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Comparative Muscle Physiology of Ringed (*Pusa hispida*), Bearded (*Erignathus barbatus*), and Spotted (*Phoca largha*) Seals from the Bering and Chukchi Seas

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Abstract

The physiological properties of marine mammal skeletal muscle are foundational in defining diving and foraging capacities. Further, these parameters can be useful when assessing the behavioral flexibility of species faced with environmental change or disturbance. Herein, we define species- and age-specific muscle physiology for three ice-associated seal species experiencing Arctic warming. Specifically, we evaluated myoglobin content ([Mb]), nonbicarbonate buffering capacity (β), and fiber type profiles of a major locomotor muscle, the longissimus dorsi. Muscle samples were obtained from subsistence harvested ringed (*Pusa hispida*; $n = 11$), bearded (*Erignathus barbatus*; $n = 41$), and spotted (*Phoca largha*; $n = 12$) seals of all ages in the Bering and Chukchi Seas. Adult ringed seals had the highest [Mb] (6.67 ± 0.20 g 100 g wet tissue^{-1}), followed by spotted (5.38 ± 0.29 g 100 g wet tissue^{-1}) and bearded (4.55 ± 0.07 g 100 g wet tissue^{-1}) seals. [Mb] increased with age for all species, but rates of increase differed by species. In contrast, β was similar for all species and age classes. We documented higher proportions of fast-twitch relative to slow-twitch fibers in these species, and fiber type proportions did not differ significantly with age. Adult bearded seals exhibited the greatest proportion of fast-twitch fibers (68.7 ± 1.5%), followed by ringed (59.0 ± 4.8%) and spotted (55.1 ± 2.1%) seals. Overall, our data suggest a strong link between muscle physiology, diving behavior, and life history strategies, and provide insight into the physiological capacities of these potentially vulnerable species.

Key Words: phocids, myoglobin, buffering capacity, fast-twitch, slow-twitch, fiber type

Introduction

Understanding the physiological capacities and constraints of ice-adapted marine mammals is of increasing importance given ongoing, unprecedented Arctic warming (Laidre et al., 2015; Rantanen et al., 2022). Skeletal muscle physiology is of particular interest as the structure and biochemistry of locomotor muscles influence species-specific diving limits and behavioral flexibility. In general, marine mammal skeletal muscle activity is fueled locally by exhaustible endogenous oxygen stores because peripheral vasoconstriction associated with the dive response ensures that blood-bound oxygen is reserved for oxygen-sensitive organs (Kooyman & Ponganis, 1998; Ponganis, 2011). Myoglobin, the oxygen-storage protein in muscle, is often used as an indicator of aerobic diving ability as it is found in greater concentrations in species with longer dive durations (Kooyman & Ponganis, 1998; Noren & Williams, 2000; Kielhorn et al., 2013). Similarly, the buffering capacity of muscle tissue can be used to assess reliance on anaerobic metabolism while diving as buffers play an important role in holding tissue pH constant as anaerobic end-products accumulate (Castellini & Somero, 1981; Noren, 2004). Muscle fiber-type composition (i.e., proportions of fast- and slow-twitch fibers) provides insight into the functional capacities and predominant metabolic pathways employed by marine mammals while swimming and diving (Peter et al., 1972; Reed et al., 1994; Kanatous et al., 2002). These parameters vary across and within species—as young

animals typically differ from adults (Dolar et al., 1999; Noren et al., 2005; Burns et al., 2007; Lestyk et al., 2009; Watanabe et al., 2009; Thometz et al., 2015)—and can differ by sex (Richmond et al., 2006; Weise & Costa, 2007).

Among pinnipeds, phocids are the most capable divers for their body size, typically exhibiting deeper and longer dives than similarly sized otariids and odobenids (Boyd, 1997; Butler & Jones, 1997; Mottishaw et al., 1999; Hochachka, 2000; Garde et al., 2018). Phocid seals also exhibit great diversity in muscle physiology related to species-specific differences in diving ability and foraging behavior. The locomotor muscles of deep, long-duration divers like elephant (*Mirounga angustirostris*) and Weddell (*Leptonychotes weddellii*) seals are almost exclusively composed of slow-twitch fibers and have particularly high myoglobin content ([Mb]) (Kanatous et al., 2002, 2008). In contrast, species with more moderate dive profiles, like generalist harbor seals (*Phoca vitulina*), have a more even mix of slow- and fast-twitch fibers and have relatively lower [Mb] in locomotor muscles (George et al., 1971; Reed et al., 1994; Watson et al., 2003). Although much is known about the muscle profiles of a few well-studied pinniped species, there are many seal species for which data are limited or lacking altogether. In particular, several Arctic seal species are data deficient in terms of their muscle physiology, making it difficult to assess their physiological capacities.

Ringed (*Pusa hispida*), bearded (*Erignathus barbatus*), and spotted (*Phoca largha*) seals are Arctic phocids that rely on sea ice as a platform for rest as well as for important life history events such as pupping, nursing, and molting (Boveng et al., 2009; Cameron et al., 2010; Kelly et al., 2010). Of these three phocids, ringed seals are the smallest (50 to 70 kg) and most ice-adapted species. Adults haul out almost exclusively within thick, persistent land-fast ice during the winter and spring (Burns, 1970), where they excavate subnivean lairs in snow drifts above breathing holes in the sea ice to give birth, care for their young, and remain hidden from predators (McLaren, 1958b; Burns, 1970). Subadults, on the other hand, move to the ice edge during winter where there are better feeding opportunities, they have no need to maintain breathing holes, and they experience less exposure to predation (Crawford et al., 2012). Adult ringed seals typically dive within 100 m of the surface for less than 10 min (Kelly & Wartzok, 1996; Gjertz et al., 2000b; Crawford et al., 2019).

Bearded seals are the largest Arctic phocid (260 to 360 kg) and are closely associated with the transition zone between fast ice and pack ice during annual spring pupping and molting periods. During these times, bearded seals haul out on broken pack ice for extended periods (McLaren, 1958a; Benjaminsen, 1973; Burns, 1981; Frost et al., 2008). Dive patterns largely reflect local bathymetry, with routine dives between 1 to 6 min to depths < 60 m (Gjertz et al., 2000b; Krafft et al., 2000; Olnes et al., 2020). Deeper and longer dives typically occur over canyons and off continental shelves (Hamilton et al., 2018; Olnes et al., 2020).

