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ORIGINAL PAPER

Links between muscle phenotype and life history: differentiation of myosin heavy chain composition and muscle biochemistry in precocial and altricial pinniped pups

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Abstract

In marine mammals, muscular development has been identified as a rate-limiting factor in achieving adult dive capacities. This study investigates the rate that myosin heavy chain (MHC) composition matures in a postural and locomotor skeletal muscle for four pinniped species with different lactation lengths: hooded seals, *Cystophora cristata*; harp seals, *Pagophilus groenlandicus*; northern fur seals, *Callorhinus ursinus,* and Steller sea lions, *Eumetopias jubatus*. The ontogeny of MHC isoform expression was compared with developmental rates of myoglobin concentrations, and aerobic (citrate synthase, β-hydroxyacyl-CoA dehydrogenase) and anaerobic (lactate dehydrogenase) enzyme activities. Within taxonomic families, species with shorter lactation periods had more mature muscles biochemically at birth, and fiber types differentiated earlier during ontogeny (Phocidae: hooded > harp seals, Otariidae: northern fur seals > Steller sea lions). Northern fur seal neonates had the most phenotypically-mature muscles in this study, with no immature MHC isoforms. The relationship between muscle biochemistry and MHC composition became more pronounced with age, and developed to reflect swimming mode and activity levels. In adults, phocids had more slow-twitch oxidative protein in their primary locomotor muscle, the *Longissimus dorsi* (*LD*), than otariids which likely reflects oxygen-sparing strategies for the phocids' longer dives. Conversely, northern fur seal muscles had higher proportions of fast-twitch MHCs in the *Pectoralis* and *LD*, likely indicative of this species' smaller size and higher mass-specific metabolic rates. Thus, muscle phenotype is linked with species life history, and a mismatch between muscle biochemistry and MHC composition at weaning has important implications for the first year of independent foraging in pinniped pups.

Keywords Myosin heavy chain · Muscle · Pinniped · Myoglobin · Enzymes · Diving physiology

Abbreviations

CS	Citrate synthase (IU g^{-1} wet tissue)
FOG	Fast-twitch oxidative glycolytic
HOAD	β-Hydroxyacyl-CoA dehydrogenase (IU g^{-1} wet tissue)
LD	Longissimus dorsi
LDH	Lactate dehydrogenase (IU g^{-1} wet tissue)
Mb	Myoglobin (mg g^{-1} wet tissue)
MHC	Myosin heavy chain
O_2	Oxygen
Pec	Pectoralis
PWF	Post-weaning fast
SO	Slow-twitch oxidative

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Introduction

During ontogeny, young animals face an energetic tradeoff between developing mature physiological traits versus allocating resources towards growth in mass and size (Ricklefs et al. 1994). Precocial animals are generally born at a larger body size and with more mature physiology and subsequently experience slower rates of neonatal growth as compared with altricial species (McLaren 1993; Choi et al. 1993; Ricklefs et al. 1994). This has ecological significance because the time needed to reach physiological maturity directly correlates with the length of the neonatal dependency period (i.e., lactation, nestling) and/or degree of maternal investment (Derrickson 1992). While maturation rates may differ among organ systems, the rate at which muscles mature may be particularly crucial because the success of both foraging and predator avoidance strategies is contingent on the newly independent juveniles having sufficiently mature skeletal muscles to perform effectively.

Across vertebrates, neonates are generally born with immature muscle phenotypes and function. This includes lower myoglobin (Mb) concentrations, mitochondrial and capillary density, and mass-specific aerobic and anaerobic enzyme activities than in adults (Condon et al. 1990; Baldwin and Haddad 2001; Richmond et al. 2006; Lestyk et al. 2009; Prewitt et al. 2010; Shero et al. 2012; Burns et al. 2007, 2015; Kanatous et al. 2008). In addition to developing the biochemical properties necessary to support oxygen (O_2) and energetic substrate delivery, the muscle's myofibrillar protein composition also determines ATPase activities and energy use for muscular contractions (Baldwin and Haddad 2001). Slow- and fast-twitch fibers are characterized by the speed of myosin cross-bridge cycling rates and contraction velocity (Baldwin and Haddad 2001), and the relative proportion of slow and fast myosin heavy chain (MHC) isoforms changes across development and in response to activity patterns (Baldwin and Haddad 2001). In vertebrates, neonatal muscles typically contain greater proportions of slow-oxidative (SO) MHCs, as well as immature Embryonic and Neonatal fiber types and MHC isoforms (d'Albis et al. 1991). Conversely, fast-twitch oxidative-glycolytic (FOG) MHCs support burst-type activities, but heavier reliance on glycolytic pathways makes these fibers more prone to fatigue (Flück 2006; Hoppeler and Flück 2002). During ontogeny, the fiber type profile of any given muscle shifts from containing more Embryonic/Neonatal and SO MHCs towards a more FOG profile. In this process, Embryonic and/or Neonatal fibers are gradually replaced by MHC I (SO), MHC IIA (FOG; primarily oxidative), and finally MHC IID/X (FOG; primarily glycolytic) and/or MHC IIB (fast-twitch glycolytic; FG) fibers. Muscles that contain more MHC I and SO isoform generally have the corresponding biochemical traits, such as high mitochondrial content, and greater citrate synthase (CS) and β-hydroxyacyl-CoA-dehydrogenase (HOAD) activities for aerobic metabolism and reliance on lipid stores (Flück 2006; Hoppeler and Flück 2002; Kanatous 1997; Kanatous et al. 1999). Conversely, muscles that contain more MHC IIA and IID/X isoform have more anaerobic potential and high lactate dehydrogenase (LDH) activities (Flück 2006; Hoppeler and Flück 2002). The regulation of this developmental process depends on multiple factors, ranging from neural stimuli and load-bearing activity, nutritional status, hormone levels, to genetic predisposition (Walker and Luff 1995).

The underwater foraging activities of adult marine mammals are facilitated by a suite of physiological adaptations that include large endogenous blood and muscle O_2 stores, and slower O_2 use rates (Hochachka and Storey 1975; Butler and Jones 1997; Kooyman and Ponganis 1998). While hematology and blood O_2 reserves of both phocid and otariid species mature relatively quickly, muscles develop more slowly (Richmond et al. 2006; Burns et al. 2007; Clark et al. 2007; Shero et al. 2012). This may be particularly impactful because during long and deep dives, peripheral vasoconstriction reduces muscular perfusion, forcing reliance on endogenous reserves and/or anaerobic metabolism (Irving et al. 1942; Scholander 1963; Ponganis et al. 2011). To compensate, pinniped muscles have Mb concentrations that are 10–20× that of terrestrial mammals and muscle fibers that are indicative of endurance capacities (Burns et al. 2007; Lestyk et al. 2009; Davis et al. 2004; Kanatous 1997; Kanatous et al. 1999). Without this mature physiology, at the onset of independent foraging pinniped pups make substantially shorter and shallower dives than their adult counterparts (Burns et al. 1999; Rehberg and Burns 2008; Fowler et al. 2006; Geiseler et al. 2013; Folkow et al. 2010). Once mature, phocid seals generally have greater O_2 stores, a more pronounced dive response, and attain much longer dive durations as compared with otariids (e.g., mean dive duration of phocids—hooded seal, *Cystophora cristata*: 14 min, Folkow and Blix 1999; harp seal, *Pagophilus groenlandicus*: 8.1 min, Folkow et al. 2004 vs. otariids—northern fur seal, *Callorhinus ursinus*: 2.2 min, Gentry et al. 1986; Steller sea lion, *Eumetopias jubatus*: 1.8 min, Rehberg et al. 2009), which is likely reflected in muscle physiological properties.

Each muscle's specific functional role further promotes development of highly differentiated phenotypes, and after birth the primary locomotor muscles mature faster and have higher aerobic capacities than muscles responsible for postural purposes (Burns et al. 2015; Lestyk et al. 2009; Choi et al. 1993). In otariids, the (forelimb) *Pectoralis* (*Pec*) is the primary locomotor muscle, while the *Longissimus dorsi* (*LD*) is primarily used for postural purposes and much less

for swimming. Conversely, phocids utilize a hind-limb swimming style and the *LD* is the primary locomotor muscle, whereas the *Pec* is used for postural purposes and steering during dives (Howell 1929). While developmental and muscle-specific shifts in biochemical properties have been characterized, the degree to which these changes are synchronized with changes in muscle MHC profiles that control contraction velocity and promote the slow and efficient use of O_2 stores is not well known. Whether maturation of biochemistry and MHC occur in concert specifically in the *Pec* and *LD* muscles in pinnipeds would be crucial to developing diving and foraging capabilities.

Among pinnipeds, there is also a wide range in the duration of lactation, which reflects different offspring provisioning strategies (Costa and Shaffer 2012). Phocid seals are typically capital breeders, relying on their on-board mass and lipid reserves (i.e., energetic capital) to provision offspring while females fast throughout the lactation period, and therefore, the nursing period is kept short (Costa et al. 1986; Kovacs and Lavigne 1992; Kovacs et al. 1991; Mellish et al. 1999; Crocker et al. 2001; Wheatley et al. 2006). In the high arctic, transient pack ice and predation pressures from polar bears (*Ursus maritimus*) further constrain lactation lengths. For example, hooded seals have the shortest lactation length of any mammal, lasting just 3–7 days before pups are weaned (Bowen et al. 1985), while the sympatric harp seal lactation period lasts 7–14 days (Sivertsen 1941). Because phocid seal pups are weaned relatively soon after birth, they then undergo a post-weaning fast (PWF) during which they remain hauled-out and inactive, catabolizing their newly acquired lipid reserves (Worthy and Lavigne 1987; Lydersen et al. 1997). The PWF is presumed to provide pups more time for physiological development prior to independent foraging (Burns et al. 2004) while decreasing the time that post-partum females are exposed to threats of predation (Stirling 1977) and reducing metabolic overhead (Crocker et al. 2001), thereby improving transfer efficiencies of maternal energy to her pup. This is in contrast to species in the otariid family which utilize an income-breeding strategy. Female otariids alternate between nursing their pups and foraging to recuperate mass, and lactation lengths in the otariid family are measured in months, as opposed to days or weeks as in phocids (Costa and Shaffer 2012). For example, northern fur seal females have the shortest lactation period of any otariid, and yet, they nurse their pups for ~4 months (Baker and Donohue 2000). In contrast, Steller sea lions have one of the longer lactation periods among the otariids, with pups nursing for a minimum of a year, and some continuing to nurse through their second and even into a third year (Calkins and Pitcher 1982). However, unlike most phocid and otariid pups, nursing Steller sea lions begin swimming and diving well before they are weaned (Rehberg and Burns 2008).

