limit (ADL) (Kooyman, 1989) and hence dictates the foraging strategies of marine mammals (Costa et al., 2001).

Odobenids [Pacific (*O. r. divergens*) and Atlantic (*Odobenus rosmarus rosmarus*) walrus] are among the largest pinniped species, which theoretically grants them an intrinsic advantage for deep diving because of their relatively low mass-specific oxygen consumption rates (e.g. reviewed in Noren and Williams, 2000).

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Fig. 1. Female Pacific walrus and calf. Photo credit: Sarah Sonsthagen, US Geological Survey.

However, a lack of data on the blood and muscle biochemistry that supports breath holding in this species makes it difficult to predict how walruses might alter foraging behaviors in response to the recent changes in the Arctic marine ecosystem. Little effort has been given in the past to examine the postnatal development of oxygen stores in walruses. Obtaining the requisite samples for such a study is difficult because of the remote Arctic distribution of walruses and the limited number of immature walruses that are taken in Alaskan Native subsistence harvests. Indeed, a previous report on the average muscle oxygen store (myoglobin) in free-ranging Pacific walruses was based on a low sample size composed of three 4–6 week old animals and two adults (Lenfant et al., 1970), and because these data were pooled, the influence of ontogeny was not considered.

In this study, we examined the ontogeny of the muscle biochemistry that supports diving in Pacific walruses to better understand their physiological breath-hold capacity, which partly determines their potential to alter foraging behaviors, as might be required in response to the rapid changes in the Arctic ecosystem. Specifically, we measured anaerobic capacity (acid-buffering capacity) and aerobic capacity (myoglobin content) in a fetus, five neonatal calves, an immature 3 month old and 20 adults. With knowledge of age-specific blood oxygen stores (S.R.N., unpublished), muscle oxygen stores (myoglobin; present study) and body size (Fay, 1982), we calculated age-specific aerobic dive limits (cADL; Kooyman, 1989). We compared our cADL estimates for adult males with recorded diving behaviors of free-ranging adult male walruses in the summer in Bristol Bay (Jay et al., 2001); this revealed that these animals appear to be diving within their physiological aerobic dive capacity. Similar comparisons for other sex and age classes of walruses would be useful, because diving at or near physiological capacity may reduce an animal's behavioral plasticity to alter foraging behaviors when prey becomes limited or more difficult to access (Costa et al., 2001). This can have marked consequences for a population, including the disproportionate starvation of immature animals (Trillmich and Limberger, 1985; DeLong et al., 1991). Thus, understanding age and sex-specific cADLs can be helpful in predicting a species' vulnerability to environmental change before declines in vital rates occur (Williams et al., 2011).

RESULTS

Ontogeny of muscle biochemistry

The acid-buffering capacity (muscle anaerobic capacity) of the longissimus dorsi appeared to be similar to that of the supraspinatus

within the single fetus and within the single 3 month old walrus. The acid-buffering capacities of these two muscle groups were also similar within neonatal calves ($t=-0.0580$, d.f.=4, $P=0.957$) and within adults ($t=-0.261$, d.f.=8, $P=0.800$). In addition, muscle acid-buffering capacities were similar between neonatal calves and adults for both the longissimus dorsi ($t=0.0145$, d.f.=23, $P=0.886$) and supraspinatus ($t=0.00242$, d.f.=12, $P=0.998$). These results indicate that buffering capacity does not vary across locomotor muscles and that the buffering capacity of these muscles is mature at birth (Table 1, Fig. 2A).

Myoglobin content (muscle aerobic capacity) varied across locomotor muscle groups. The myoglobin content of the longissimus dorsi was similar to that of the supraspinatus within the single fetus and within neonatal calves ($t=0.448$, d.f.=4, $P=0.678$). However, soon after birth, the myoglobin content of the longissimus dorsi surpassed that of the supraspinatus, as evidenced by the single 3 month old walrus, which had twice as much myoglobin in the longissimus dorsi than in the supraspinatus. Even into adulthood, the myoglobin level of the longissimus dorsi remained greater than that of the supraspinatus ($t=4.808$, d.f.=6, $P=0.0003$), with myoglobin levels in the longissimus dorsi representing 1.13× the levels in the supraspinatus.

Myoglobin content also varied within muscle groups throughout ontogeny. Myoglobin content in neonatal calves was significantly lower than that in adults within both the longissimus dorsi ($t=-7.968$, d.f.=23, $P<0.001$) and the supraspinatus ($t=-5.933$, d.f.=10, $P<0.001$; Fig. 2B, Table 1). However, myoglobin content matured quickly in the longissimus dorsi. By 3 months post-partum, the myoglobin content of the longissimus dorsi of the single 3 month old walrus was 79% of the levels found in the longissimus dorsi of adults.

Table 1. Biochemistry of locomotor muscles in Pacific walruses

Age class (N)	Mean±s.e.m.	Minimum	Maximum
Acid-buffering capacity (slykes)			
Longissimus dorsi			
Fetus (1)	42.06 (102%)	–	–
Calf (5)	41.86±2.89 (101%)	34.93	47.78
Immature (1)	54.14 (131%)	–	–
Mature adult (20)	41.30±1.76	29.54	59.19
Supraspinatus			
Fetus (1)	46.93 (112%)	–	–
Calf (5)	42.01±2.25 (100%)	36.54	49.33
Immature (1)	45.38 (108%)	–	–
Mature adult (9)	42.00±1.95	32.90	49.33
Mb content (g 100 g ⁻¹ wet muscle)			
Longissimus dorsi			
Fetus (1)	0.92 (24%)	–	–
Calf (5)	1.68±0.11 [‡] (44%)	1.32	1.98
Immature (1)	3.01 (79%)	–	–
Mature adult (20)	3.80±0.13*	3.00	4.71
Supraspinatus			
Fetus (1)	0.88 (26%)	–	–
Calf (5)	1.64±0.19 [§] (49%)	1.05	2.18
Immature (1)	1.48 (44%)	–	–
Mature adult (7)	3.35±0.208*	2.70	4.14

Values in parentheses are the percentage of adult levels. At $P<0.05$, no differences in acid-buffering capacity were found across the two muscle groups, while myoglobin (Mb) content across the two muscle groups only differed within the adult age class (denoted by *). At $P<0.05$, no differences in acid-buffering capacity were found across age classes, while Mb content in longissimus dorsi and supraspinatus of neonatal calves was significantly lower than that of the respective muscle in adults (denoted by ‡ and §, respectively). A sample size of 1 precluded comparisons within and among the fetus and immature age classes. See Results for statistics.