While ringed and bearded seals have circumpolar distributions, spotted seals are found only in the North Pacific basin and are intermediate in size (60 to 110 kg). They give birth, rear their pups, and molt on open ice floes in the Bering Sea near the margins between loosely packed sea ice and open water (Burns, 1970; Fay, 1974). During summer and autumn, they seem to prefer nearshore areas and coastal land-based haulouts (Frost et al., 1993; Lowry et al., 1998, 2000). Spotted seals generally dive to less than 70 m for 1 to 8 min at a time (Ziel et al., 2011, 2012; London et al., 2014; Quakenbush et al., 2019).

Due to their close association with sea ice, ringed, bearded, and spotted seals are vulnerable to Arctic warming (Kovacs et al., 2011; Laidre et al., 2015; Reimer et al., 2019). Continued ice loss and retreat will result in the spatial separation of seal haul-out and foraging areas (Laidre et al., 2008; Moore & Huntington, 2008; Kovacs et al., 2011; Von Duyke et al., 2020) and alter prey availability on ecosystem scales (Grebmeier et al., 2006; Bluhm & Gradinger, 2008). These changes may force individuals to modify routine behavior during much of the year, yet we know little about their physiological capacity to do so. To better understand this issue, we examined the structure and biochemical properties of locomotor muscle in ringed, bearded, and spotted seals from the Bering and Chukchi Seas. Herein, we report the [Mb], nonbicarbonate buffering capacity (β), and fiber type profiles of the longissimus dorsi muscle for these three phocid species, across a range of age classes. We compare their distinct muscle physiology with that of other Arctic pinnipeds and consider how species-specific muscle physiology may influence behavioral flexibility.

Methods

Sample Collection

Samples of the longissimus dorsi muscle were obtained opportunistically from ringed (n = 11), bearded (n = 41), and spotted (n = 12) seals of all age classes from the Bering and Chukchi Seas. Most samples were obtained by the Alaska Department of Fish & Game (ADF&G) from seals harvested during legal subsistence activities. Authorization for collection of samples was

granted by the National Marine Fisheries Service (NMFS) under Marine Mammal Research Permits 15324, 18902, and 20466 with expressed support from the Ice Seal Committee, an Alaska Native co-management organization. All bearded seal samples were collected from subsistence harvested seals at Point Hope, Alaska, from 2016 to 2019, except for one sample that was collected during the necropsy of a 1-y-old bearded seal under human care. The death of this seal was reported under NMFS Permit 18902 and also to the Institutional Animal Care and Use Committee (IACUC) at the University of California at Santa Cruz. Ringed and spotted seal samples were obtained from subsistence harvested seals in the communities of Point Hope, Shishmaref, and Gambell, Alaska, from 2016 to 2018 (Figure 1). One sample was collected at Utqiaġvik, Alaska, from a spotted seal that died during research capture activities in 2016. The death of this seal was authorized and reported under NMFS Permit 15324 and also reported to the ADF&G IACUC. In addition to muscle, a tooth and/or claw was collected from each seal for aging, and standard length was measured for each seal as a straight line from the nose to the tip of the tail while a seal was on its back (Committee on Marine Mammals, 1967). Permission to receive and evaluate samples was granted by the NMFS West Coast Region under Letter of Authorization 151401WCR2017PR00012 issued to CR.



Figure 1. Sampling locations of Alaskan ice seals obtained for this study. Opportunistic sample collection of the longissimus dorsi muscle from a total of 64 seals (11 ringed [*Pusa hispida*], 41 bearded [*Erignathus barbatus*], and 12 spotted [*Phoca largha*] seals) occurred over four consecutive summers (2016 to 2019).

Subsistence harvested seals were sampled shortly after death, typically within 24 h. Carcasses remained outside at or near freezing temperatures prior to sampling, minimizing the potential for tissue degradation. During sample collection, muscle samples (≥ 50 g) were wrapped in foil, placed in plastic freezer bags, and placed in a -20°C freezer. Following each field sampling effort, samples were shipped frozen in totes to Fairbanks, Alaska, and stored at -80°C. Tissue samples were subsequently shipped on dry ice overnight to the University of San Francisco and Hendrix College (Conway, AR, USA) and stored at -80°C until analyses could be performed, typically within 6 mo of receipt of samples.

Seals can be reliably aged by either the formation of dentinal annuli or by counting the number of growth ridges on claws (Laws, 1953; Fisher, 1954; McLaren, 1958b). Cementum annuli were counted by Matson's Laboratory (Manhattan, MT, USA), and claw annuli were analyzed by ADF&G personnel. Growth ridges on claws can be used dependably for aging up to 8 to 10 y of age, after which time the wear on claws makes them reliable indicators of an individual's minimum age only (McLaren, 1958b; Burns, 1981). As age determinations made with claws are known to be biased low for older age classes, we used tooth ages when available (n = 58) and claw age for the remainder of individuals (n = 6). Seals were divided into three age classes: pup (< 1 y), subadult (1 to 4 y), and adult (≥ 5 y). While there are some differences in maturity by species and sex (Boveng et al., 2009; Quakenbush et al., 2009, 2011a, 2011b; Cameron et al., 2010; Kelly et al., 2010), we elected to use common criteria based on age in years to define age classes for the target species.

Biochemical Analyses

Myoglobin Content—We determined the [Mb] of longissimus dorsi muscle for 11 ringed, 41 bearded, and 12 spotted seals of all age classes. [Mb] was measured following the technique of Reynafarje (1963). To prepare muscle samples for analysis, they were thawed and cut, avoiding connective tissue, and weighed into 0.3 g subsamples using an analytical scale. Each subsample was minced in a 0.04 M phosphate buffer (pH = 6.6), placed on ice, and sonicated (Sonifier Cell Disruptor SFX550; Branson Ultrasonics, Brookfield, CT, USA) for 150 s to fully disrupt cell membranes. Homogenates were spun in a refrigerated ultracentrifuge (Avanti J-E; Beckman Coulter, Brea, CA, USA) at 28,000 G for 50 min at 2°C, and 3 mL of the resulting supernatant was placed in a culture tube. Each tube was bubbled with carbon monoxide (CO) for 8 min, and then 0.025 g of sodium hydrosulfite was added to facilitate binding of

CO to available myoglobin. Sample tubes were then bubbled with CO for an additional 2 min to ensure complete binding of myoglobin. Finally, the absorbance of each sample was measured at 538 and 568 nm with a spectrophotometer (UV-1900; Shimadzu Corporation, Nakagyō-ku, Kyoto, Japan). The differential absorbance between the wavelengths was used to calculate [Mb] in units of g 100 g wet tissue^{-1}. Samples were run in triplicate for each seal along with a control sample of known [Mb] to validate methods. Triplicate values were averaged to obtain individual mean and standard deviation (SD) values; SD values > 0.5 were used to screen for outliers within replicates. No outliers were identified.