This study characterizes the development of both the biochemical properties and MHC profiles of pinniped skeletal muscles in four species with different lactation lengths and divergent swimming and diving patterns. First, we test the hypothesis that the rate at which muscles mature is inversely correlated with the length of the dependent period (i.e., faster development is associated with a shorter lactation period). In addition, we test the hypothesis that differences in muscular properties and developmental rates reflect the manner in which the muscles are used during locomotor activities, with locomotor muscles displaying mature MHC composition before postural muscles. Finally, this work evaluates whether the biochemical and MHC (contractile) components of muscles develop in concert, to identify factors that limit dive capabilities in young pinnipeds. Such physiological constraints in muscular efficiency and O_2 use has important implications for first-year survival when mortality rates are highest and marine mammals are just beginning to forage independently (Hastings 1996; Hastings et al. 2011; Baker and Thompson 2007).

Methods

Animal handling

For this comparative study of muscular biochemistry (Mb and enzyme kinetic activities) and MHC characteristics, neonatal and adult individuals from two phocid (harp and hooded seals) and two otariid (northern fur seals and Steller sea lions) species were handled. These species were selected because of their divergent developmental, swimming, and diving strategies (Fig. 1). Individuals were categorized by life-history stages (i.e., fetal, nursing neonate, weaned pup, and adult) to standardize cross-species comparisons.

Within the phocid family, hooded seals and harp seals were captured in the Gulf of St. Lawrence, Canada from 2005 to 2008. Hooded seals were classed as either nursing neonates (1–2 days old), weaned (~ 7 days, pup unaccompanied), or adult, while harp seals were nursing neonates (1–2 days oldyellowcoats, thin whitecoats), early and late weaned (~ 12 vs. ~ 21 days), or adult. Animals were sacrificed according to approved methods for scientific harvest in Canada (Burns et al. 2007). Within the otariid family, northern fur seals from the Lovushki Island rookery in the Kuril Islands of far eastern Russia were handled in 2008. Nursing neonates (< 1 month) and adult females were captured by hoop net, and transported to a research vessel (Shero et al. 2012). Steller sea lions were captured throughout the Alaska range in 2001–2004. Age was determined using morphometric and canine tooth eruption and annuli (Richmond et al. 2006). Both species were anesthetized with isoflurane gas for muscle sampling, except for three adult sea lions

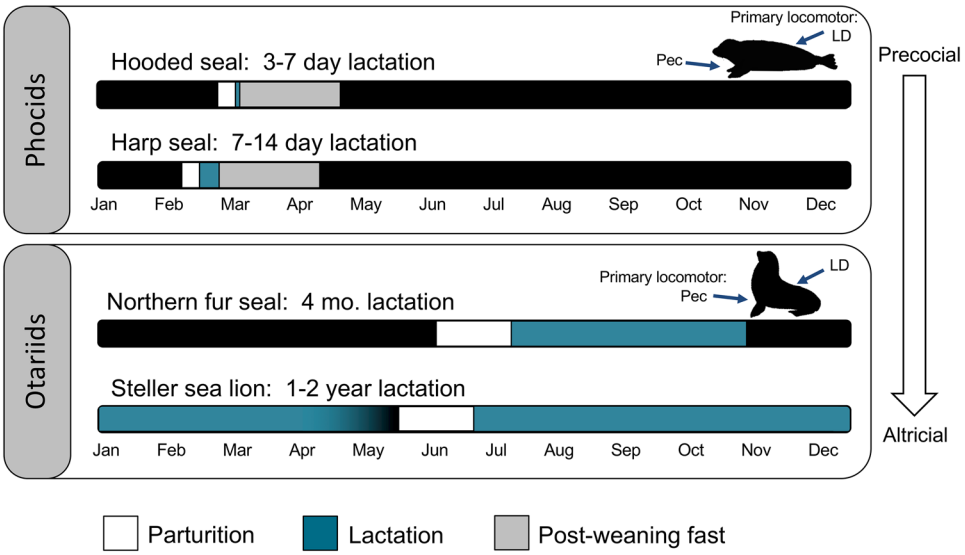


Fig. 1 Annual life history calendar for the four pinniped species included in this study, showing the length of lactation (neonatal dependency period) and the post-weaning fast (PWF) ranked from precocial to altricial status. Muscles sampled are diagrammed, with the *Longissimus dorsi* being the primary locomotor muscle in phocids and the *Pectoralis* is the primary locomotor muscle in otariids

which were opportunistically sampled following harvests. Skeletal muscle samples (*Pectoralis [Pec]* and *Longissimus dorsi [LD]*) were collected from sacrificed animals (hooded and harp seals) or with a 4–6 mm biopsy punch (northern fur seals and Steller sea lions) and stored at − 80 °C until analyses could be performed (for sample sizes *see* Table 1).

Muscle myosin heavy chain composition and biochemistry

Myosin heavy chain isoforms were separated using the SDS-PAGE technique as described by Blough et al. (1996) and Reiser and Kline (1998). All muscle samples (5–15 mg) were homogenized in gel sample buffer (8 M urea, 2 M thiourea, 0.05 M Trizma base, 0.075 M dithiothreitol, 3% (w/v) SDS, at pH 6.8, and 0.004 (w/v) bromophenol blue) (Blough et al. 1996) with 60 µL buffer mg^{-1} wet muscle. The homogenate was heated for 2 min at 65–95 °C, chilled on ice, and centrifuged. The supernatant was collected and further diluted by 5× in gel sample buffer. Gels (0.75 mm thick) were prepared with a separating gel: 7% acrylamide:bis-acrylamide (50:1), 30% glycerol, 200 mM Tris buffer (pH 8.8 at 8 °C), 100 mM glycine, and 0.4% SDS (w/v) and stacking gel: 4% acrylamide:bis-acrylamide (50:1), 5% glycerol, 70 mM Tris buffer (pH 6.8 at 8 °C), 4 mM glycine, 4 mM EDTA (pH 6.8), and 0.4% SDS (w/v). Three µL of prepared sample was loaded into each well. The gels ran with upper running buffer: 100 mM Tris base, 150 mM glycine, 0.1% SDS, and 800 mM β-mercaptoethanol, and lower buffer: 50 mM Tris base, 75 mM glycine, and 0.05% SDS. Gels were run for 20–24 h on a Hoefer standard vertical electrophoresis unit (model SE600, Hoefer, Inc., Holliston, MA, USA) with a PS300-B power supply set at constant 300 V and cooling system at 8 °C. Following completion of the run, gels were silver-stained and developed as described by Blough et al. (1996). Gels were then scanned and imaged using digitizing software (UN-SCAN IT gel v 6.1).

Seal muscle samples were run with a rat muscle standard consisting of (50:25:25) plantaris:EDL:diaphragm using SDS-PAGE, and the rat MHC isoforms were identified by comparison with previously published studies using identical analytical techniques (Reiser and Kline 1998). Relative migrations of rat and seal bands were used for preliminary protein identification. Bands were excised from Coomassie stained gels and sent to the Ohio State University Mass Spectrometry and Proteomics Facility, where in-gel protease digestion using trypsin was performed, followed by nano-liquid chromatography–mass spectrometry (LC–MS/MS) for analysis of peptides. A Mascot Daemon (Matrix Science v. 2.3.2, Boston, MA, USA) search against the SwissProt Database was performed to identify significant protein matches.

Myoglobin (Mb) concentrations, aerobic enzymes proportional to metabolic rate and marking the entrance to the citric acid cycle (citrate synthase; CS), aerobic markers of reliance on fatty acids for fuels (*β*-hydroxyacyl-CoA dehydrogenase; HOAD), and anaerobic enzyme (lactate dehydrogenase; LDH) activities were measured in these same individual animals and reported previously (Burns et al. 2015; Lestyk et al. 2009; Richmond et al. 2006; Shero et al. 2012), except for Steller sea lion enzyme activities due to insufficient sample. Enzyme activities were measured for a few Steller sea lion muscles, different from specimens with MHCs in this study (Table 2).