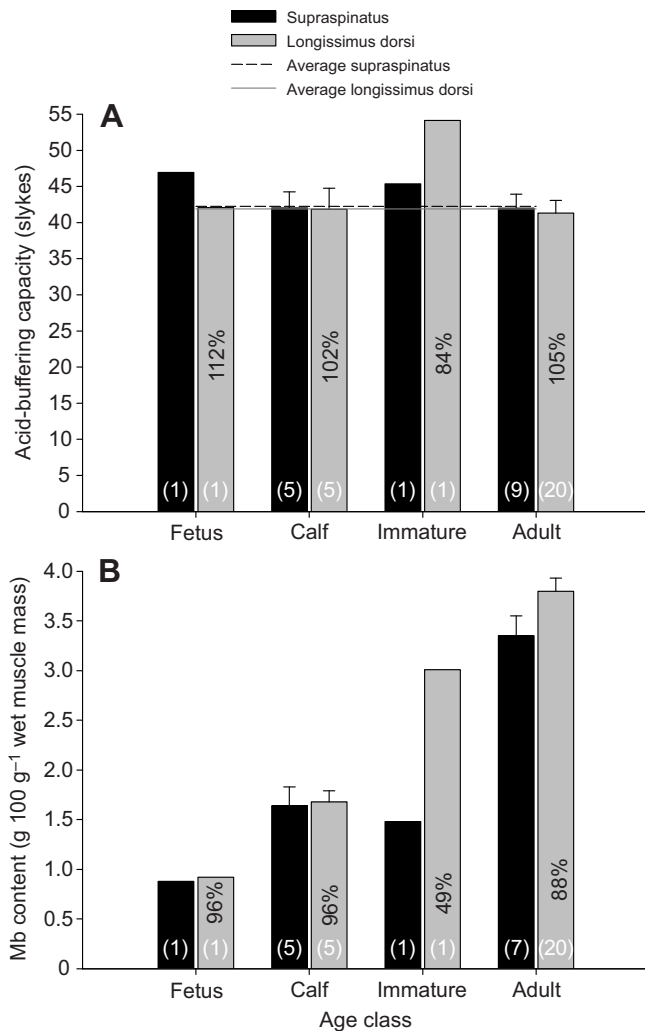


Fig. 2. Muscle acid-buffering capacity and myoglobin content in Pacific walrus muscle throughout ontogeny. (A) Muscle acid-buffering capacity (anaerobic capacity) and (B) myoglobin (Mb) content (aerobic capacity). The proportion of the muscle biochemistry in the supraspinatus in relation to the longissimus dorsi is shown by percentages in the longissimus dorsi bars for each age class. The number in parentheses within each bar represents the number of individuals analyzed for that muscle and age class. See Results and Table 1 for statistics.

Even though the developmental trajectory of buffering capacity and myoglobin were different, some still might argue that the postnatal development of myoglobin drives changes in buffering capacity throughout maturation because myoglobin contributes to the buffering capacity of muscle. Similar to Castellini and Somero (1981) and Noren (2004), we analyzed the relationship between myoglobin content and the ratio of buffering capacity to myoglobin content in each of the muscle groups. Acid-buffering capacity of the muscle was constant across ontogeny; thus, as myoglobin content increased during development, the proportion of acid-buffering capacity (AB) in relation to myoglobin content ($[Mb]$) decreased in both the longissimus dorsi ($AB[Mb]^{-1}=60.59^{-0.47Mb}$, d.f.=26, $F=150.4584$, $P<0.0001$) and supraspinatus ($AB[Mb]^{-1}=75.35^{-0.59Mb}$, d.f.=13, $F=112.9958$, $P<0.0001$). These relationships demonstrate that muscles with low myoglobin content had an extremely high ratio of buffering capacity to myoglobin content compared with myoglobin-rich muscle, implying that myoglobin is not the predominate buffering agent of

the muscle in walrus (Fig. 3). This result concurs with previous investigations (Noren, 2004a; Lestyk et al., 2009; Burns et al., 2010; Noren et al., 2014a).

Total body oxygen stores and cADLs

Because the 3 month old specimen had already achieved 79% of adult myoglobin content in the longissimus dorsi, we assumed that walrus ≥ 1 year old had achieved adult myoglobin content in the longissimus dorsi for the purpose of calculating body oxygen stores. As myoglobin content in the longissimus dorsi increased with maturity from 0 to 1 year post-partum, calculated total mass-specific oxygen stores in the muscle increased from approximately $7.54 \text{ ml O}_2 \text{ kg}^{-1}$ in neonatal calves, to $14.31 \text{ ml O}_2 \text{ kg}^{-1}$ in the 3 month old walrus, to $21.58\text{--}22.26 \text{ ml O}_2 \text{ kg}^{-1}$ in adults (Fig. 4). The mass-specific oxygen storage capacity in the lung and blood remained relatively constant throughout ontogeny because we assumed that mass-specific lung capacity did not change with age and the oxygen storage capacity in the blood (hemoglobin levels) remained constant throughout growth (S.R.N., unpublished). Thus, as the oxygen storage capacity of the muscle increased with maturity, the relative contribution of the muscle oxygen store to total body oxygen stores (lung, blood and muscle) increased (Fig. 4).

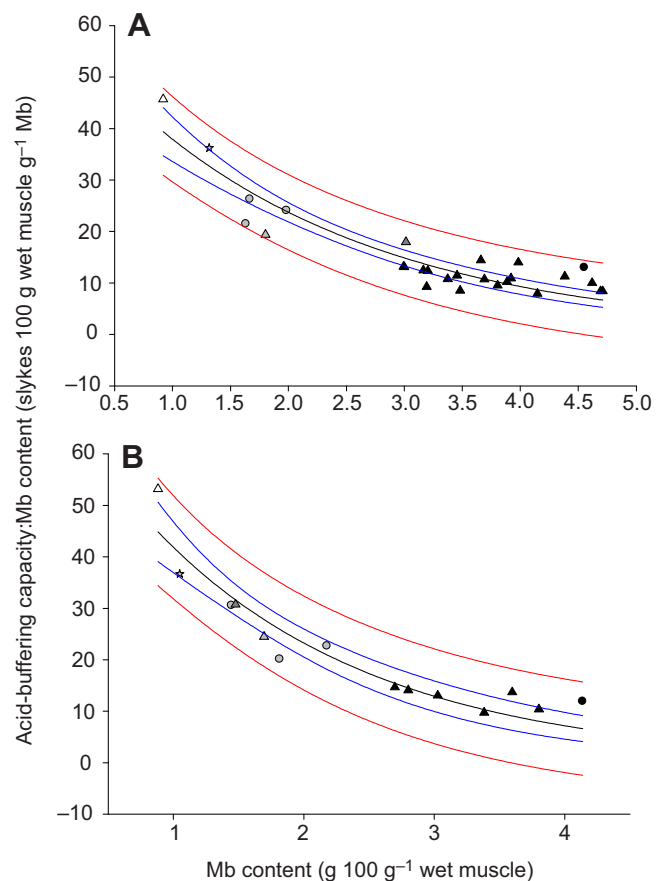


Fig. 3. Relationship between myoglobin content and acid-buffering capacity to myoglobin content ratio in Pacific walrus muscle throughout ontogeny. Data are given in relation to myoglobin content in the longissimus dorsi (A) and supraspinatus (B). Each point represents the mean for an individual specimen that was analyzed in triplicate. Age classes are as follows: fetus (white), calf (light gray), immature (3 month old; dark gray) and adult (black). Males and females are denoted by triangles and circles, respectively. The star represents an animal of unknown sex. See Results for statistics.