Buffering Capacity—We determined the β due to nonbicarbonate buffers of the longissimus dorsi muscle samples for 11 ringed, 41 bearded, and 12 spotted seals of all age classes. Buffering capacity was measured following the methods of Castellini & Somero (1981). Muscle samples were thawed, cut, and weighed via an analytical scale into 0.5 g subsamples, avoiding connective tissue. In scintillation vials, samples were minced in 10 mL of 0.9% NaCl and homogenized via sonication (Sonifier cell disruptor SFX550, Branson Ultrasonics) while on ice for 150 s. Vials were capped, and homogenates were equilibrated in a 37°C water bath to mimic typical mammalian internal body temperature (Clarke & Rothery, 2008). Samples were then titrated (accumet AB150 pH benchtop meter equipped with an accumet pH/ATC epoxy body combination electrode; Fisher Scientific, Pittsburgh, PA, USA) with NaOH over an approximate range of pH 6 to 7. Buffering capacity was calculated in units of slykes (β), defined as the μmoles of NaOH needed to change the pH of a sample 1 pH unit per gram wet muscle (Van Slyke, 1922). Samples were run in triplicate and averaged to give a final mean β value and associated SD for each individual; SD values > 5 were used to identify outliers within triplicates, which warranted inspection and potential removal of an erroneous replicate value or reanalysis of the sample. A single erroneous replicate was identified for one individual due to high amounts of connective tissue observed during processing and was removed from the final average. Control samples of known β were analyzed to ensure accuracy of the titration.

Muscle Histochemistry

We determined fiber type profiles of longissimus dorsi muscle for 10 ringed, 23 bearded, and 10 spotted seals of all age classes. For each seal, eight 0.5 g subsamples were mounted on cork blocks covered in aluminum foil using Tissue Freezing Medium (TFM-5™ TBS®; General Data, Cincinnati, OH, USA). Subsamples were submersed and frozen in isopentane cooled to -150°C with liquid nitrogen. Subsamples were frozen for 5, 10, 15, or 20 s (two replicates per freezing time) and placed in vials that had been cooled on dry ice for at least 30 min. Vials were left open until isopentane evaporated and then were capped and stored in a -70°C freezer until further processing could be performed.

To prepare for cutting, frozen samples were placed in a -20°C cryostat (Leica CM1860; Leica Biosystems, Wetzlar, Germany) for a minimum of 1 h. Each sample was mounted to a cryostat chuck using Tissue Freezing Medium and quick frozen (Super Friendly Freeze'It Spray, Fisherbrand, Fisher Scientific). After the freezing time that yielded the best tissue morphology was identified for each sample, either eight 10 μm and eight 12 μm thick sections or eight 9 μm and eight 11 μm sections were cut, and pairs were placed on slides (Superfrost Plus, Fisherbrand). Five slides were used for myosin ATPase staining, and three slides were saved for myosin heavy-chain antibody staining when further classification of fast-twitch fibers was necessary. Slides were left at room temperature for 45 min to 1 h and then stained for their myosin ATPase activity or moved to a -20°C freezer until they could be antibody-stained.

Myosin ATPase Staining—To identify fast- and slow-twitch fibers in the muscle tissue of individual seals, five slides were used for myosin ATPase staining following the methods of Hermanson & Hurley (1990). Sections were pre-incubated in a series of four basic solutions with pH from 10.1 to 10.5 in 0.1 increments for 10 min at 37°C and rinsed for 3 min in deionized water (pH 8.5 to 9.0). Sections were then incubated for 30 min in a freshly prepared ATP solution with pH 9.4 at 37°C. Sections were subsequently run through a series of 3-min rinses in deionized water, 2% calcium chloride, deionized water, 2% calcium chloride, and then stained for 3 min in 1% ammonium sulfide. Following staining, the sections were rinsed in cold running water for 5 min, dehydrated in an ascending series (70, 80, 90, and 100%) of alcohols, and coverslipped with a xylene-based mounting media. Additional details of these methods can be found in Thometz et al. (2018).

Myosin Heavy-Chain Antibody Staining—Initial inspection of sections stained for their myosin ATPase activity revealed that a small proportion of fibers in the bearded seal samples were staining intermediately for ATPase activity after alkaline pre-incubation and that these fibers appeared in the muscles of all age classes. Intermediate staining was not observed in ringed or spotted seal samples. Thus, to confirm the bearded seal muscle fiber types initially identified through myosin ATPase staining, three additional

slides were used per bearded seal sample for myosin heavy-chain antibody staining. Sections of rat gastrocnemius muscle of known fiber type proportions were run alongside bearded seal muscle samples as a control.

Prior to antibody staining, slides were removed from the freezer and brought to room temperature for 5 min. Circles were drawn around each muscle section with a mini PAP pen (Invitrogen, Waltham, MA, USA). After circles were dry (~2 min), sections were rehydrated with phosphate buffered saline (PBS) (26.8 mmol KCl, 14.7 mmol KH_2PO_4, 1.45 L NaCl, 79.5 mmol $H_{15}Na_2O_{11}P$) for 15 min. Sections were then exposed to 2.5% horse serum (Vector Laboratories, Burlingame, CA, USA) in a hydrated box at room temperature for 20 min.

After blocking, sections were incubated with one of two primary antibodies: (1) A4.951 (type I [slow] myosin antibody; Blau) or (2) SC-71 (type IIa [fast] myosin antibody; Schiaffino) at a concentration of 3.5 µg/mL for 1 h in a hydrated box at room temperature. The antibodies were produced by hybridomas (developed by Blau and Schiaffino) obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the National Institute of Child Health and Human Development (NICHD) and maintained by the University of Iowa (Department of Biology, Iowa City, IA, USA). After exposing sections to primary antibodies, slides were rinsed three times (5 min/rinse) in PBS. Sections were then exposed to the secondary antibody (ImmPRESS® HRP Horse Anti-Mouse IgG Polymer Detection Kit [peroxidase], Vector Laboratories) and diluted 1:2 in PBS for 30 min. Sections were rinsed again three times (5 min/rinse) in PBS and exposed to 3,3'-diaminobenzidine (DAB) (Vector Laboratories). Development of the stain was monitored and, when the appropriate darkness level was reached, sections were placed in PBS to stop the reaction. Sections were then rinsed three times (5 min/rinse) in PBS.

Antibody staining was followed by hematoxylin and eosin (H&E) counterstaining (Presnell & Schreibman, 1997). After H&E counterstaining, sections were rinsed under running water for 9 min, dehydrated in an ascending series (70, 80, 90, and 100%) of alcohols (3 min/alcohol), rinsed in xylene (3 min), and coverslipped with xylene-based mounting medium.