Statistical analyses

Data were assessed for normality using a Shapiro–Wilk test, and MHC percentages were arcsine transformed. For

Table 1 Study sample sizes and animal body mass (mean ± SE) by species and age class

	Animal *n*	Mass (kg)
Phocids		
Hooded seal (3–7-day lactation)		
Nursing (< 2 days)	8 (2F: 6M)	24.0 ± 0.8
Wean and fasting (5–14 days)	8 (4F: 4F)	47.8 ± 1.2
Adult	9 (6F: 3M)	275.0 ± 18.6
Harp seal (7–14-day lactation)		
Fetus	6 (4F: 2M)[a]	8.3 ± 0.5
Nursing (< 2 days)	9 (6F: 3M)	9.0 ± 0.6
Wean and fasting (early)	5 (0F: 5M)	42.0 ± 2.4
Wean and fasting (late)	8 (4F: 4M)	32.9 ± 1.6
Adult	8 (8F: 0M)	115.6 ± 6.5
Otariids		
Northern fur seal (4-month lactation)		
Nursing (< 1 month neonate)	8 (4F: 4M)[a]	7.6 ± 0.2
Adult	9 (9F: 0M)	38.3 ± 2.4
Steller sea lion (1–2-year lactation)		
Fetus	1 (1F: 0M)	7
Nursing (< 1 month neonate)	8 (2F: 6M)[a]	24.2 ± 3.2
Nursing (5 months)	2 (1F: 1M)	56.2 ± 4.1
Nursing (9 months)	11 (5F: 6M)[a]	89.6 ± 7.3
Wean (17 months)	3 (1F: 2M)[a]	117.8 ± 4.3
Wean (21 months)	6 (2F: 4M)	124.0 ± 4.5
Adult (> 24 months)	7 (0F: 7M)[a]	498.1 ± 114.9

Animal sex is shown in parentheses

[#]Masses could not be obtained from 2 Steller sea lion 1-month neonates and 2 adults

[a]Note that not all animals had a biopsy taken from both muscles. Harp seal fetus had $n = 5$ for the *Pec* and $n = 6$ for the *LD*. Northern fur seal < 1 month Neonate had $n = 8$ for the *Pec* and $n = 6$ for the *LD*. Steller sea lion Nursing (< 1 month neonate) had $n = 7$ for each muscle type; Nursing (9 months) had $n = 9$ for each muscle type; Wean (17 months) had $n = 3$ for *Pec* and $n = 2$ for *LD*; Wean (21 months) had $n = 5$ for *Pec* and $n = 4$ for *LD*; Adult (> 24 months) had $n = 5$ for each muscle type

each species, MHC composition changes among age classes and between muscles were first tested using linear mixed effect (LME) models with animal ID as a random effect, to account for the two types of muscle samples taken from the same individual. However, the age × muscle interaction was always significant, and therefore, a student's *t* test or a one-way ANOVA with Bonferroni post hoc comparisons was performed to test for intra-specific age differences for each muscle type. If data were still not normally distributed after transformation, a Kruskal–Wallis *H* test with pairwise Wilcoxon rank sum comparisons were used instead. Interspecific comparisons were conducted for the youngest nursing neonates and adult age classes.

Because the intra-specific changes in muscle biochemical properties for these species have been reported previously, our analysis focuses on the correlations between muscle biochemical properties and MHC composition. We predicted that as both muscle biochemistry and MHC profiles matured, the two muscle types (swimming vs. postural) would become more distinct as each develops to suit its functional role. To test whether muscle biochemistry and MHC profiles developed at similar rates, the relationship between MHC composition and muscle Mb/biochemistry was tested using LME models with age class and muscle type as covariates and animal ID as a random effect. However, because there was multicollinearity among variables, a principal component analysis (PCA) was used to create an ordination of muscle parameters for each species and to assess their similarity (or dissimilarity), based on all measured MHC and biochemical components in 2-D space. PCAs were performed using the princomp function in R with a correlation matrix. PCA ordination plots were constructed showing the different age and muscle groups. Within each species, LME analyses tested whether each principal component differed by age class and muscle. All analyses were conducted in R (v. 3.5.1) and significance was set as $\alpha = 0.05$.

Results

MHC isoform identification

Six different MHC isoforms were identified in the *Pec* and *LD*, most of which have been reported previously in other mammals and in these species specifically [Figs. 2, 3; (Shero et al. 2012, 2015)]. MHC isoforms identified include the slow-twitch MHC I isoform, as well as fast-twitch MHC IIA, IID/X, and an unknown isoform with glycolytic IIB and Neonatal properties (Shero et al. 2012, 2015). Immature-type bands were confirmed by proteomic analysis at the Ohio State University proteomics laboratory. The slowest migrating band (top) in this study was confirmed from a Steller sea lion sample as Embryonic MHC. A second previously uncharacterized protein band was identified from a harp seal sample as the Neonatal myosin isoform (*see* Table S1). Because the Neonatal and Unknown isoforms were identified in relatively few animals, and when present, comprised small proportions of overall MHC isoforms (4.7 ± 1.0% and 4.2 ± 0.8%, respectively), they were not included in age and species-related comparisons of MHC profiles. There were considerable changes in MHC isoform composition during ontogeny in both muscles, as well as differences in profile and developmental pattern among the four species.

Table 2 Field metabolic rate and muscle biochemistry (myoglobin concentrations, citrate synthase, β-hydroxyacyl-CoA dehydrogenase, and lactate dehydrogenase activities) across development in the *Pectoralis* and *Longissimus dorsi* skeletal muscles

	FMR (mL O_2 kg^{-1} min^{-1})	FMR references	Pectoralis				Longissimus dorsi				Biochemistry references
			Myoglobin (mg g $tissue^{-1}$)	CS (IU g $tissue^{-1}$)	HOAD (IU g $tissue^{-1}$)	LDH (IU g $tissue^{-1}$)	Myoglobin (mg g $tissue^{-1}$)	CS (IU g $tissue^{-1}$)	HOAD (IU g $tissue^{-1}$)	LDH (IU g $tissue^{-1}$)	
Hooded seals											
Nursing neonate	23.7	Lydersen et al. (1997)	27.2 ± 0.7[a]	56.3 ± 1.8[a] (2.37 ± 0.08)	68.0 ± 5.6 (2.87 ± 0.24)[1]	1305.1 ± 57.2[a] (55.1 ± 2.41)[1]	33.5 ± 0.8[a]	59.7 ± 2.6[a] (2.52 ± 0.11)[1]	84.1 ± 5.3[a] (3.54 ± 0.22)	1159.6 ± 72.9[a] (48.9 ± 3.1)[1]	Burns et al. (2015)
Weaned	12.4	Lydersen et al. (1997)	23.4 ± 1.0	60.1 ± 3.7 (4.85 ± 0.30)	63.0 ± 2.7 (5.08 ± 0.22)	1152.7 ± 77.0 (93.0 ± 6.21)	30.8 ± 1.4	63.4 ± 3.6 (5.11 ± 0.29)	90.7 ± 4.6 (7.31 ± 0.37)	1059.6 ± 68.7 (85.5 ± 5.5)	
Adult	5.0	2× Kleiber	52.2 ± 1.6[a]	30.7 ± 1.8[a] (6.13 ± 0.36)[1]	26.4 ± 1.6[a] (5.28 ± 0.32)	1490.2 ± 105.9[a] (289.0 ± 21.2)[1]	88.6 ± 1.6[a]	37.4 ± 1.4[a] (7.49 ± 0.27)[1]	65.2 ± 2.7[a] (13.0 ± 0.54)[1]	1197.9 ± 79.4[a] (239.6 ± 15.9)[1]	
Harp seals											
Fetus	21.4	Used neonate value	9.7 ± 1.6	40.2 ± 3.0 (1.88 ± 0.14)	47.2 ± 2.6 (2.21 ± 0.12)	546.7 ± 42.5 (25.5 ± 1.99)	12.3 ± 1.2	44.8 ± 4.5 (2.09 ± 0.21)	48.3 ± 2.7 (2.26 ± 0.12)	596.4 ± 27.0 (27.9 ± 1.3)	Burns et al. (2015)
Nursing neonate	21.4	Lydersen nd Kovacs (1996)	18.5 ± 1.2[b]	49.8 ± 2.3[a] (2.33 ± 0.11)	60.3 ± 4.2 (2.82 ± 0.20)[1]	799.5 ± 96.3[b] (37.4 ± 4.50)[2]	23.9 ± 1.0[b]	55.3 ± 2.3[a] (2.58 ± 0.11)[1]	71.5 ± 1.6[a] (3.34 ± 0.08)	855.3 ± 82.2[b] (40.0 ± 3.8)[1,2]	
Early weaned	7.3	Worthy and Lavigne (1987)	26.7 ± 1.8	67.4 ± 1.5 (9.24 ± 0.20)	62.4 ± 8.5 (8.54 ± 1.17)	1620.9 ± 81.7 (222.0 ± 11.2)	29.1 ± 2.2	67.4 ± 0.8 (9.23 ± 0.11)	87.8 ± 3.0 (12.0 ± 0.41)	1156.7 ± 84.8 (158.5 ± 11.6)	
Late weaned	6.6	Worthy and Lavigne (1987)	33.9 ± 1.8	67.8 ± 2.8 (10.3 ± 0.42)	77.0 ± 4.6 (11.7 ± 0.69)	1205.0 ± 113.8 (182.6 ± 17.2)	41.5 ± 2.5	71.7 ± 3.4 (10.9 ± 0.52)	86.4 ± 9.0 (13.1 ± 1.36)	1126.3 ± 68.4 (170.7 ± 10.4)	
Adult	3.2	Aarseth et al. (1999)	51.1 ± 2.9[a]	31.6 ± 1.6[a] (9.89 ± 0.51)[2]	18.2 ± 1.2[b] (5.69 ± 0.37)	1275.0 ± 43.1[a,b] (398.4 ± 13.5)[2]	83.3 ± 4.0[a]	31.0 ± 2.6[b] (9.69 ± 0.66)[2]	34.0 ± 2.6[b] (10.6 ± 0.80)[2]	1146.6 ± 143.1[a] (452.1 ± 44.7)[2]	
Northern fur seals											
Nursing neonate (1 month)	15.3	Donohue et al. (2000)	12.4 ± 0.9[c]	31.8 ± 1.7[b] (2.08 ± 0.11)	56.6 ± 3.4 (3.70 ± 0.22)[2]	598.0 ± 45.2[b] (39.2 ± 2.96)[2]	10.6 ± 0.6[c]	25.3 ± 2.7[b] (1.66 ± 0.18)[2]	44.1 ± 3.27[b] (2.89 ± 0.21)	442.6 ± 51.1[c] (29.0 ± 3.3)[2]	Shero et al. (2012)
Adult	9.1	2× Kleiber	35.8 ± 2.2[b]	41.8 ± 2.2[b] (4.57 ± 0.24)[3]	42.5 ± 2.4[c] (4.65 ± 0.27)	988.7 ± 61.1[b] (108.1 ± 6.69)[3]	34.5 ± 1.2[b]	24.0 ± 1.6[c] (2.63 ± 0.17)[3]	33.2 ± 1.3[b] (3.63 ± 0.14)[3]	681.7 ± 51.8[b] (74.6 ± 5.7)[3]	
Steller sea lions											
Fetus	15.8	Used neonate value	–	9.72 (0.62)	24.2 (1.53)	304.7 (19.3)	–	6.60 (0.42)	17.5 (1.11)	265.0 (16.8)	Richmond et al. (2006)
Nursing neonate (1 month)	15.8	Hoopes et al. (2004)	5.7 ± 0.1[d]	26.7 ± 0.16 (1.69 ± 0.01)	48.3 ± 0.3 (3.06 ± 0.02)	477.1 ± 103.3 (30.2 ± 6.54)	7.2 ± 0.1[c]	24.8 ± 5.9 (1.57 ± 0.4)	41.4 ± 5.9 (2.62 ± 0.37)	509.6 ± 109.4 (32.3 ± 6.92)	Enzyme data, unpublished
Nursing 5 months	11.9	Hoopes et al. (2004)	12.9 ± 0.3	–	–	–	10.4 ± 0.1	–	–	–	
Nursing 9 months	8.9	Hoopes et al. (2004)	18.6 ± 0.8	–	–	–	14.9 ± 1.0	–	–	–	
Weaned 17 months	7.2	Hoopes et al. (2004)	24.6 ± 0.4	–	–	–	16.3 ± 0.3	–	–	–	
Weaned 21 months	6.8	Hoopes et al. (2004)	21.9 ± 1.0	30.5 ± 2.7 (4.49 ± 0.40)	23.3 ± 2.1 (3.43 ± 0.31)	806.6 ± 121.8 (118.6 ± 17.9)	11.4 ± 0.5	14.1 (2.07)	14.4 (2.12)	368.6 (54.2)	
Adult	2.7	Hoopes et al. (2004)	34.8 ± 0.5[a,b]	23.7 (8.78)	20.3 (7.52)	778.5 (288.3)	20.7 ± 0.4[b]	13.6 (5.04)	15.5 (5.74)	593.9 (220.0)	