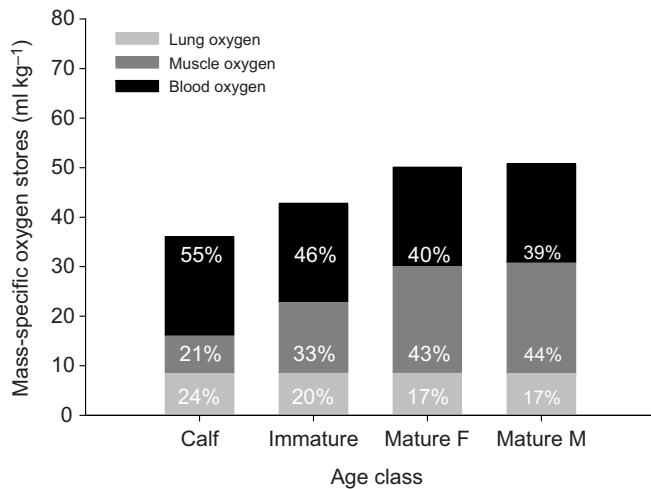


Fig. 4. Mass-specific oxygen stores of neonatal calves, immature (3 month old) and adult Pacific walruses. Total mass-specific oxygen stores are represented by the bars, which are divided into three colors to represent each of the oxygen compartments: lung, muscle and blood. M, male; F, female. Percentages within the bars show the proportion of the total oxygen stores that each compartment represents. See Materials and methods for the assumptions made for these calculations and the references that support these assumptions.

Increasing muscle oxygen stores and decreasing mass-specific metabolic rate associated with increasing body size resulted in an increase in cADL with age (Fig. 5). As breath-hold capacity increases with maturity, walruses are afforded more submergence

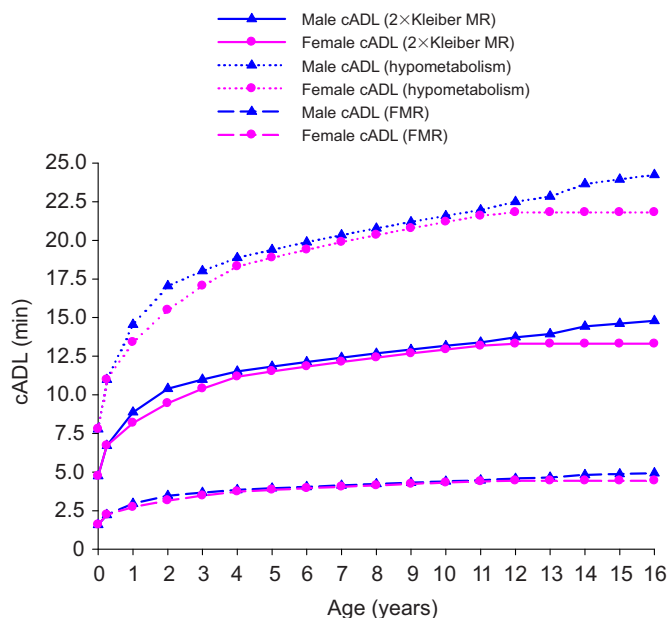


Fig. 5. Calculated aerobic dive limit (cADL) in relation to age for female and male Pacific walruses. Three metabolic rates (MRs) were assumed. The dotted lines represent cADLs based on hypometabolism [$2\times$ Kleiber MR (BMR; Kleiber, 1975) reduced by 39%], which likely overestimates physiological limits. The solid lines represent cADLs based on a MR equivalent to $2\times$ Kleiber MR, which has been shown to provide the best estimate of the diving capacity of mature marine mammals. The dashed lines represent cADLs based on field MR (FMR, equivalent to $6\times$ Kleiber MR), which likely underestimates the diving capacity of mature marine mammals, but could approximate those of immature marine mammals, which typically have elevated metabolism. See Materials and methods for the assumptions made for these calculations and the references that support these assumptions.

time. For example, when assuming a diving metabolism of $2\times$ Kleiber metabolic rate, a neonatal walrus can only dive aerobically for approximately 5 min, while a weaned 2–3 year old walrus can dive for 9–11 min. This compares with an ADL of 13–15 min for a fully grown adult female and male walrus, which affords deeper depth dives and longer bottom times to search for prey along the seafloor. Nonetheless, there is a trade-off between dive depth and bottom time (Fig. 6). For example, a newly weaned 2 year old walrus has 8–9 min of bottom time at a depth of 41 m, 6–7 min of bottom time at 80 m, 5–6 min of bottom time at 102 m, and only 1–2 min of bottom time at 200 m. This compares with 12–13 min, 10–11 min, 9–11 min and 5–6 min of bottom time for fully grown adult female and male walruses diving down to the same depths.

DISCUSSION

Although there have been several recent investigations on the ontogeny of myoglobin in pinnipeds (phocids and otariids; Table 2), the development of the muscle biochemistry that