Post-Processing Digital Analysis—Stained slides for both myosin ATPase and myosin heavy-chain antibody methods were imaged microscopically (Zeiss Axiolmager A1 microscope and Zeiss AxioCam MRC color camera, Carl Zeiss AG, Oberkochen, Germany; *AxioVision*, Version 4.7, software). Images were taken from identical regions of the tissue for each staining process. Images of each sample stained for myosin ATPase activity were printed and manually analyzed to obtain fiber type profiles. Fast- and slow-twitch fibers were identified on each image (1 to 7 images/sample) until at least 450 fibers were counted for each sample. The proportion of each fiber type on each image was calculated and used to determine the overall average percentages of fast- and slow-twitch fibers across all images for each seal. The average percentage of fast-twitch fibers for each species was calculated using the individual means. The diameters (Ellipse: minor axis) of 25 fibers of each type for each individual (images = 2) were measured on the myosin ATPase images to 0.01 microns using *ImageJ*, Version 1.8.0_172 (bundled with *Java*). The mean diameter of each fiber type was calculated for each seal, and these values were used to determine the mean diameter of fast- and slow-twitch fibers for each species.

For spotted and ringed seals, fibers were classified as either fast- or slow-twitch using myosin ATPase staining only (Figure 2). Classification of bearded seal fiber types using myosin ATPase staining alone was confounded by unusual intermediate staining of some bearded seal fibers. Therefore, we used myosin heavy-chain antibody staining to classify these intermediately staining fibers (from myosin ATPase) as either fast- or slow-twitch (Figure 3). We identified four types of fibers within bearded seal muscle samples based on myosin ATPase staining and reactions to two myosin heavy-chain antibodies. Most fibers stained classically as either slow-twitch (Figure 3, fiber 1) or fast-twitch IIA (Figure 3, fiber 2). However, some fast-twitch IIA fibers, identified by their reaction to the SC-71 antibody, stained intermediately after myosin ATPase basic pre-incubation (Figure 3, fiber 3). Finally, a small number of slow-twitch fibers were hybrids (see Medler, 2019) that reacted to both A4.951 and SC-71 antibodies (Figure 3, fiber 4). We elected to group traditional slow-twitch and hybrid fibers together (Figure 3, fibers 1 & 4), and traditional fast-twitch IIA fibers with the nontraditional IIA fast-twitch fibers (Figure 3, fibers 2 & 3).

Although antibody staining can be used to identify subtypes of fast-twitch fibers (e.g., type IIA, type IIB), here we only used this technique to confirm the primary fiber type profiles of bearded seal samples. Available data suggest that pinniped locomotor muscles typically lack type IIB (i.e., fast glycolytic) fibers (Kanatous et al., 2002; Watson et al., 2003; Moore et al., 2014), meaning we can be reasonably confident that the fast-twitch fibers identified in this study are type IIA (i.e., fast oxidative glycolytic). Thus, we report the fiber type profiles of all three species as general percentages of fast- and slow-twitch fibers without further distinguishing fast-twitch subtypes.

Statistical Analyses

We used factorial ANOVAs to examine differences in [Mb]; β; percentage fast-twitch fibers; and diameter of fast- and slow-twitch fibers by species, age class, and sex, as well as the interaction between species and sex. Percentage data (e.g., fast-twitch fiber data) were logit transformed before analysis. When significant differences were identified ($p < 0.05$), Tukey's HSD post-hoc comparisons were used to determine specific differences by species and age class, while Student's *t* tests were used to examine the effect of sex. Multiple linear regression was used to describe changes in [Mb] and β as a function of standard length by species, with standard length used as a proxy for age. Data were screened for outliers and normality prior to analysis, and residuals screened for homoscedasticity. Statistical analyses were completed using *JMP 14* software (SAS Institute Inc., Cary, NC, USA). Results were considered significant if $p < 0.05$.

Results

Muscle Biochemistry

Ringed seals exhibited the highest [Mb] of all species, followed by spotted and bearded seals ($F(2,52) = 43.33$; $p < 0.0001$; Table 1). Further, [Mb] differed by age class ($F(2,56) = 32.01$; $p < 0.0001$), with pups exhibiting the lowest [Mb] and adults exhibiting the highest. Sex did not

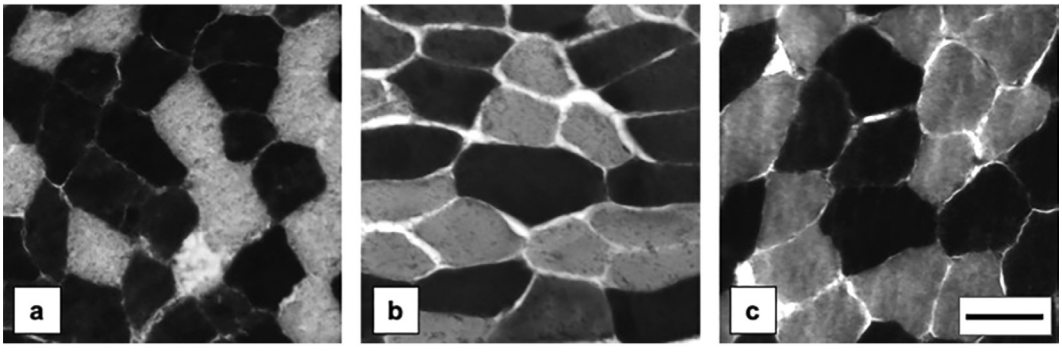


Figure 2. Representative images of longissimus dorsi muscle cross-sections of adult (a) ringed, (b) bearded, and (c) spotted seals stained for their myosin ATPase activity after basic pre-incubation. Slow-twitch fibers appear light, and fast-twitch fibers appear dark. Scale bar = 50 microns.