Enzyme activities scaled to field metabolic rate (FMR) are shown in parentheses. See references for muscle and age-related differences in biochemistry; *different letters* = significant differences in Mb and absolute enzyme activities among species within an age group for that muscle, while *different numbers* = significant difference in scaled enzyme activities. Note that Steller sea lion enzyme activities were acquired from different individuals than Mb and MHC measurements (fetus: $n = 1$; 1 month: $n = 2$; 21 months: Pec $n = 4$, LD $n = 1$; adult: $n = 1$) and Steller sea lion enzyme activities were not included in cross-species comparisons

Ontogeny of MHC profiles

All species experienced shifts in MHC composition across ontogeny, and transitions in the muscle contractile apparatus tended to be more pronounced in altricial species within each taxonomic family, in order to achieve mature function. For example, both phocid species included in this study exhibited shifts from immature to adult MHC isoforms (Fig. 2). In the precocial hooded seal and relatively altricial harp seal, Embryonic MHC declined during development in both the *Pec* and *LD* (Fig. 2). As expected, slow-twitch MHC I protein was replaced with fast-twitch MHC IID/X during development, but only in the *Pec* muscle. In the *LD*, MHC I content tended ($P < 0.1$) to increase with age in both phocid species, but did not change significantly. MHC IIA content did not shift with age in the hooded or harp seal *Pec* muscles. In the hooded seal *LD*, MHC IIA content decreased with age, whereas the proportion of MHC IIA increased with age in the harp seal *LD*. As a result, across ontogeny in phocid seals, the postural *Pec* muscle always contained significantly more MHC IID/X isoform than the *LD*, characteristic of fast-twitch primarily glycolytic fibers. The only exception was the fetal harp seal muscles which had very low MHC IID/X content and showed no difference between muscles. Within each age class, the two muscles contained similar proportions of MHC IIA. Conversely, the locomotor *LD* muscle was poised for greater endurance capacities and always had significantly greater slow-type, oxidative MHC I content than the postural *Pec* (all $F > 10$, $P < 0.05$).

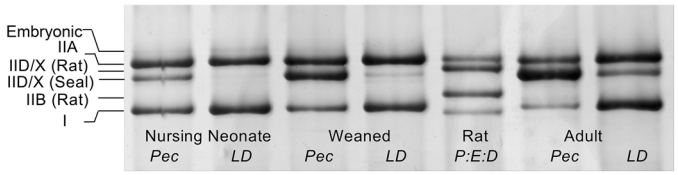
While northern fur seals have a longer lactation period than either phocid species in this study, their lactation period is much shorter than all other otariids, including the Steller sea lion. The northern fur seal was the only species in this study for which no muscle samples contained any Embryonic or Neonatal MHC protein (Fig. 3), demonstrating that this species was born with the most phenotypically mature muscles. However, both immature MHC isoforms were present in altricial Steller sea lion pups and even in an adult. In the single Steller sea lion fetus sample, Embryonic MHC content was 5–6× greater than in 1-month-old neonates, and MHC I and MHC IIA were present in substantially lower proportions. Embryonic MHC content decreased with age in both muscles in the Steller sea lion. Similar to developmental shifts in phocid muscles, MHC I content declined with age in the *Pec* of both otariid species, and in the northern fur seal *LD*. Correspondingly, FOG MHC IID/X content increased with age in both species' *Pec* muscles, but not in the *LD*. Within muscle type, MHC IIA content remained relatively constant across ontogeny in both species. Consequently, in both otariid species, at birth, the locomotor *Pec* muscles already had significantly higher MHC IID/X content than the postural *LD*, whereas the postural *LD* had greater MHC I than the locomotor *Pec* (all $F > 10$, $P < 0.05$). Northern fur seal neonates' *LD* muscles had greater IIA content at birth than the locomotor *Pec* muscle; however, it was not until adulthood that this pattern emerged in Steller sea lion muscles.

Inter-specific comparisons in MHC profiles

Among the youngest nursing neonates, there were inter-specific differences in the relative proportion of MHC isoforms in both muscles, with the more precocial species being born with less immature and slow-twitch MHC isoform content in their muscles. Correspondingly, relatively precocial species had a greater proportion of fast-twitch protein composition than more altricial species, within each taxonomic family and in both muscle types. For example, nursing hooded seals had a lower proportion of Embryonic MHC protein than seen in Steller sea lions or harp seals, and this was true for both *Pec* and *LD* muscles (*Pec*: $\chi^2 = 21.4$, $P < 0.001$, hooded seal: 2.1 ± 1.2%, harp seal: 15.3 ± 1.9%, Steller sea lion: 11.5 ± 4.1%; *LD*: $F_{3,28} = 17.5$, $P < 0.001$, hooded seal: 3.0 ± 1.7%, harp seal: 13.1 ± 2.5%, Steller sea lion: 11.6 ± 3.3%). In Steller sea lions, which have the longest lactation period, *Pec* muscles of nursing pups contained the highest proportion of MHC I (39.7 ± 1.7%) of all species ($F_{3,28} = 6.8$, $P = 0.001$; northern fur seal: 28.5 ± 2.5%, $P = 0.009$, hooded: 28.8 ± 1.6%, $P = 0.014$; harp: 26.8 ± 2.3%, $P = 0.002$). Despite the fact that the primary locomotor muscle differs, within each taxonomic family the more precocial species had greater proportions of MHC IID/X in the *Pec* muscle than the more altricial species (hooded and northern fur seals > harp seals and Steller sea lions; $F_{3,28} = 9.6$, $P < 0.001$) indicating inherent differences between muscle type are present even in utero and/or just after birth in the precocial hooded seal and in northern fur seals that have the shortest lactation length of otariid species. The relative proportion of MHC I or IID/X isoform in the *LD* muscle did not vary by species within the youngest nursing neonates. However, because the neonatal hooded seal muscles had lower immature MHC content at birth and northern fur seal muscles did not contain any immature MHCs, these species had the highest proportions of mature MHC IIA in the *LD* muscle at birth ($F_{3,28} = 4.6$, $P = 0.010$; hooded seal: 48.7 ± 3.2%, northern fur seal: 49.6 ± 2.0%, harp seal: 41.5 ± 1.3%, Steller sea lion: 38.8 ± 2.8%). Surprisingly, there were no inter-specific differences in the relative proportion of MHC IIA in the neonatal *Pec* muscles.