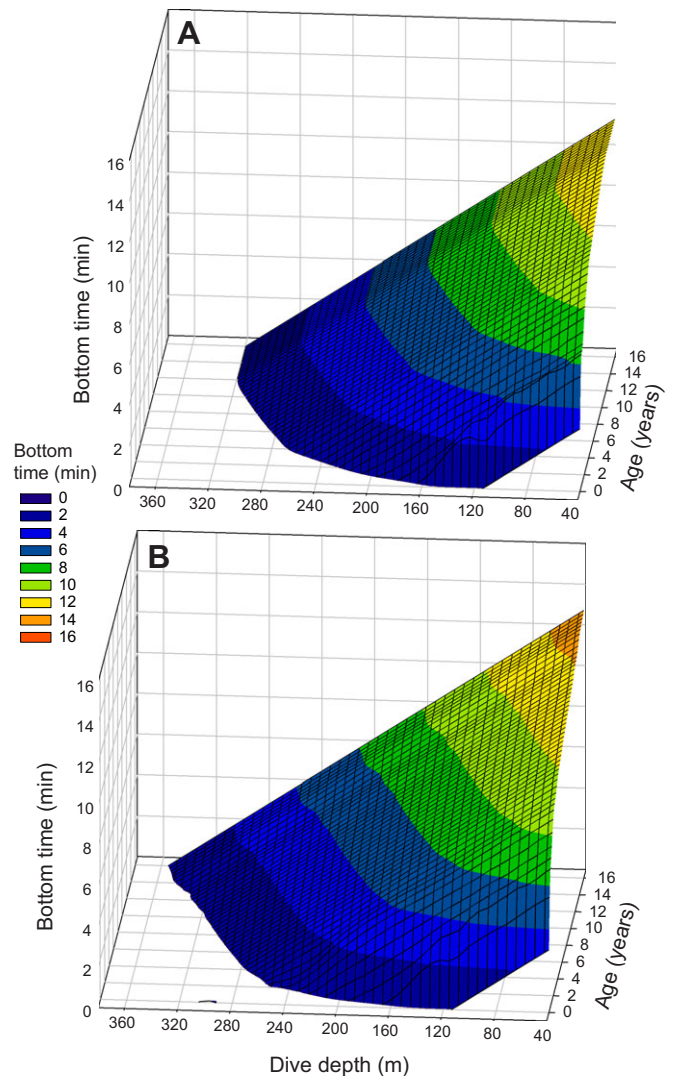


Fig. 6. Theoretical bottom time in relation to age and dive depth for Pacific walruses. (A) Female, (B) male. An ascent and descent swim speed of 0.8 m s^{-1} and a calculated aerobic dive time based on a diving metabolism of $2\times$ Kleiber MR were assumed to set the limits for bottom time at each depth. See Materials and methods for the assumptions made for these calculations and the references that support these assumptions.

Table 2. Ontogeny of myoglobin content in pinnipeds, with species listed by family according to increasing duration of dependency period

Species and Mb reference	Age class	Time into DP (%)	Mb content (g 100 g ⁻¹ muscle)
Phocids			
Harbor seal, <i>Phoca vitulina</i> DP=28 days Burns et al., 2005	Neonate (<7 days)	11	1.56 (41%)
	Nursing pup	50	2.28 (60%)
	Weaned pup	100	2.72 (71%)
	Yearling		4.22 (110%)
	Adult female and male (NS different)		3.83
Hooded seal, <i>Cystophora cristata</i> DP=35 days Burns et al., 2007	Neonate (<2 days)	3	3.15 (33%)
	Nursing (2–4 days)	9	2.93 (31%)
	PWF (5–14 days)	29	2.99 (32%)
	Yearling (12 months)	100	7.65 (81%)
	Adult female		9.48
Gray seal, <i>Halichoerus grypus</i> DP=45 days Noren et al., 2005	Newborn (3 days)	7	1.7 (43%)
	Weaned (17 days)	38	2.1 (53%)
	Mid-PWF (29 days)	64	2.5 (63%)
	Late PWF (41 days)	91	2.7 (68%)
	Yearling (1 year)	100	3.2 (80%)
	Adult female (>6 years)		4.0
Harp seal, <i>Pagophilus groenlandicus</i> DP=47 days Burns et al., 2007	Neonate (<2 days)	2	2.1 (24%)
	Nursing (3–12 days)	17	2.67 (31%)
	PWF (14–28 days)	45	3.42 (40%)
	Yearling (10–11 months)	100	5.7 (66%)
	Adult female		8.6
Northern elephant seal, <i>Mirounga angustirostris</i> DP=90 days Thorson, 1993	Nursing pup (<2 weeks)	8	2.9 (43%)
	Weaned (4 weeks)	31	3.4 (51%)
	Mid-PWF (8 weeks)	62	4.5 (67%)
	Late PWF (12 weeks)	93	5.1 (76%)
	Juveniles (8–10 months)	100	5.7 (85%)
	Adult female (3–12 years)		6.7
	Sub-adult male (3–6 years)		6.1
Adult male (8–12 years)		6.4	
Otariids			
California sea lion, <i>Zalophus californianus</i> DP=8 months Weise and Costa, 2007	5 months	42	2.36 (53%)
	9 months	75	2.41 (55%)
	Small juvenile (1.5–2.5 years)	100	2.95 (67%)
	Large juvenile		3.1 (70%)
	Adult female		4.42
	Sub-adult males		2.93
	Adult males		3.52
Australian sea lion, <i>Neophoca cinerea</i> DP=12 months Fowler et al., 2007	6 months	26	0.8 (30%)
	14.5 months	100	1.3 (48%)
	22.6 months		1.6 (59%)
	3 years		2.2 (81%)
	Adult female		2.7
Australian fur seal, <i>Arctocephalus pusillus doriferus</i> DP=12 months D. P. Costa (University of California, Santa Cruz, USA), personal communication	1 month	8	0.38 (9%)
	5 months	42	0.63 (15%)
	7 months	58	0.89 (21%)
	9 months	75	0.96 (23%)
	Adult female		4.16
Steller sea lion, <i>Eumetopias jubatus</i> DP=12 months Richmond, 2004	1 month	8	0.57 (20%)
	5 months	42	1.29 (45%)
	9 months	75	2.02 (70%)
	19 months	100	2.46 (86%)
	21 months		2.73 (95%)
	29 months		3.11 (108%)
	Adult female		2.87
	Adult males		4.9

Dependency period (DP) is the nursing interval plus any post-weaning fast (PWF). When a range for nursing durations is provided, the shortest period was assumed.

Values in parentheses are the percentage of the levels in adult females.

Harbor seal: 4 week nursing (Reeves et al., 1992), swim and dive at birth (Greaves et al., 2005); hooded seal: 4 day nursing (Reeves et al., 1992), 1 month PWF (Bowen et al., 1987); gray seal: 17 day nursing, 4 week PWF (Reeves et al., 1992); harp seal: 12 day nursing (Reeves et al., 1992), 5–6 week PWF (Sivertsen, 1941); Northern elephant seal: 28 day nursing, 2 months PWF (Reeves et al., 1992); California sea lion: 4–8 months, but frequently >1 year nursing (Reeves et al., 1992); Australian sea lion: at least 1 year nursing (Reeves et al., 1992); Australian fur seal: at least 1 year, rarely 2 year nursing (Reeves et al., 1992); Steller sea lion: 1, 2 and sometimes 3 year nursing (Reeves et al., 1992).

supports the diving breath hold in odobenids (the third group of pinnipeds) has received little attention. Unlike the majority of newborn pinnipeds, which primarily remain on land until they are weaned from their mother's milk, walrus enter the water within about 2 days of birth (Fay, 1982) and are often observed nursing underwater (Reeves et al., 1992). Walrus also have one of the longest nursing and maternal dependency periods among pinnipeds, as they have been observed nursing up to 35 months post-partum and are attended by their mothers for 2–3 years (Fay, 1982; Kovacs and Lavigne, 1992). Thus, odobenids have a unique life history pattern among pinnipeds in that they are precocial swimmers at birth despite having a prolonged maternal dependency period. Typically, pinniped pups demonstrate a tight correlation between the timing of muscle maturation and independence from their mother, marking the time that the pup must enter the water to forage (Table 2). Unlike the majority of other pinnipeds, entry into the water and maternal independence are uncoupled in the walrus. This provides a novel system to explore the mechanism(s) that might drive the maturation of these biochemical properties as well as to consider the ecological consequences for mothers that must forage alongside physiologically constrained offspring.