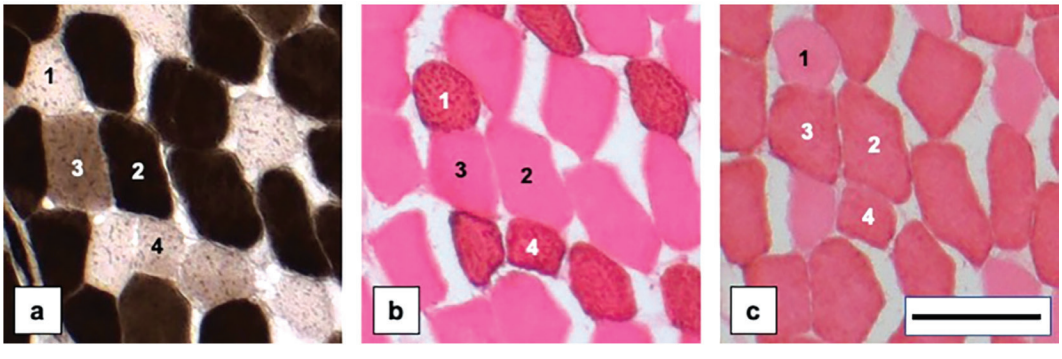


Figure 3. Representative images of bearded seal longissimus dorsi muscle cross-sections stained for (a) myosin ATPase activity after basic pre-incubation and reaction to (b) anti-slow (A4.951) and (c) anti-fast type IIA (SC-71) myosin antibodies. The same four representative fibers are identified in each panel by number. Fiber 1 is a classic slow-twitch fiber that stains light for myosin ATPase, dark for A4.951, and light for SC-71. Fiber 2 is a traditional IIA fast-twitch fiber that stains dark for myosin ATPase, light for A4.951, and dark for SC-71. Fiber 3 is a nontraditional IIA fast-twitch fiber that stains intermediately for myosin ATPase, light for A4.951, and dark for SC-71. Fiber 4 is a hybrid fiber that stains light for myosin ATPase, but darkly for both A4.951 and SC-71. To determine general proportions of fast- and slow-twitch fibers for bearded seals, we grouped fibers that stained similarly to fibers 1 and 4 in these images as slow-twitch fibers, and fibers that stained similarly to fibers 2 and 3 in these images as fast-twitch fibers. Scale bar = 100 microns.

Table 1. Myoglobin content ([Mb]) and buffering capacity (β) for individuals included in this study, grouped by species and age class. Standard length (SL) range indicates variability of individuals sampled, and *n* denotes number of samples per age class. Means are reported ± standard error.

Species	Age class	SL range (cm)	*n*	[Mb] (g 100 g wet tissue^{-1})	β (slykes)
Ringed seal (*Pusa hispida*)	Pup, < 1 y	63-98	7	5.18 ± 0.37	74.69 ± 4.47
	Subadult, 1-4 y	112	1	6.57	84.36
	Adult, ≥ 5 y	120-138	3	6.67 ± 0.20	84.37 ± 1.01
Bearded seal (*Erignathus barbatus*)	Pup, < 1 y	134-145	4	3.28 ± 0.09	69.24 ± 1.93
	Subadult, 1-4 y	161-201	10	4.17 ± 0.13	78.42 ± 2.05
	Adult, ≥ 5 y	184-233	27	4.55 ± 0.07	80.69 ± 0.91
Spotted seal (*Phoca largha*)	Pup, < 1 y	66-112	3	3.86 ± 0.76	68.49 ± 3.46
	Subadult, 1-4 y	127-149	5	5.40 ± 0.22	82.17 ± 1.82
	Adult, ≥ 5 y	141-171	4	5.38 ± 0.29	77.14 ± 0.65

significantly influence [Mb] ($F(1,56) = 3.85$; $p = 0.05$); however, we did find a significant species-by-sex interaction ($F(2,56) = 5.63$; $p = 0.006$). Post-hoc comparisons revealed that spotted seals were the only species to exhibit a significant difference in [Mb] by sex, with males showing higher [Mb] than females. Given that standard length can be used as a proxy for age in seals, we documented strong positive relationships between [Mb] and standard length for all three species (Figure 4), although the increase in [Mb] by length varied by species ($F(2,58) = 10.76$; $p = 0.0001$). Ringed and spotted seals exhibited the largest changes in [Mb] with increasing length, while bearded seals exhibited the smallest.

In contrast to [Mb], buffering capacity did not differ by species ($F(2,56) = 2.80$; $p = 0.07$) or sex ($F(1,56) = 0.02$; $p = 0.90$), and did not demonstrate a significant interaction of species-by-sex ($F(2,56) = 1.54$; $p = 0.22$). However, buffering capacity did vary by age class ($F(2,56) = 13.08$; $p < 0.0001$), with pups having lower β values than subadult and adult seals, which were comparable to one another (Table 1). Buffering capacity was positively correlated with standard length for all species (Figure 5), and developmental patterns generally mirrored those reported for [Mb]. Ringed seals showed the largest change in β across ontogeny, with bearded and spotted seals exhibiting more subtle changes as they matured ($F(2, 58) = 3.98$; $p = 0.02$).

Muscle Histochemistry

The muscles of all three species exhibited greater proportions of fast-twitch (type II) fibers relative to slow-twitch (type I) fibers (Table 2), with significant differences observed in the percentages of fast-twitch fibers by species ($F(2,35) = 16.02$; $p < 0.0001$). Bearded seals, followed by

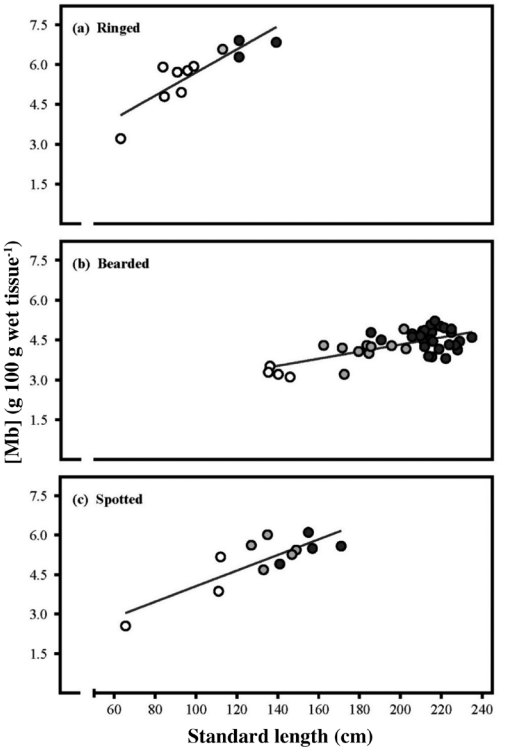


Figure 4. Myoglobin concentration [Mb] in relation to standard body length for (a) ringed, (b) bearded, and (c) spotted seals by age class (pup = white; subadult = gray; adult = black). Each point represents the mean value for an individual specimen analyzed in triplicate. [Mb] and standard length were positively correlated for all species (ringed: $n = 11$, $Y = 0.04383*X + 1.353$, $r^2 = 0.7585$, $p = 0.0005$; bearded: $n = 41$, $Y = 0.01344*X + 1.665$, $r^2 = 0.4497$, $p < 0.0001$; spotted: $n = 12$, $Y = 0.02808*X + 1.257$, $r^2 = 0.6343$, $p = 0.0019$). Excluding the smallest ringed seal from the analysis did not significantly alter the observed trend.