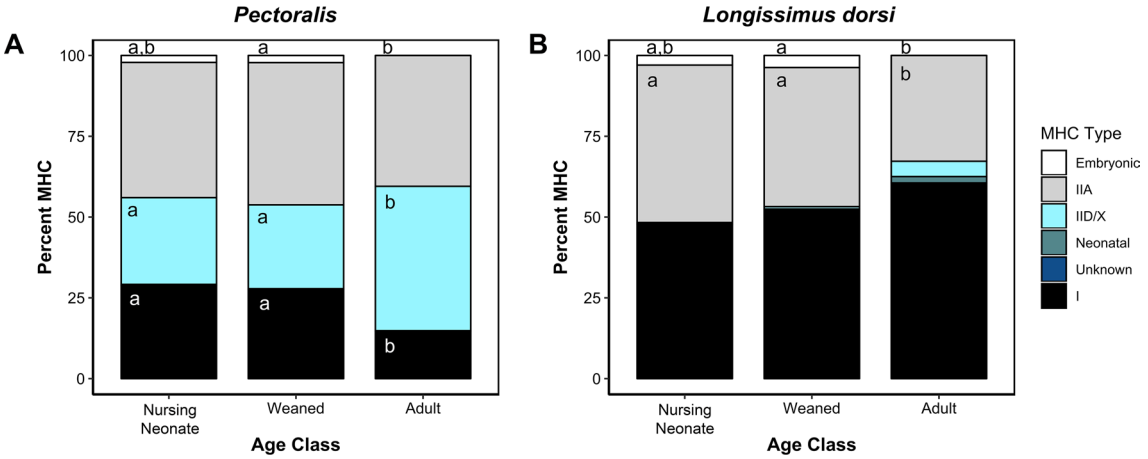
To test whether mature MHC profiles were more reflective of muscle use patterns and functional role, inter-specific differences in MHC profiles were also characterized among adults. No adult *Pec* samples contained the Embryonic MHC isoform, and only one adult Steller sea lion *LD*

Hooded seals

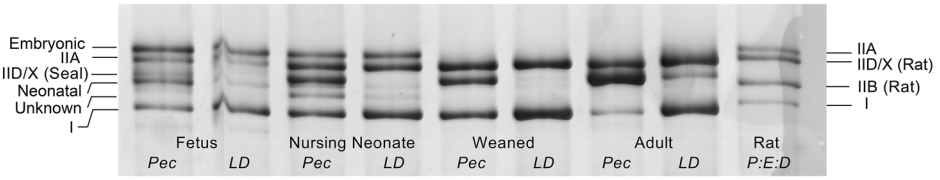


Embryonic: χ^2 = 6.6, *P* = 0.037 *
MHC I: $F_{2,22}$ = 12.2, *P* < 0.001 *
MHC IIA: $F_{2,22}$ = 0.4, *P* = 0.672
MHC IID/X: $F_{2,22}$ = 8.2, *P* = 0.002 *

Embryonic: χ^2 = 9.9, *P* = 0.007 *
MHC I: $F_{2,22}$ = 3.1, *P* = 0.064
MHC IIA: $F_{2,22}$ =10.1, *P* = 0.001 *
MHC IID/X: χ^2 = 1.1, *P* = 0.580

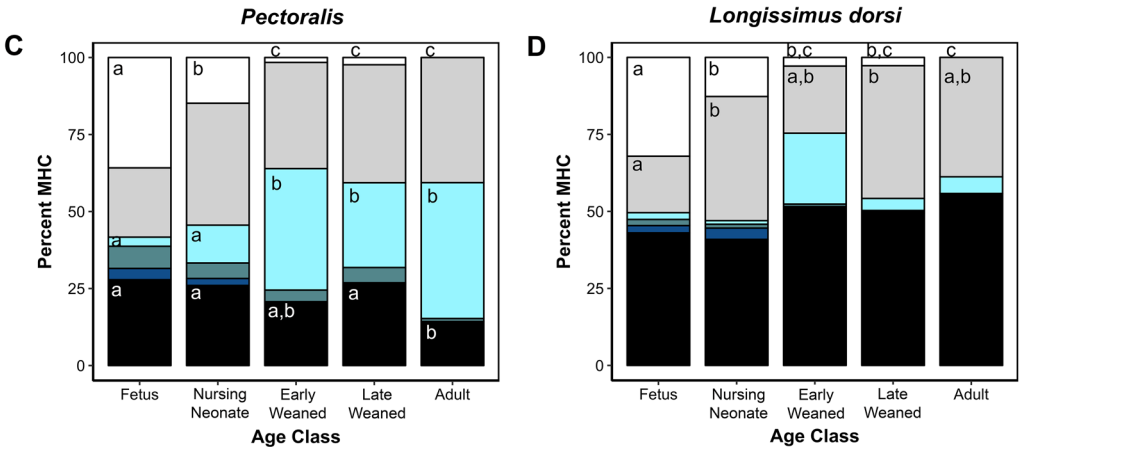


Harp seals



Embryonic: χ^2 = 29.0, *P* < 0.001 *
MHC I: $F_{4,30}$ = 5.9, *P* = 0.001 *
MHC IIA: χ^2 = 5.8, *P* = 0.215
MHC IID/X: χ^2 = 23.3, *P* < 0.001 *

Embryonic: χ^2 = 28.0, *P* < 0.001 *
MHC I: $F_{4,31}$ = 2.2, *P* = 0.094
MHC IIA: χ^2 = 18.3, *P* = 0.001 *
MHC IID/X: χ^2 = 8.0, *P* = 0.093



◂**Fig. 2** Myosin heavy chain composition across development in phocid seals (hooded seal, harp seal) in a postural muscle (*Pec*; **a**, **c**) and locomotor muscle (*LD*; **b**, **d**). MHC isoform age effects are displayed for each muscle; *different letters* indicate significant differences between age classes for the respective MHC isoform. Note that pinniped muscles did not contain any MHC IIB isoform; the seal IID/X isoform ran further in the gel than rat IID/X

(postural) contained any Embryonic MHC (8% total protein). Conversely, 8 adult harp seal postural *Pec* muscles still contained the immature Neonatal MHC (only < 5% of total MHC protein content), and one adult hooded and one harp seal locomotor *LD* contained Neonatal MHC (18% and < 1%, respectively). Among the primary "adult" fiber types (MHC I, MHC IIA, MHC IID/X), Steller sea lion adults had the highest proportion of MHC I protein in the *Pec* (overall species effect—$F_{3,27} = 6.2$, $P = 0.002$; pairwise comparison—Steller sea lion: 30.6 ± 4.7% > harp and hooded seals: 14.1 ± 1.1% and 14.7 ± 2.7%; both $P < 0.01$, slightly higher than northern fur seal: 20.1 ± 1.9%), which fits with their primary use of this muscle in locomotion, and the use of O_2-sparing strategies. Similarly, adult phocids had greater MHC I content in their primary locomotor muscle, the *LD* (overall species effect—$\chi^2 = 13.4$, $P = 0.004$; pairwise comparison—harp seal: 55.8 ± 3.3%, hooded seal: 60.6 ± 5.4%, and Steller sea lion: 54.0 ± 3.1% > northern fur seal: 38.3 ± 2.3%, all $Ps < 0.05$). While adults of all species had relatively similar proportions of MHC IIA in the *Pec* muscle (38.6 ± 2.0%), the amount of MHC IIA in the *LD* was again highest in northern fur seals (overall species effect—$F_{3,26} = 7.5$, $P < 0.001$; pairwise comparison—northern fur seal: 53.5 ± 2.5% > harp and hooded seals: 38.8 ± 4.7% and 32.7 ± 2.7%, both $Ps < 0.05$), but not statistically different from Steller sea lions (44.7 ± 1.4%). The relative proportion of fast-twitch primarily glycolytic MHC IID/X did not vary across species for either muscle but was overall significantly higher in the adult *Pec* (42.8 ± 2.3%) than the *LD* muscle (5.1 ± 1.4%; overall effect of muscle type—$\chi^2 = 42.7$, $P < 0.001$).

Linking MHC composition with muscle biochemistry

As has been reported previously, muscle Mb increased with age, and concentrations were generally higher in the primary locomotor muscle of each species (Fig. 4; Table 2; *see* Lestyk et al. 2009; Richmond et al. 2006; Shero et al. 2012). In addition, the relative maturity of muscle Mb load in the primary locomotor muscle was greater in neonates with shorter nursing periods. Indeed, in the otariid locomotory *Pec* muscle, northern fur seal neonates had Mb concentrations that were 34% of adult values, while neonatal Steller sea lion *Pec* muscles only contained 16% of adult Mb. Similarly, in the phocid locomotory *LD*, hooded seal neonates already had developed 38% of adult Mb concentrations whereas concentrations had only reached 29% of adult values in harp seals (Fig. 4).

Within phocids, Mb concentrations and MHC composition were often directly correlated, as might be expected based on reliance of MHC I fibers on oxidative metabolism (Fig. 5). However, the relationship changed throughout development (both species $F > 20$, $P < 0.001$). For example, as hooded seals reached maturity, there was a significant positive correlation between the proportion of MHC I and Mb concentrations in both muscles of weaned pups ($F_{1,8.6} = 30.9$, $P < 0.001$). But in adult hooded seals, the relationship between Mb and MHC I also differed between muscles (MHC I × muscle interactive effect; $F_{1,18} = 45.9$, $P < 0.001$). The positive correlation between Mb and MHC I existed only in the adult hooded seal *Pec* muscle ($F_{1,8} = 10.6$, $P < 0.014$), whereas in the *LD*, Mb content appeared to plateau at higher concentrations and exhibited no correlation with MHC I. Similarly, there was a significant positive correlation between Mb concentrations and proportions of MHC I in harp seal: nursing neonates, late-weaned pups, and adults (LME *all* $Ps < 0.01$), with no effect of muscle type.

In contrast, there was not a clear linear relationship between Mb concentrations (and thus O_2-stores) and MHC I (indicative of oxidative metabolism) within otariids. MHC I was significantly positively correlated with Mb only in 1-month-old Steller sea lion neonates ($F_{1,10} = 9.6$; $P = 0.011$), but did not correlate with MHC composition in any other otariid groups. As reported previously, enzyme activities (scaled to metabolic rate) increased with age, and muscles also became more differentiated to suit their functional role (Burns et al. 2015; Shero et al. 2012) with primary locomotor muscles developing higher enzyme activities (Table 2).

Because many muscle physiological parameters were correlated with one another (Fig. 6a), a PCA analysis further elucidated the extent that muscles differentiated during development in precocial and altricial species (Fig. 6b). Muscle biochemical properties (i.e., Mb and enzyme activities) tended to account for the most variation across ontogeny and muscle type, as indicated by the first principal component's loadings (PC1; Fig. 6c; Table 3). The second principal component (PC2) consistently measured changes in MHC composition (Fig. 6c; Table 3).