Development of muscle acid buffering

To date, the full developmental trajectory of the acid-buffering capacity of the locomotor muscle has only been investigated in four marine mammals [cetaceans: *Tursiops truncatus* (Noren, 2004) and *Phocoena phocoena* (Noren et al., 2014a); pinnipeds: *Cystophora cristata* and *Pagophilus groenlandicus* (Lestyk et al., 2009; Burns et al., 2010)]. Based on this limited dataset, maturation of acid-buffering capacity in marine mammals appears to be protracted. Adult levels are attained after the age of weaning, at least 25 days post-partum in pinnipeds (Lestyk et al., 2009) to greater than 1.5 years post-partum in cetaceans (Noren, 2004; Noren et al., 2014a). Of the species measured to date, Pacific walrus are unique in that they are born with a mature locomotor muscle acid-buffering capacity (Table 1, Fig. 2A).

For pinnipeds, it appears as though impending exposure to exercise and hypoxia in the aquatic environment was the evolutionary driver that promoted locomotor muscle readiness, as is the case for the walrus that must follow its mother into the water soon after birth. Oddly, results from studies on cetacean species do not align with this assumption. The postnatal maturation period required for cetaceans (dolphins, porpoises and whales) to attain adult levels of muscle acid-buffering capacity is prolonged, despite cetacean neonates being born directly into the ocean (Noren, 2004; Noren et al., 2014a). The disparate results between walrus and cetaceans may be associated with differences in buffering capacity among adults. The acid-buffering capacity of the longissimus dorsi is substantially lower in adult walrus (41.30 slykes; Table 1) compared with adult cetaceans (63.70–94.50 slykes; Noren, 2004) and other adult pinnipeds (81.1–81.9 slykes; Burns et al., 2010). In addition, the acid-buffering capacity of walrus muscle is low even when compared with the acid-buffering capacity of the locomotor muscle of adult terrestrial mammals (49.70–66.9 slykes; Castellini and Somero, 1981). Thus, postnatal development of this biochemical property may not be necessary in walrus because the acid-buffering capacity of their locomotor muscle is not above levels found in terrestrial mammals. It is possible that the swim speeds needed to capture sedentary or slow-moving benthic prey, and the short dive durations (Jay et al., 2001) for their large body size, have not

asserted adequate evolutionary pressure for increasing muscle acid-buffering capacity in walrus. Indeed, marine mammal species that exhibit fast swim speeds and/or prolonged dive durations typically have increased muscle acid-buffering capacity (Noren, 2004; Velten, 2012).

Development of muscle myoglobin

The demands of exercise and hypoxia have also been thought to promote the maturation of myoglobin content (Morrison et al., 1966; Stephenson et al., 1989; MacArthur, 1990; Saunders and Fedde, 1991; Kanatous et al., 2009; Geiseler et al., 2013). Detailed studies of the ontogeny of myoglobin content in aquatic birds (Hagglom et al., 1988), penguins (Weber et al., 1974; Ponganis et al., 1999; Noren et al., 2001) and pinnipeds (Thorson, 1993; Noren et al., 2005; Burns et al., 2005, 2007; Richmond, 2004; Fowler et al., 2007; Weise and Costa, 2007) have demonstrated that myoglobin content increased as immature animals increased exposure to physical activity, thermal demands and hypoxia (Noren et al., 2001). Yet, these factors fail to explain patterns observed for cetaceans, which have prolonged myoglobin maturation periods despite facing the demands of activity, thermoregulation and hypoxia immediately at birth (for review, see Noren et al., 2014a). Interestingly, species-specific myoglobin maturation durations among odontocetes were correlated with the calf nursing interval, suggesting that the rate of muscle maturation evolved to match maternal dependency periods (Noren et al., 2014a).

Closer examination of myoglobin maturation patterns across pinnipeds demonstrates a delineation between phocids (true seals) and otariids (fur seals and sea lions). Phocids typically show more rapid development of myoglobin content compared with otariids (Table 2). The rapid muscle development of phocids was attributed to early entry into water and short maternal dependency periods (nursing plus any post-weaning fast: 28–90 days; Table 2) compared with the protracted muscle development of otariids, thought to be a consequence of late entry into water and prolonged maternal dependency periods (>1 year; Table 2; Burns et al., 2004; Noren et al., 2005; Richmond et al., 2006). However, in these two groups, pup independence is generally coupled with entry into the water, making it difficult to discern which of the two factors (maternal dependency period versus exposure to hypoxia) has driven the pattern of muscle development over evolutionary time.

Walrus demonstrate rapid myoglobin maturation (Table 1, Fig. 2B). Within a short period after birth, the myoglobin content of the longissimus dorsi of walrus neonatal calves represents 44% of adult levels, and by 3 months post-partum, the levels are 79% of adult levels. This is one of the most rapid developmental trajectories of myoglobin among pinnipeds, and is similar to the developmental patterns observed for all phocid species studied to date (see Table 2 for review). The results from walrus suggest that perhaps exposure to physical activity, thermal demands or hypoxia, but not the duration of the maternal dependency period, may define species-specific myoglobin maturation patterns among pinnipeds. Alternative hypotheses have suggested that inter-specific variation in myoglobin maturation across species is due to differences in early growth rates (Burns et al., 2004) and iron availability associated with fasting (Burns and Hammill, 2008). Yet, despite dolphins and walrus both having prolonged growth phases and being consistently nursed, the myoglobin maturation period of dolphins is prolonged (Noren et al., 2001) while that of walrus is truncated (present study). Additional research is required to elucidate the mechanism(s) behind the disparate maturation patterns found across aquatic air-breathing animals.

Limited samples from females precluded us from analyzing sex-specific differences in the developmental trajectory of myoglobin in Pacific walrus. Of the studies that have examined myoglobin maturation in pinnipeds, only four have examined sex-specific differences (Table 2). Within phocids, adult myoglobin levels are similar across males and females for both the harbor seal (*Phoca vitulina*) and the sexually dimorphic Northern elephant seal (*Mirounga angustirostris*). In contrast, sex-specific differences in myoglobin levels were identified in two sexually dimorphic otariid species (Table 2), but the results were inconsistent. For Stellar sea lions (*Eumetopias jubatus*), adult males had 1.7× more myoglobin per gram of muscle than female conspecifics, but in California sea lions (*Zalophus californianus*), adult females had 1.3× more myoglobin per gram of muscle than male conspecifics. It is likely that sex-specific foraging behaviors and the magnitude of sexual dimorphism work in concert to influence the magnitude of the onboard muscle oxygen store.