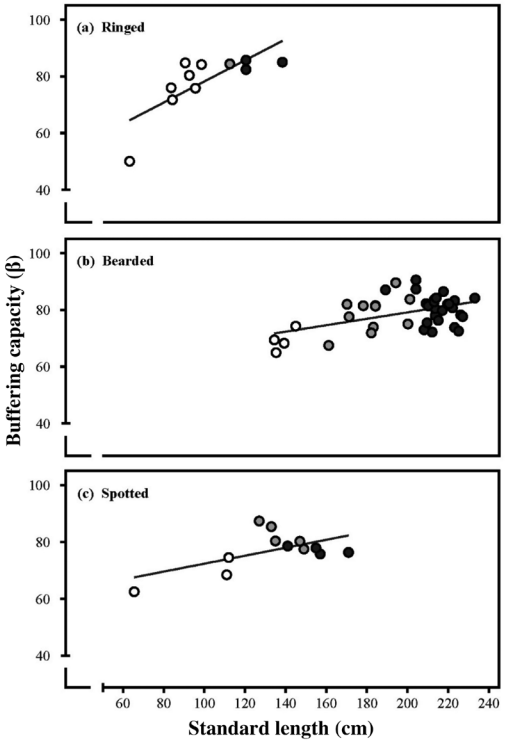


Figure 5. Nonbicarbonate buffering capacity (β; slykes) in relation to standard body length for (a) ringed, (b) bearded, and (c) spotted seals by age class (pup = white; subadult = gray; adult = black). Each point represents the mean value for an individual specimen analyzed in triplicate. The relationship between standard length and β was positively correlated for all species (ringed: $n = 11$, $Y = 0.3708*X + 41.33$, $r^2 = 0.5750$, $p = 0.0068$; bearded: $n = 41$, $Y = 0.1131*X + 56.53$, $r^2 = 0.2451$, $p = 0.0010$; spotted: $n = 12$, $Y = 0.1398*X + 58.39$, $r^2 = 0.3380$, $p = 0.0474$). Excluding the smallest ringed seal from the analysis did not significantly alter the observed trend.

ringed seals, had the greatest proportion of fast-twitch fibers for juvenile and adult age classes. Spotted seal pups had a greater proportion of fast-twitch fibers than ringed seal pups. Spotted seals possessed the most even mix of fast- and slow-twitch fibers (Table 2). Fiber type profiles did not differ by age class ($F(2,35) = 2.01$; $p = 0.15$), although this result may be an artifact of our limited sample sizes for some age classes. Similarly, there were no differences in fiber type profiles by sex ($F(1,35) = 3.56$; $p = 0.07$) nor a significant interaction of species-by-sex ($F(2,35) = 1.01$; $p = 0.38$).

In addition to typical fast- and slow-twitch muscle fibers, bearded seal muscle contained two fiber types not routinely observed in the muscles of ringed and spotted seals: (1) fast-twitch fibers staining intermediately for their myosin ATPase activity after alkaline preincubation and (2) hybrid slow-twitch fibers. Intermediately staining fibers (Figure 3, fiber 3) included in the fast-twitch category accounted for 29.9 ± 1.6% of the fast-twitch fibers and 20.5 ± 1.2% of the total bearded seal fibers counted. Hybrid fibers (Figure 3, fiber 4) included in the slow-twitch category made up 9.0 ± 1.8% of the slow-twitch fibers and only 2.9 ± 0.6% of the total bearded seal fibers counted.

The diameter of both fast- and slow-twitch fibers differed by species (fast-twitch: $F(2,35) = 6.90$; $p = 0.003$; slow-twitch: $F(2,35) = 5.56$; $p = 0.008$) and age class (fast-twitch: $F(2,35) = 6.47$; $p = 0.004$; slow-twitch: $F(2,35) = 3.40$; $p = 0.045$). Post-hoc comparisons indicated bearded seal fast- and slow-twitch fibers were larger in size (diameter) than those observed in spotted seals. There were no significant differences between the diameters of fast- or slow-twitch fibers in the muscles of males and females (fast-twitch: $F(1,35) = 0.23$;

Table 2. Proportion of fast-twitch fibers (%) and average diameters (microns) of fast- and slow-twitch fibers for a subset of individuals included in this study. Samples are grouped within species by age, and *n* denotes number of samples per age class. Means are reported ± standard error.

Species	Age class	*n*	Fast-twitch fibers (%)	Diameter (μm)	
				Fast-twitch	Slow-twitch
Ringed seal	Pup, < 1 y	6	53.65 ± 3.36	26.37 ± 3.17	28.25 ± 2.53
	Subadult, 1-4 y	1	56.00	40.00	35.10
	Adult, ≥ 5 y	3	58.96 ± 4.86	40.80 ± 2.88	39.87 ± 2.92
Bearded seal	Pup, < 1 y	2	59.18 ± 3.82	36.45 ± 6.45	35.70 ± 8.90
	Subadult, 1-4 y	7	69.18 ± 1.75	38.37 ± 2.34	35.49 ± 1.36
	Adult, ≥ 5 y	15	68.65 ± 1.46	48.65 ± 2.00	43.66 ± 1.80
Spotted seal	Pup, < 1 y	2	55.67 ± 8.83	32.15 ± 2.85	37.50 ± 0.90
	Subadult, 1-4 y	3	53.17 ± 1.44	33.00 ± 3.63	30.00 ± 3.41
	Adult, ≥ 5 y	4	55.10 ± 2.06	31.55 ± 3.71	28.45 ± 2.70

$p = 0.63$; slow-twitch: $F(1,35) = 0.90$; $p = 0.35$) and no species-by-sex interactions (fast-twitch: $F(2,35) = 0.26$; $p = 0.77$; slow-twitch: $F(2,35) = 0.21$; $p = 0.81$).

Individual physiological parameters and associated metadata for each seal included in this study can be found in Table S1 (the supplemental table for this article is available on the *Aquatic Mammals* website).

Discussion

This study provides a comprehensive assessment of the physiological properties and ontogenetic changes in locomotor muscle for several Arctic seals. Skeletal muscle profiles differed markedly by species in sexually mature adults. For the youngest individuals, a period of skeletal muscle development was apparent after birth, with the degree of change varying by species and parameter. In the following sections, we review the muscle biochemistry and fiber type profiles of the target species relative to other pinnipeds; relate these physiological properties to routine dive behavior and life history characteristics; and briefly explore the implications of our findings relative to changing environmental conditions.