Both PC1 and PC2 exhibited an age and muscle effect in all species in this study, further indicating that numerous biochemical and contractile changes occur before muscles reach maturity. In hooded and northern fur seals, muscle types formed distinct groupings even in young animals (Fig. 6), showing that biochemical and MHC properties had developed at an early age, leading to muscle differentiation in neonates (and the *Pec* and *LD* exhibited little overlap in the ordination plot, showing muscles were dissimilar; Fig. 6b). This was due to significant age × muscle interactions in both

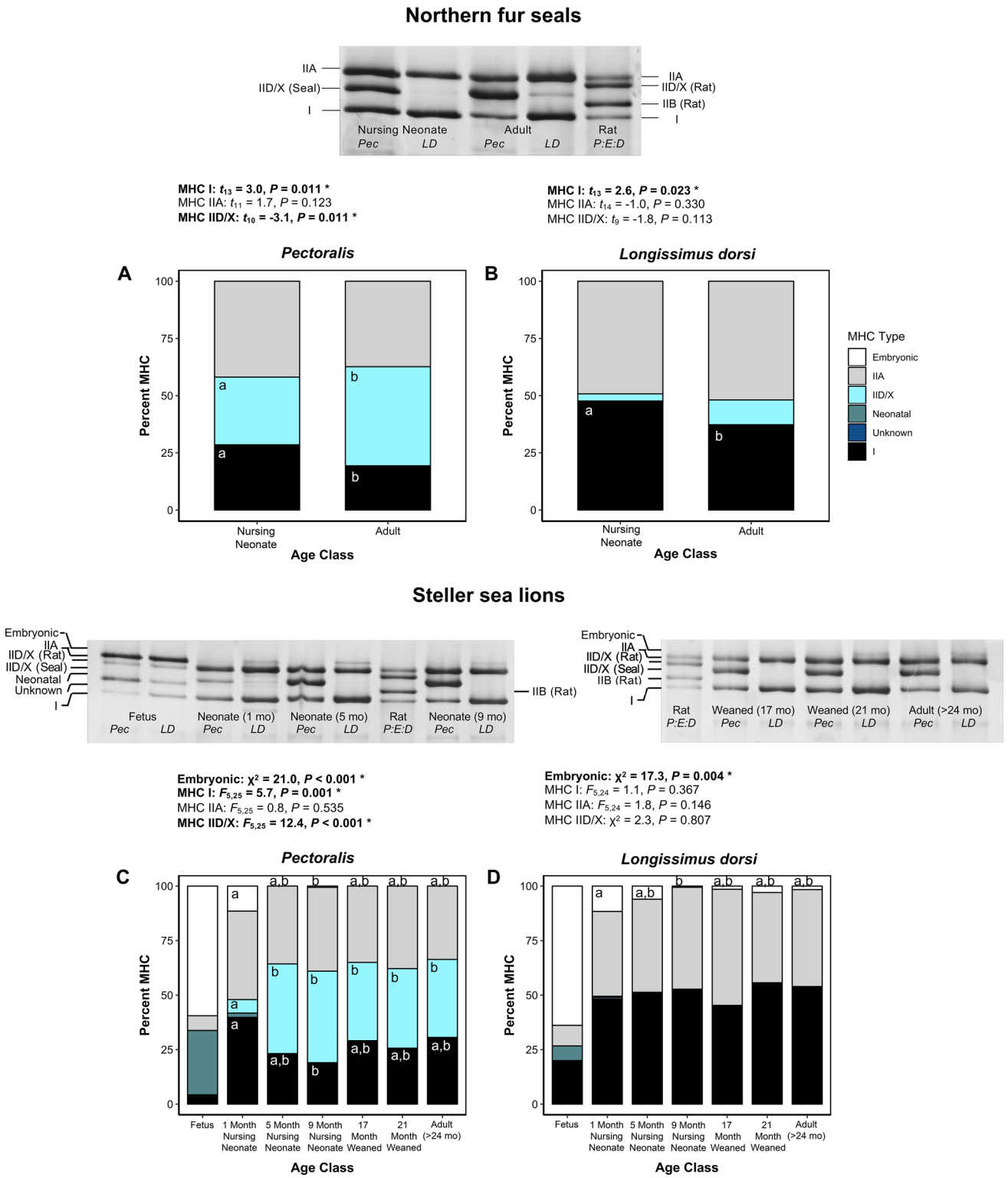


Fig. 3 Myosin heavy chain composition across development in otariid seals (northern fur seal, Steller sea lion) in a locomotor muscle (*Pec*; **a**, **c**) and postural muscle (*LD*; **b**, **d**). MHC isoform age effects are displayed for each muscle; *different letters* indicate significant differences between age classes for the respective MHC isoform. Note that pinniped muscles did not contain any MHC IIB isoform; the seal IID/X isoform ran further in the gel than rat IID/X

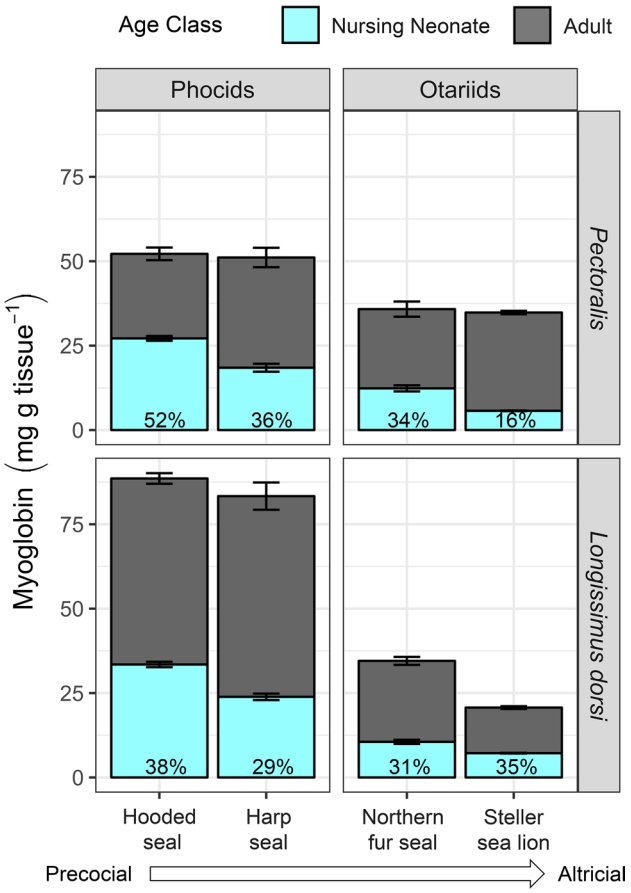


Fig. 4 Myoglobin concentrations in the *Pec* and *LD* muscles of otariid and phocid species. Percentages indicate nursing neonate myoglobin loads relative to adult values

PC1 and PC2 in the hooded seal (interactive effect—PC1: $F_{2,22}=61.3$, $P<0.001$; PC2: $F_{2,22}=31.8$, $P<0.001$). Only PC1 exhibited an interactive effect in northern fur seals ($F_{1,11}=8.0$, $P=0.016$). In the relatively altricial harp seal, neonatal skeletal muscles were less differentiated by function, and even as adults some individuals had similar properties in their *Pec* and *LD* muscles (indicated by more overlap between muscle types; Fig. 6b). Therefore, biochemical and MHC components both developed by an earlier age in precocial species, but the pattern of differentiation and mature phenotype appeared specific to each taxonomic family (rates of differentiation: hooded seal > northern fur seal > harp seal).

Discussion

This study shows that substantial development of muscle biochemistry and the underlying myosin isoform expression occurs prior to, or soon after independent foraging begins in pinnipeds. While all species in this study were born with immature muscles, the muscles of precocial species had MHC profiles more similar to those of adults at birth than did altricial species. However, the pattern was specific to each taxonomic family. Within the phocid family, hooded seals were born with greater proportions of MHC IID/X characteristic of fast-twitch fibers for burst-type activities, and less Embryonic and Neonatal MHC, suggesting that hooded seal muscles underwent more in utero development (Close 1972; Walker and Luff 1995; Singer and Mühlfeld 2007) than harp seals. Still, despite having the shortest nursing period of any mammal, hooded seal pup muscles were not functionally mature (Burns et al. 2007, 2015; Lestyk et al. 2009) nor did they have mature contractile properties as indicated by MHC composition. Similarly, among the otariids, northern fur seal muscles were more mature at birth than those of the relatively altricial Steller sea lion. Surprisingly, neonatal northern fur seals had the most phenotypically mature muscles of all the species in this study, despite the fact that their nursing period was substantially longer than either of the phocid species.

In the phocid species, the muscles of weaned pups did not contain all the biochemical properties or MHC profiles indicative of contractile potential, as in adults. Thus, weaned pups must start their first year of independent foraging with a disadvantage in performance that persists until their muscles have time to further develop to reach adult phenotypes. In combination, pups are weaned with lower O_2 stores (Richmond et al. 2006; Shero et al. 2012; Burns et al. 2005, 2007; Clark et al. 2007), less cardiovascular control with which to partition O_2 during dives (i.e., bradycardia and peripheral vasoconstriction) (Lapierre et al. 2004; Greaves et al. 2005), higher mass-specific metabolic rates (Donohue et al. 2000; Boily and Lavigne 1997), and immature muscle fibers (*this study*; Kanatous et al. 2008) that continue to consume O_2 without generating propulsive force as effectively, as compared with adults. This study was unable to distinguish precisely when northern fur seal muscle MHC composition reaches maturity; however, Steller sea lions had mature muscles at weaning, because they were weaned at a much older age relative to all other species in this study. While the protracted lactation period in Steller sea lions would pose great energetic costs to the adult female (Boyd 1998), additional time for the offspring's physiology and musculature to develop prior to the first year of independent foraging would likely increase survival rates and fitness.