Diving capacity of walrus

Walrus are among the largest pinniped species, which should grant them an intrinsic advantage for prolonged breath holds because the oxygen-carrying capacity of the body increases with body mass by the power of 1.0 while metabolic rate increases with body mass by the power of 0.75 (Kleiber, 1975). However, contrary to this expectation, the recorded maximum dive duration of adult male Pacific walrus is only 15.2 min (Jay et al., 2001) and they are rarely observed to venture into water deeper than 80 m (Vibe, 1950; Fay, 1982). It is likely that Pacific walrus are physiologically capable of deeper dives; indeed, Fay and Burns (1988) observed that Pacific walrus killed on ice floes over deep water (102–117 m) had fresh, undigested infaunal and epifaunal mollusks and crustaceans in their stomachs. Therefore, it is likely that walrus choose to stay in shallow water because their preferred prey is most abundant on the shallow continental shelf (Fay and Burns, 1988; Born et al., 2005; Jay et al., 2012). This behavior is in agreement with the idea that it is advantageous for benthivores to stay in shallow water to minimize transit time to the benthos and thereby maximize foraging time on the seafloor, because foraging time decreases with water depth (Costa and Gales, 2003).

To determine whether the physiology of Pacific walrus can support longer dive durations and deeper dive depths than previously observed, we calculated ADL. Based on calculations that assumed a diving metabolism of 2× Kleiber metabolic rate, a mature 830 kg female and 1200 kg male can dive aerobically for up to 13.3 and 14.8 min, respectively (Fig. 5). When ascending and descending at a speed typical of diving walrus (0.8 m s^{-1} ; Gjertz et al., 2001; Jay et al., 2001), females and males can achieve maximum dive depths of 319 and 355 m, respectively (Fig. 6). This calculation is supported by observations of dive depths of >250 m in Atlantic walrus (Born et al., 2005). However, deep dives such as these would not allow walrus much time to search for prey on the seafloor (Fig. 6). Thus, there is a trade-off between achieving greater foraging depths and having adequate submergence time to locate, handle and consume benthic prey (Fig. 6). Indeed, the median foraging depth recorded for four adult male Pacific walrus in Bristol Bay, AK, USA, was 41 m, and at this depth they spent a median of 5–6 min along the benthos (Jay et al., 2001). According to the calculated ADL determined in this study for a 1200 kg mature adult male walrus, 13 min of bottom time would be permitted at this depth (Fig. 6). This suggests that these walrus had considerable flexibility to alter behavior, in terms of increasing search time along the benthos.

Assuming that myoglobin levels are similar between males and females, as found for the sexually dimorphic Northern elephant seal (Thorson, 1993), the greater body size of male walrus could provide males with an advantage over females when competing for food in resource-limited habitats. This could occur when males and females overlap in distribution during the winter breeding season (Fay, 1982). Indeed, differences in diving behavior have been documented in other sexually dimorphic pinnipeds, such as gray (*Halichoerus grypus*; Beck et al., 2003) and southern elephant (*Mirounga leonina*; McIntyre et al., 2010) seals, which supports the hypothesis that body size is a limiting factor on the physiological capacity for diving. For elephant seals, sex-specific differences in dive depths were associated with differences in prey selection between males and females; this could be a mechanism to minimize inter-sexual competition (McIntyre et al., 2010). For walrus, there is no evidence of resource partitioning between sexes when they overlap in distribution during the winter. Thus, inter-sexual competition could be problematic for female walrus if prey availability decreases in the Arctic because the typical response of pinnipeds to low prey availability is to increase dive duration (Feldkamp et al., 1989; Crocker et al., 2006; Melin et al., 2008), and breeding females will be most at risk as they have elevated energetic requirements because of the costs of lactation (Noren et al., 2012, 2014b). In addition, maternal foraging behaviors could be constrained by the physiological limits of their progeny. Although neonatal calves accompany their mothers almost all the time, including during foraging trips (Kovacs and Lavigne, 1992; Charrier et al., 2010), there is no evidence that calves dive alongside their mothers all the way to the seafloor. If neonatal calves must accompany their mothers to the seafloor, maternal foraging behaviors would be constrained by the physiological limits of their calves, which only have a cADL of 4.7 min. This limit provides 3 min of bottom time for a 41 m dive and only 1 min of bottom time for an 80 m dive. However, this cADL does support the submergence times required for the calf to nurse underwater, which last from about 0.5 to 2.0 min (Miller and Boness, 1983).

Even by the age of weaning (approximately 2 years post-partum; Fay, 1982), the dive capacity of immature walrus is considerably lower than that of adults (Fig. 6). Differences in diving ability at this stage are associated with differences in body size because, by weaning, the blood oxygen stores of walrus are mature (S.R.N., unpublished), and it is likely that the muscle oxygen store is mature, based on the observation that the longissimus dorsi myoglobin content was already 79% of adult levels by 3 months post-partum (Table 1). Although there are no published accounts of the diving behaviors of immature walrus for comparison, studies on other pinnipeds have demonstrated shorter dive durations, shallower dive depths and a lower percentage of time spent diving by immature seals compared with adult conspecifics (Thorson, 1993; Lydersen and Hammill, 1993; Lydersen et al., 1994; Horning and Trillmich, 1997; McCafferty et al., 1998; Bowen et al., 1999; Burns, 1999; Greaves et al., 2005; Fowler et al., 2006; Rehberg and Burns, 2008). The dive durations of immature pinnipeds increase with age as body size increases (Kooyman et al., 1983; Lydersen and Hammill, 1993; Thorson, 1993; Lydersen et al., 1994; Burns and Castellini, 1996; Burns et al., 1998; Horning and Trillmich, 1997; Burns et al., 1998; Costa et al., 1998; McCafferty et al., 1998; Burns, 1999), and these ontogenetic differences in diving capacity have been associated with inter-age class variations in diet (Bowen et al., 1999; Field et al., 2007; Jeglinski et al., 2012). Yet, unlike these other pinnipeds, independent juvenile walrus consume the same prey as adults, as suggested by their stomach contents (Fay, 1982), which could result

in competition between juvenile and adult walrus for food if prey became limited. The lower breath-hold capacity and inexperience with foraging of independent immature walrus could result in their being disproportionately affected during periods of low prey availability, as has been observed in other immature pinnipeds (Trillmich and Limberger, 1985; DeLong et al., 1991).