Species Comparisons

Adult ringed seal muscle had the greatest [Mb] of the studied species, followed by spotted and bearded seals. Of note, we found higher [Mb] for adult ringed seals in Alaskan waters than previously reported for ringed seals in Svalbard (Lydersen et al., 1992). This difference (6.67 g 100 g wet tissue^{-1} vs 4.1 g 100 g wet tissue^{-1}) may be due to variation at the population level or a consequence of multiple muscle locations being sampled, measured, and averaged by Lydersen et al. Despite clear differences between ringed, bearded, and spotted seals documented in the present study, these species exhibit lower absolute [Mb] than reported values for other Arctic seals (Table 3).

Bearded seals have the lowest [Mb] of any phocid reported to date. They more closely resemble odobenids (Fay, 1982; Fay et al., 1984) and shallow-diving otariids than other Arctic phocids (Figure 6a). Both bearded seals and walruses (*Odobenus rosmarus*) are benthic foragers that typically exhibit shallow, short-duration dives. In contrast, harp (*Pagophilus groenlandicus*), hooded (*Cystophora cristata*), and ribbon (*Histriophoca fasciata*) seals are deep-diving, pelagic foragers and exhibit the greatest [Mb] among Arctic phocids (Lenfant et al., 1970; Burns et al., 2007, 2010). Ringed and spotted seals tend to dive to intermediate, epipelagic depths (Born et al., 2004; Boveng et al., 2009) and exhibit [Mb] values that are intermediate between benthic foragers and deep divers (Lenfant et al., 1970; Burns et al., 2007, 2010). The correspondence of [Mb] to diving and foraging strategies in Arctic pinnipeds supports the hypothesis that diving behavior is a strong driver of this physiological attribute (Kooyman & Ponganis, 1998).

The β values reported herein are similar for mature ringed, bearded, and spotted seals and to those reported for other Arctic phocids but roughly double the value reported for walruses (Noren et al., 2015). Such consistency in buffering ability for different phocid species is likely attributable to overarching trends in muscle physiology. In general, marine mammals that exhibit faster swim speeds and/or prolonged dive durations have increased muscle buffering capacity (Castellini & Somero, 1981; Noren, 2004). Phocids are not particularly fast swimmers among marine mammals as they typically swim at slower speeds to minimize oxygen consumption and increase dive durations (Williams, 1999). Thus, we would not expect differences in the buffering capacity of phocid locomotor muscles based on small differences in routine swimming speeds. Further, we do not observe any correlation between β and diving strategy or duration (Figure 6b). As phocid seals typically dive within their aerobic limits (Castellini et al., 1981; Kooyman et al., 1983; Ponganis, 2011), the development of distinct buffering capacities among species with varying dive profiles is not necessary.

Spotted seals show the most balanced distribution of fast- and slow-twitch muscle fibers in our study, with only a slight predominance of fast-twitch fibers (~55%), similar to the ~53% fast-twitch fibers in epaxial muscle of the closely related harbor seal (Watson et al., 2003). Ringed seals display a marginally higher proportion of fast-twitch fibers (~59%), which may reflect minor differences in routine diving behavior of ringed seals relative to spotted and harbor seals (Kelly & Wartzok, 1996; Eguchi & Harvey, 2005; Ziel et al., 2012). These species are midwater foragers that typically consume a variety of schooling fish; therefore, an even mix of fast- and slow-twitch fibers likely facilitates the suite of movements necessary to search for and capture small mobile prey in the water column.

Bearded seals display a distinct muscle fiber-type profile that includes hybrid slow-twitch fibers and unique, nontraditional fast-twitch fibers. The functional significance of these muscle fibers requires further exploration, but their existence reveals fundamental differences in the structure of bearded seal skeletal muscle relative to previously studied species. Further, bearded seals exhibit the highest proportion of fast-twitch

Table 3. Average body size (mass and standard length [SL]) and routine diving behavior (average dive duration and depth) of adult Arctic pinnipeds in relation to the myoglobin content ([Mb]) and buffering capacity (β) of their locomotor muscles.

Species	Mass (kg)	SL (cm)	Dive duration (min)	Dive depth (m)	[Mb] (g 100 g wet tissue^{-1})	β (slykes)
Ringed seal	50-70[a]	120-140[a]	< 10[h]	20-100[h]	6.7†	84†
Spotted seal	60-110[b]	140-170[b]	1-8[i]	< 70[i]	5.4†	77†
Ribbon seal	90-150[c]	150-180[c]	8-16[j]	200-600[j]	8.1[o]	--
Harp seal	120-140[d]	150-180[d]	2-15[k]	50-300[k]	8.6[p]	~82[q]
Hooded seal	150-350[e]	200-260[e]	5-25[l]	100-600[l]	9.5[p]	~82[q]
Bearded seal	260-360[f]	200-250[f]	1-6[m]	10-60[m]	4.6†	81†
Walrus	1,200-1,900[g]	150-360[g]	4-6[n]	30-70[n]	3.8[o]	41[r]

†This study; [a]Kelly et al., 2010; [b]Boveng et al., 2009; [c]Boveng et al., 2013; [d]Innes et al., 1981; [e]Kovacs, 2009; [f]Cameron et al., 2010; [g]Fay, 1982; [h]Kelly & Wartzok, 1996; Gjertz et al., 2000a; Born et al., 2004; Crawford et al., 2019; [i]Lowry et al., 1998; Olnes et al., 2023; [j]London et al., 2014; [k]Lydersen & Kovacs, 1993; Folkow et al., 2004; [l]Folkow & Blix, 1999; [m]Gjertz et al., 2000b; Krafft et al., 2000; Olnes et al., 2020; [n]Born & Knutsen, 1997; Nowicki et al., 1997; Gjertz et al., 2001; Jay et al., 2001; [o]Lenfant et al., 1970; [p]Burns et al., 2007, 2010; [q]Lestyk et al., 2009; Burns et al., 2010; and [r]Noren et al., 2015

fibers (~68%) of any seal species reported to date. Although we did not differentiate fast-twitch fibers into fast oxidative glycolytic (type IIA) or fast glycolytic (type IIB) subtypes, previous studies suggest that other seals lack type IIB fibers altogether (Kanatous et al., 2002; Watson et al., 2003; Moore et al., 2014). Given this comparative finding and the characteristic shallow-diving, slow-swimming behavior of bearded seals (J. Goodwin, pers. comm., 25 September 2014; Meranda, 2023), we presume that the fast-twitch fibers documented here are oxidative. Thus, the high proportion of fast-twitch fibers observed in this species may simply reflect an extension of the more typical mixed fiber profiles observed for shallow-diving pinnipeds; however, further investigation into the unusual muscle physiology of bearded seals is warranted.