Of species included in this study, the hooded seal achieves the longest and deepest dives, whereas neonatal otariids are the most active on land. These attributes may further account for the inter-specific variation in the maturity of neonatal muscles. In mammals, the environment in which the embryo develops plays a critical role in shaping myogenesis and muscle phenotype at birth (Maltin et al. 2001), and maternal nutritional status and foraging success

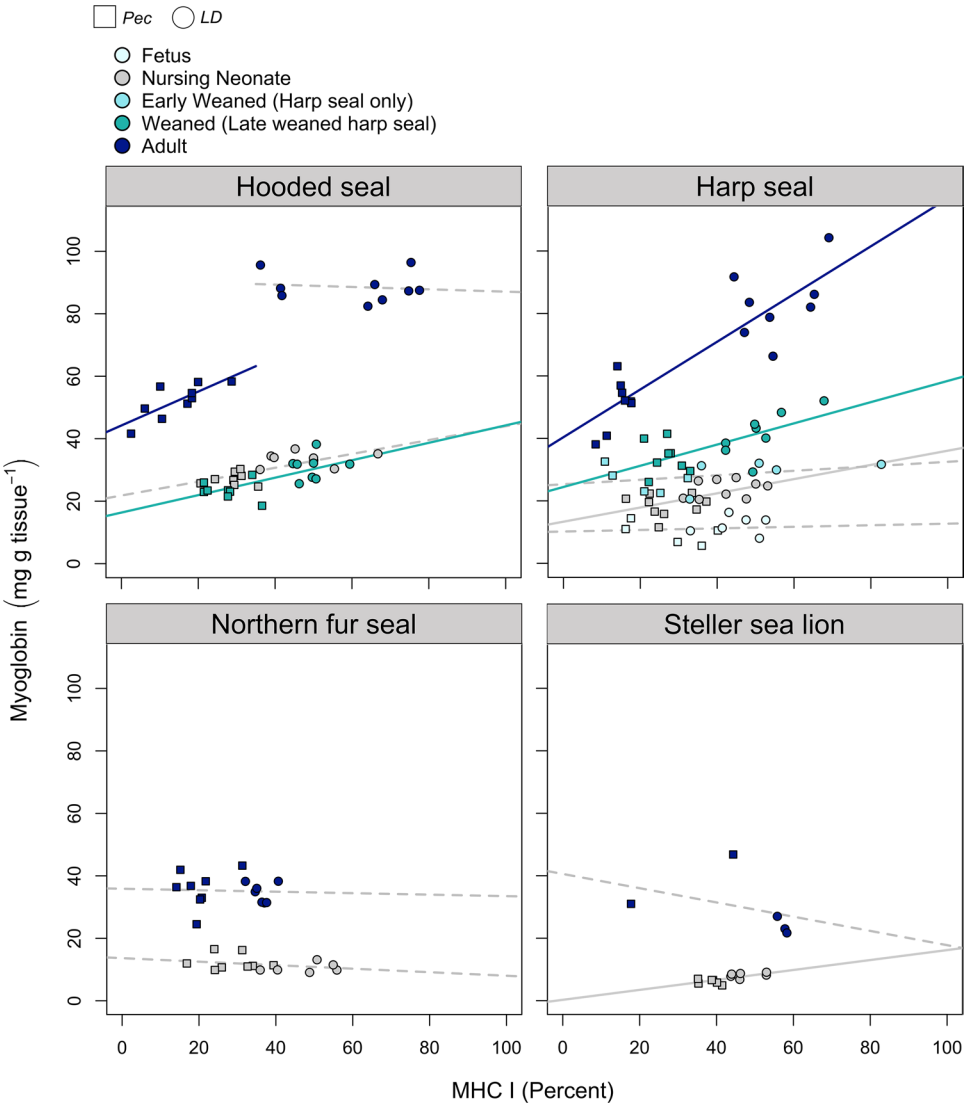


Fig. 5 The relationship between muscle myoglobin and MHC I changed across age classes, for hooded seals, harp seals, northern fur seals, and Steller sea lions (squares = *Pectoralis* and circles = *Longissimus dorsi*). Solid lines show significant correlations, dashed indicate non-significant relationships

can have life-long impacts on offspring muscle fiber composition and metabolic machinery (Hillier et al. 2007; Pettitt and Knowler 1998). For example, in utero hypoxia exposure, such as occurs while the pregnant female is diving, may also promote some prenatal development of muscle biochemistry and fiber composition (Hoppeler and Vogt 2001). Profound bradycardia and vasoconstriction reduce O_2 use rates and delivery to the working muscles during dives (Butler and Jones 1997; Butler 2004; Elsner 1969), and also decreases the maternal–placental O_2 gradient and arterial O_2 (Pa_{O2}) tension in the fetus (Liggins et al. 1980). The greater magnitude and duration of hypoxia exposure in species that are capable of making longer dives may promote earlier muscle development.

Exercise (i.e., load-bearing activity) is also required for muscle maturation in pinnipeds, which has been demonstrated by delayed Mb maturation in hooded seals denied access to swimming pools (Geiseler et al. 2013) and that pinniped myocytes cultured under hypoxic conditions still fail to develop Mb and enzyme activities equivalent to wild animals (De Miranda et al. 2012; Kanatous and Mammen 2010). Hypoxia and exercise both stimulate NFAT/MEF-2, hypoxia inducible factor (HIF)-1, and Sp1 pathways, ultimately resulting in the production of endogenous O_2-binding proteins, glycolytic enzymes, and the transition to mature fiber phenotype (Hochachka et al. 1998; Hochachka and Somero 2002; Halvorsen and Bechensteen 2002; Hoppeler and Vogt 2001; Haddad et al. 2003). Particularly in northern fur seals, physical activity on land at neonatal rookeries may stimulate these pathways and promote faster maturation of muscle phenotypes. Thus, species that start load-bearing activities earlier in development and/or to a greater degree are likely to undergo more rapid muscle maturation.

Concomitant life history events that occur directly post-weaning may also help facilitate muscle maturation just prior to the first year of independent foraging. For example,

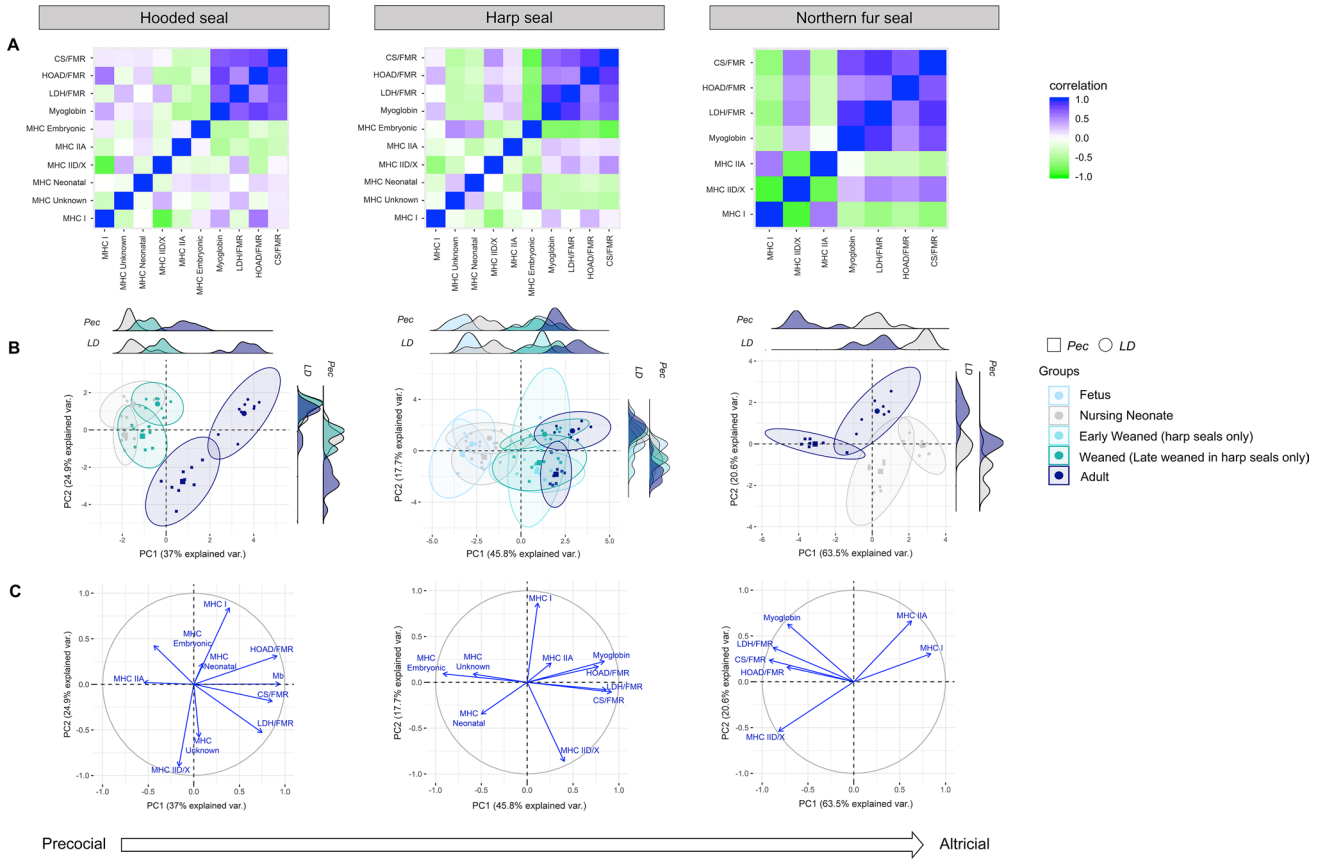


Fig. 6 **a** Heat map correlations, and **b**, **c** Principal component analyses showing a 2-D ordination of muscle MHC composition and biochemistry across development in hooded, harp, and northern fur seals. Ellipses show 95% CI for each age and muscle; biplots show contribution of physiologic measure to PC. Ordinations show that muscle types diverge in their physiology across development (squares = *Pectoralis* and circles = *Longissimus dorsi*), and to varying degrees among species. Steller sea lions were not included due to the lack of muscle biochemistry and MHC data for the same individuals

the high mass-specific metabolic rates of neonates, preferential catabolism of fats during the PWF, and neonatal molt (which occurs in utero in hooded seals) are all associated with increased circulating thyroid hormone concentrations (Atkinson et al. 2011; Boily 1996; Cox 2010; Somo et al. 2015; Oftedal et al. 1991). These endocrine factors are potent regulators for transcription of skeletal muscle genes associated with Mb and aerobic enzymes (dos Santos et al. 2001). Furthermore, these same hormones are critical in pathways responsible for decreasing expression of Embryonic and Neonatal MHC isoforms and for up-regulation of fast-twitch MHC and fiber types (Baldwin and Haddad 2001).