Conclusions

Among the pinniped species with prolonged maternal dependency periods, Pacific walrus demonstrate the most rapid maturation of the muscle biochemistry that supports breath hold while diving. Pacific walrus are unique in that they are born with a mature locomotor muscle acid-buffering capacity; however, the level attained by adulthood is low compared with that of adult cetaceans and other pinnipeds. The development of myoglobin in walrus is one of the most rapid among pinnipeds. Additional research is required to elucidate the mechanism(s) behind the disparate muscle maturation patterns found across aquatic air-breathing animals. As for other young marine mammals, the smaller body size of independent immature walrus could make them competitively disadvantaged, in terms of diving capacity, in a resource-limited environment. In addition, the extreme sexual dimorphism in this species grants adult males a competitive advantage over adult females in terms of breath-hold capacity to support prey exploitation.

MATERIALS AND METHODS

Specimen and muscle collection

Muscle samples were collected during subsistence harvest of free-ranging Pacific walrus. Neonatal calves ($N=5$; 1 male, 3 females, 1 unknown) were harvested from Gambell, AK, USA. Sexually mature adults were harvested from Gambell, AK, USA ($N=2$; 1 male, 1 female) and at Round Island, AK, USA ($N=18$ males). The US Geological Survey's Walrus Research program is permitted to collect and possess biological samples from walrus salvaged from the Alaska Native subsistence harvest under the US Fish and Wildlife Service Marine Mammal Scientific Research permit no. MA801652-7, which is issued by the Office of Management Authority under regulation 50 CFR 18.31 of the US statute 16 USC 1371 (a) (1). Additional samples were taken from a full-term, stillborn male fetus at Six Flags Discovery Kingdom (Vallejo, CA, USA) and an orphaned immature male walrus (3 months old) that was euthanized at the Alaska SeaLife Center (Seward, AK, USA). Collection and laboratory protocols were approved under University of California, Santa Cruz (UCSC) IACUC NORES1306.

Whole carcasses were typically sampled immediately after death. Whole carcasses that could not be sampled immediately were frozen in a 'snow cellar', with sampling occurring up to a maximum of 12 days postmortem. A minimum of 10 g of muscle was sampled from the major swimming muscle (longissimus dorsi). This muscle was sampled at a location approximately three finger widths above the hip; the same site was sampled from phocids, which also use hind flipper propulsion (Noren et al., 2005). For a subset of walrus (the fetus, all neonatal calves, the immature walrus and 9 adults), the supraspinatus was also sampled. This site was also sampled from otariids that use front flipper propulsion (Weise and Costa, 2007). Muscle samples from the subsistence hunts were kept chilled with ice and snow in a cooler and put into a -7°C freezer, shipped frozen to UCSC, and stored in a -20°C freezer until muscle biochemical analyses were performed within 6 months of collection. Muscle samples obtained from the fetus and immature animal were immediately frozen in a -20°C freezer after sampling.

Muscle biochemical analyses

To examine a component of the anaerobic capacity of the muscle, we explored the ability of the muscle to buffer against lactic acid. The muscle buffering capacity (β) due to non-bicarbonate buffers was determined using procedures of Castellini and Somero (1981), adapted by Noren (2004).

Briefly, thawed muscle samples (approximately 0.5 g) were minced in 10 ml normal saline solution (0.9% NaCl), and sonicated (Sonifier Cell Disrupter Model 450, Branson Ultrasonics Corporation, Danbury, CT, USA) for 3 min on ice. Samples were maintained at 37°C by immersion of the test flask in a warm water bath and titrated with 0.2 N NaOH. Buffering capacity was measured in slykes (μmoles of base required to raise the pH of 1 g wet muscle mass by one pH unit, over the range of pH 6.0 to pH 7.0). Changes in pH were measured using an Accumet basic pH $\text{mV}^{-1} \text{ }^{\circ}\text{C}^{-1}$ meter (AB15+, Fisher Scientific) with an Accumet liquid-filled, glass-body single-junction combination pH Ag/AgCl Electrode (13-620-285, Fisher Scientific) and separate ATC probe (13-620-19, Fisher Scientific). All samples were run in triplicate with a muscle sample obtained from a harbor porpoise (*Phocoena phocoena*), which served as a control because the acid-buffering capacity of this specimen was determined previously (Noren et al., 2014a).

To examine the oxygen storage capacity in the muscle, myoglobin content ($[\text{Mb}]$, reported in $\text{g Mb } 100 \text{ g}^{-1}$ wet muscle) was determined using the procedure of Reynafarje (1963), which was adapted for marine mammals by Noren and Williams (2000). Approximately 0.5 g of thawed muscle was minced in a low ionic strength buffer (40 mmol l^{-1} phosphate, pH 6.6) and then sonicated (Sonifier Cell Disrupter Model 450, Branson Ultrasonics Corporation) for 2–3 min on ice. The buffer to tissue ratio was 19.25 ml buffer g^{-1} wet tissue. The samples were centrifuged at -4°C and 28,000 g for 50 min (Sorvall RC-5C Plus superspeed refrigerated centrifuge, DuPont Instruments). The clear supernatant was extracted and then bubbled at room temperature with pure CO for approximately 8 min. To ensure a complete reduction, 0.02 g of sodium dithionite was added. The absorbance of each sample was read at room temperature at 538 and 568 nm on a spectrophotometer (Shimadzu UV – visible recording spectrophotometer UV-160, Shimadzu Corporation, Kyoto, Japan). All samples were run in triplicate with a muscle sample obtained from a harbor porpoise (*P. phocoena*), which served as a control because the myoglobin content of this specimen was determined previously (Noren et al., 2014a).

Modeling breath-hold limits

The cADL was determined by dividing calculated total body oxygen stores by estimates of diving metabolic rate following methods described in Kooyman (1989). The cADL can accurately predict experimentally determined ADL (Kooyman, 1989; Kooyman and Ponganis, 1998) when estimates of body oxygen stores and metabolic rate are reliable (Ponganis et al., 1997). We maximized the reliability of our calculations by utilizing species-specific oxygen store data and we considered a range of metabolic rates. Details regarding the assumptions that were made for these calculations are provided below.

The calculations for the oxygen storage capacity of the blood (in l) are as follows:

$$\text{Arterial O}_2 = (0.33 \times \text{BV} \times M) ([\text{Hb}] \times 0.00134) (0.95 - 0.20 \text{ saturation}), \quad (1)$$

$$\text{Venous O}_2 = (0.67 \times \text{BV} \times M) ([\text{Hb}] \times 0.00134) (0.95 \text{ saturation} - (0.05 \times 0.95 \text{ saturation})), \quad (2)$$

where 0.33 and 0.67 are the estimated proportions of arterial and venous blood, respectively (Lenfant et al., 1970), the blood volume (BV) of walrus is $0.106 \text{ l blood kg}^{-1}$ (Lenfant et al., 1970), and age-specific body mass (M) was derived from fig. 21 in Fay (1982), which provided a graphical representation of age versus measurements of body mass from free-ranging Pacific walrus. S.R.N. (unpublished) found that the hemoglobin content ($[\text{Hb}]$) of walrus did not vary with age; therefore, the average value of $163.5 \text{ g Hb l}^{-1}$ of blood was used in the calculation for all age classes. The oxygen-binding capacity of hemoglobin is $1.34 \text{ ml O}_2 \text{ g}^{-1} \text{ Hb}$ (Kooyman, 1989), and the proportion of saturation and depletion of arterial and venous oxygen reserves is described in detail in Ponganis (2011).