In addition to variation in the rate of muscular development during ontogeny, the degree of differentiation between the postural and locomotor muscles became more pronounced with age and likely reflects a balance between the need for endurance versus sprint activities, as well as the differences in ATP yields between aerobic and anaerobic metabolism (Bass et al. 1969). Once again, in the more precocial species, the *Pec* and *LD* portrayed distinctive biochemical properties and MHC profiles earlier during ontogeny, in a family-specific manner. The precocial hooded seal and northern fur seal postural and locomotor muscles were more differentiated at a young age (evidenced by age, muscle, and age × muscle differences in principal components), whereas the *Pec* and *LD* did not exhibit substantial differences in the relatively altricial harp seal until adulthood.

Overall, there were inherent differences in MHC profiles between muscle type, suggesting that muscle contractile properties were constrained. Regardless of muscle function, the *Pec* muscles always had greater fast-twitch myosin composition, and more of the IID/X isoform characteristic of glycolytic fibers, as compared with the *LD* muscles in all species. In contrast, muscle biochemistry appeared to be more plastic and developed to be most reflective of muscle use (Choi et al. 1993; Ricklefs et al. 1994; Shea et al. 2007). Adults of all species tended to have higher Mb and aerobic and

Table 3 Principal components with the highest five loadings (eigenvalues) from PCA ordination of muscle biochemistry and MHC composition, for species, where all data were available (hooded seal, harp seal, northern fur seal)

Strongest relationship	Hooded seal			
	PC 1 (37.0%)		PC 2 (24.9%)	
	Parameter	Loading	Parameter	Loading
1	Mb	−0.490	MHC IID/X	−0.569
2	HOAD/FMR	−0.474	MHC I	0.532
3	CS/FMR	−0.447	MHC Unknown	−0.366
4	LDH/FMR	−0.389	LDH/FMR	−0.335
5	MHC IIA	0.284	MHC Embryonic	0.269
Strongest relationship	Harp seal			
	PC 1 (49.9%)		PC 2 (15.0%)	
	Parameter	Loading	Parameter	Loading
1	CS/FMR	0.430	MHC I	0.649
2	MHC Embryonic	−0.428	MHC IID/X	−0.648
3	LDH/FMR	0.403	MHC Neonatal	−0.260
4	Mb	0.393	Mb	0.172
5	HOAD/FMR	0.362	MHC IIA	0.158
Strongest relationship	Northern fur seal			
	PC 1 (63.5%)		PC 2 (20.6%)	
	Parameter	Loading	Parameter	Loading
1	CS/FMR	−0.437	MHC IIA	0.553
2	LDH/FMR	−0.418	Mb	0.524
3	MHC I	0.398	MHC IID/X	−0.450
4	MHC IID/X	−0.388	LDH/FMR	0.311
5	HOAD/FMR	−0.346	MHC I	0.254

The proportion variance accounted for by each PC is shown in parentheses

anaerobic enzyme activities in the primary locomotor muscle. In phocids, the *LD* generally had higher Mb and enzyme activities, and this pattern was entirely reversed in otariids with biochemical parameters being higher in the *Pec* (Lestyk et al. 2009; Burns et al. 2015; Shero et al. 2012; Kanatous et al. 1999). Considering muscle development across multiple levels of organization revealed that muscle biochemical properties (i.e., [Mb], CS, HOAD, and LDH activities) were highly correlated, and thus the pathways facilitating Mb and enzyme maturation are likely up-regulated simultaneously. This is consistent with the notion that enzyme groups within the same metabolic systems develop in constant proportions (Bass et al. 1969). However, metabolic properties were not always correlated with changes in MHC profiles suggesting a mismatch between the development of muscle biochemistry versus contractile capacity (Shero et al. 2012).

In this study, the phenotype of mature muscles differed by species in ways predicted based on their diving abilities and swimming styles. For example, all muscle biochemical components (i.e., Mb and enzyme activities) were lower in the otariids as compared with the longer/deeper diving phocid adults, suggesting lower aerobic potential for generating propulsive power. Conversely, adult phocid seals had the highest MHC I content in the *LD*, the primary locomotor muscle, and having predominantly slow-contracting characteristics (SO fibers) likely facilitates slower O_2 use for enhanced endurance and longer dive durations. These muscles were poised for maintaining aerobic, lipid-based metabolism in hypoxic conditions during long dives (Kanatous et al. 1999). The adult northern fur seal had lower proportions of slow MHC I than all other species in this study, and greater FOG content in the *LD* as compared with the two phocid species. This suggests the northern fur seals have greater oxidative fast-twitch (burst) capacities, perhaps reflecting this species' higher activity levels at rookeries and faster metabolic rate than the phocid species included in this study (*see* Table 2). Variation in musculature that is reflective of use is also well characterized among breeds in domestically bred large mammals. For example, thoroughbred horses are selectively bred for enhanced burst-speed activities (i.e., fewer

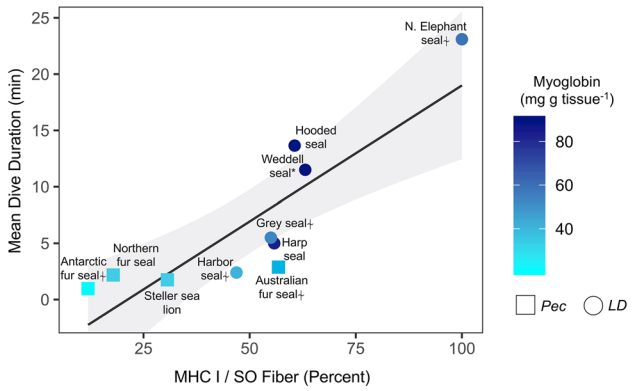


Fig. 7 Both myoglobin O_2 stores and slow use rates via more MHC I and slow-oxidative muscle fibers are critical to increasing dive durations across pinniped species. MHC I content or the proportion of slow-oxidative fibers in the locomotor muscle for each species (*LD* for phocids; *Pec* for otariids) was significantly correlated with mean dive duration (Eqn: Mean Dive Duration = 0.24(MHC I/SO Fiber Percent) – 5.13; $F_{1,8} = 22.3$, $P = 0.001$; $R^2 = 0.703$). "†" denotes species for which the value comes from SO fiber counts; for all other species MHC I content was determined using SDS-PAGE or western blot. "*" signifies that the same value was obtained by SO fiber counts and also MHC protein separation. Data from: *This study*; Arnould and Hindell 2001; Bajzak et al. 2009; Beck et al. 2000; Boyd and Croxall 1992; Burns et al. 2007, 2015; Gentry et al. 1986; Hastings et al. 2004; Kanatous et al. 2008; LaRosa et al. 2012; Lestyk et al. 2009; Moore et al. 2014; Reed et al. 1994; Rehberg et al. 2009; Richmond et al. 2006; Robinson et al. 2012; Shero et al. 2012, 2015, 2018; Spence-Bailey et al. 2007

slow-oxidative muscle fibers), 'double muscled' cattle were bred for exaggerated hyperplasia during development resulting in more meat, and others are selected for meat quality (more SO fibers and fat 'marbling' in the tissue) (Wegner et al. 2000; McPherron and Lee 1997; Maltin et al. 2001; More O'Ferrall and Cunningham 1974). Thus, whether it is under artificial conditions or in the wild, differences in selective pressures result in markedly different mature muscle phenotypes. Across Pinnipedia, species with longer dive durations had locomotor muscles with a higher proportion of SO MHC/SO fibers and greater myoglobin concentrations at maturity (Fig. 7; Lestyk et al. 2009). This demonstrates that both high O_2 stores, as well as slow and effective use of O_2 are crucial to maximizing dive capacities. Therefore, the mismatch in development of muscle biochemistry and MHC composition would constrain underwater foraging times in newly weaned pups, as compared to adults with coordinated muscle biochemical and MHC profiles.

In summary, this study characterized the development of MHC composition for muscular contraction, and biochemical properties of substrate and O_2 use in multiple species representing precocial and altricial animals across the pinniped lineage. The longest and deepest-diving pinniped species had higher aerobic potential (Mb and enzyme activities) and more endurance-type SO MHC content at maturity, indicating that effective O_2 management in muscle fibers is crucial to reaching greater depths (i.e., more strokes) and extending the duration of underwater foraging efforts. Across ontogeny, the transitions to mature fiber types and contractile apparatus appeared to be prioritized, and were followed by protein production to build Mb-O_2 stores and enzyme capacities specific to muscle function. Pups of species that exhibit slower development and/or a greater degree of mismatch between muscle biochemistry and MHC composition at the time of weaning, will likely have less flexibility in the foraging strategies they can utilize (Burns et al. 1999; Rehberg and Burns 2008; Fowler et al. 2006; Geiseler et al. 2013; Folkow et al. 2010). Constraints in diving and foraging behaviors of newly weaned pups are likely to make these species particularly vulnerable to unpredictable changes in prey availability, due to either climate regime shifts or anthropogenic disturbance, during the first year of independent foraging.

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