The calculation for the oxygen storage capacity of the muscle (in l) is as follows:

$$\text{Muscle O}_2 = ([\text{Mb}] \times 0.00134) M_m, \quad (3)$$

where [Mb] is myoglobin content in g per 100 g wet muscle mass, $1.34 \text{ ml O}_2 \text{ g}^{-1} \text{ Mb}$ is the oxygen-binding capacity of Mb (Kooyman, 1989) and M_m is total muscle mass in the body. Total muscle mass was calculated as $M_m = 0.2410 \times M^{1.084}$ (equation derived from dissection of various aged male and female Atlantic walrus; Knutsen and Born, 1994), where M was determined as described above. Observations of swimming walrus suggest that hind flipper propulsion is the dominant form of locomotion (Fay, 1985); thus, following the approach of studies of phocids, which also use hind flipper propulsion, age-specific Mb levels from the longissimus dorsi were assumed for all muscle groups for the purpose of calculating total muscle oxygen storage capacity (i.e. Noren et al., 2005). Although the approach of assuming one myoglobin level across all muscle groups is consistent with previous studies that have calculated the muscle oxygen stores of marine mammals, this approach can potentially overestimate the muscle oxygen store as non-swimming muscles of marine mammals generally have lower myoglobin levels (e.g. Polasek and Davis, 2001; Lestyk et al., 2009).

The calculation for the oxygen storage capacity of the lung (in l) is as follows:

$$\text{Lung O}_2 = M \times \text{TLV} \times 0.50 \times 0.15, \quad (4)$$

where body mass (M) was determined as described above and total lung volume (TLV) is 0.116 l kg^{-1} based on the measured lung capacity of three, 4–6 week old walrus calves (Lenfant et al., 1970). We assumed that the diving lung volume for a pinniped is 50% of TLV, with 15% representing the oxygen concentration of the air in the lungs which is extracted during the dive (Ponganis, 2011). Because of a lack of data on how lung volume changes with age in walrus, we assumed a constant mass-specific lung volume as in previous pinniped studies (e.g. Noren et al., 2005). Future research on how lung volume changes with maturation is warranted as recent studies on other marine mammals show opposite trends; the mass-specific lung volume of sea otters (*Enhydra lutra*; Thometz et al., 2015) decreases with maturation while the mass-specific lung volumes of two phocid species increase with age (J.M.B., unpublished).

Across air-breathing, diving vertebrates, including three Odontocete species (Noren et al., 2012), shallow-diving emperor penguins (*Aptenodytes forsteri*; Ponganis et al., 2010) and freely diving Weddell seals (*Leptonychotes weddelli*; Castellini et al., 1992; Ponganis et al., 1993), a cADL that assumed an oxygen consumption rate of $2 \times$ Kleiber basal metabolic rate (BMR; Kleiber, 1975) best approximated experimentally determined ADLs. Based on these findings, we assumed a metabolic rate of $2 \times$ BMR for our cADLs to estimate age-specific bottom times at various dive depths, where BMR (in $\text{l O}_2 \text{ min}^{-1}$) is $0.0101 \times M^{0.75}$ (Kleiber, 1975). To calculate bottom time during a dive, we assumed a swim speed of 0.8 m s^{-1} , which is the typical ascent and descent swim speed of foraging adult walrus (Gjertz et al., 2001; Jay et al., 2001). Thus, total bottom time at each depth was calculated according to:

$$\text{Bottom time} = \text{cADL} - (0.8 \times 2d), \quad (5)$$

where cADL is the calculated ADL (in s), 0.8 is swim speed (in m s^{-1}) and d is the depth of the dive (in m).

Without empirical data on the age-specific diving metabolism of walrus, additional cADLs were determined, to provide a range for the diving capacities of walrus. cADLs that assumed hypometabolism during submergence were considered based on the observation that California sea lions (*Zalophus californianus*) can reduce resting-surface metabolic rates of $2 \times$ BMR by 39% when sedentarily submerged (Hurley and Costa, 2001). This assumption likely overestimates cADLs as free-ranging diving walrus are not sedentary. cADLs that assumed oxygen consumption rates during activity were also determined by using the average field metabolic rate (FMR) of two adult male Atlantic walrus (approximately $6 \times$ BMR; Acquarone et al., 2006). However, the use of a FMR could underestimate cADLs because marine mammals use oxygen-conserving strategies while diving, such as the dive response (Scholander, 1963) and stroke and glide propulsion (Williams et al., 2000). Nonetheless, an elevated diving metabolism may be the best model for the diving capacity of

immature walrus as Weddell seals (*Leptonychotes weddelli*) demonstrate a strong developmental effect on diving metabolism, where experimentally determined ADLs for pup, yearling and adult Weddell seals were best approximated by cADLs that assumed a diving metabolism of $4 \times$ BMR, $2 \times$ BMR and $1 \times$ BMR, respectively (for review of these data, see Schreer et al., 2001).

Statistics

Limited samples from females precluded us from analyzing sex-specific differences in the developmental trajectory of muscle biochemistry within Pacific walrus. Thus, samples across males and females were combined for statistical analyses. Differences in muscle acid-buffering capacity and myoglobin content between the supraspinatus and longissimus dorsi within the neonatal calf and adult age classes were tested using paired Student's t -tests. Differences in muscle acid-buffering capacity and myoglobin content of the supraspinatus and longissimus dorsi between the neonatal calf and adult age classes were tested using Student's t -tests. Only one fetus and one immature 3 month old walrus were sampled, so they were not included in the statistical comparisons. The interaction between the ratio of acid-buffering capacity to myoglobin content with age-related changes in myoglobin content in the supraspinatus and longissimus dorsi was examined with non-linear regression analyses. All statistical analyses were conducted using SigmaStat 3.5 (Systat Software, Inc.).

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Competing interests

The authors declare no competing or financial interests.

Author contributions

S.R.N. developed the approach, performed the majority of the laboratory and data analyses, and prepared the manuscript. C.V.J. secured funding for the study and helped prepare the manuscript. J.M.B. performed the laboratory analyses for the adult muscle samples. A.S.F. went into the field in Alaska to secure the muscle samples from the neonatal calves and adult walrus.

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