The fiber type data reported herein fill important data gaps and raise interesting evolutionary questions for true seals (Family Phocidae). Our findings suggest a potential pattern in fiber type proportions between the Phocinae and Monachinae subfamilies. Hooded and ribbon seals are now the only Phocinae seals for which comparable fiber type data remain unavailable, but species from this lineage typically have a predominance of fast-twitch fibers in locomotor muscles (George et al., 1971; Reed et al., 1994; Watson et al., 2003). This pattern is opposite to that exhibited by the few Monachinae species that have been measured (i.e., elephant and Weddell seals), which have primarily slow-twitch fibers (Kanatous et al., 2002, 2008; Moore et al., 2014). Data from the remaining species in each clade would resolve whether seal fiber type profiles are more associated with phylogeny or diving behavior. For example, if trends in muscle structure are predominantly supported by evolutionary relationships, hooded and ribbon seals would have greater proportions of fast-twitch fibers as seen in other Phocinae seals. Alternately, if behavior is the primary driver, these deep-diving species would show a predominance of slow-twitch fibers as seen in the Monachinae seals studied to date.

Developmental Trends

The developmental trends in muscle physiology for our study species generally fit with reported patterns in other phocids. Precocial species that begin diving and foraging at younger ages typically exhibit more mature muscle parameters at birth (Burns & Castellini, 1996; Burns et al., 2005, 2007; Noren et al., 2005; Kuhn et al., 2006). Bearded seals have the shortest period of maternal dependency of the target species—entering the water within days of birth, diving regularly during the nursing period (Kovacs et al., 1996; Gjertz et al., 2000b), and consuming live prey prior to weaning (Burns, 1981)—and are born with relatively mature muscle physiology. Ringed seal pups have high [Mb] relative to adult conspecifics and rapid maturation of muscle physiology early in development, both of which are beneficial for a species that enters the water early and

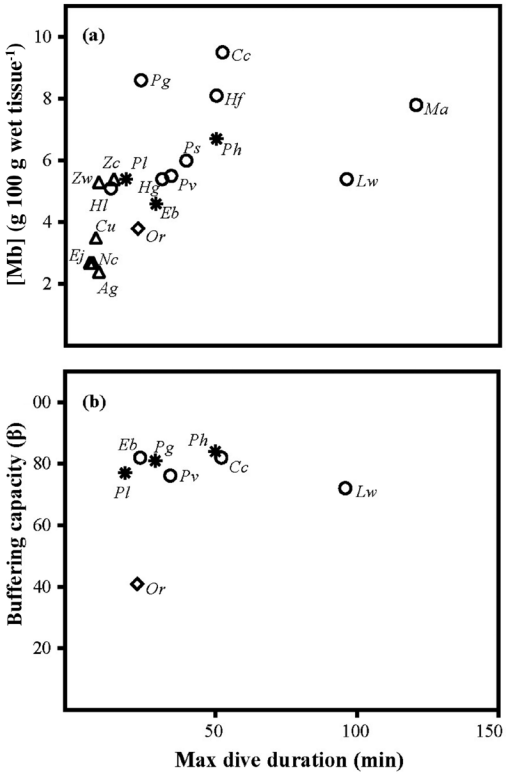


Figure 6. Comparisons of maximum dive durations to (a) known myoglobin concentrations and (b) buffering capacities across clade Pinnipedia. Symbols represent three taxonomic groups: (1) phocids (circles), (2) otariids (triangles), and (3) odobenids (diamonds). Asterisks represent the phocid data presented in this study. Where available, matched myoglobin and buffering capacity values are shown for each species. Species and source data are as follows: *Ag* = Antarctic fur seal (Boyd & Croxall, 1992; Reed et al., 1994), *Cc* = hooded seal (Folkow & Blix, 1999; Burns et al., 2007, 2010), *Cu* = northern fur seal (Lenfant et al., 1970; Sterling & Ream, 2004), *Eb* = bearded seal (Gjertz et al., 2000b; Olnes et al., 2020), *Ej* = Steller sea lion (Merrick & Loughlin, 1997; Richmond et al., 2006), *Hf* = ribbon seal (Lenfant et al., 1970; London et al., 2014), *Hg* = gray seal (Thompson & Fedak, 1993; Reed et al., 1994), *Hl* = leopard seal (Kuhn et al., 2006; Nordøy & Blix, 2009), *Lw* = Weddell seal (Ponganis et al., 1993; Heerah et al., 2013), *Ma* = northern elephant seal (Stewart & DeLong, 1995; Hassrick et al., 2010), *Nc* = Australian sea lion (Fowler et al., 2006, 2007), *Or* = walrus (Lenfant et al., 1970; Gjertz et al., 2001), *Pg* = harp seal (Folkow et al., 2004; Burns et al., 2007, 2010), *Ph* = ringed seal (Lydersen et al., 1992; Gjertz et al., 2000a), *Pl* = spotted seal (Boveng et al., 2009), *Ps* = Baikal seal (Neshumova & Cherepanova, 1984; Stewart et al., 1996), *Pv* = harbor seal (Lenfant et al., 1970; Eguchi & Harvey, 2005), *Zc* = California sea lion (Weise & Costa, 2007; Melin et al., 2008), and *Zw* = Galapagos sea lion (Villegas-Amtmann & Costa, 2010).

dives throughout the nursing period (Lydersen & Hammill, 1993). In contrast, spotted seals typically do not enter the water or forage during the dependency period (Burns, 2002), and they exhibit more gradual changes in muscle physiology across dependency compared to ringed seals.

The muscle physiology of subadult seals appears to be fully mature for each species, and observations of wild individuals support this finding. Dive profiles obtained from satellite-tagged ringed seals in the Bering, Chukchi, and Beaufort Seas show that subadult ringed seals often dive deeper than adults (Crawford et al., 2019), suggesting that subadults are not physiologically limited in their diving capacities in comparison to adult seals. Further, limited tagging data from subadult and adult bearded seals in Kotzebue Sound, Alaska, indicate similar diving behavior between these age groups (Boveng & Cameron, 2013). Diet studies for these seal species also indicate no difference in diet for subadult and adult age classes (Quakenbush et al., 2009; Crawford et al., 2015).

Compared to other Arctic pinnipeds, the documented proportions of [Mb] in ringed, bearded, and spotted seal pups is greater than expected (72 to 78% of adult values), while β values are similar (85 to 88% of adult values) to what was expected. For example, the [Mb] of harp and hooded seal pups is only 30% of the adult average, but they have β values that are about 75% of adults (Lestyk et al., 2009). In walruses, calf [Mb] is 44 to 49% of adult values, whereas individuals are born with β values similar to adults (Noren et al., 2015). Further, in temperate-living gray (*Halichoerus grypus*) and harbor seals, pup [Mb] is 30 to 60% of adult values (Burns et al., 2005; Noren et al., 2005; Clark et al., 2007). The relatively small differences between pup and adult [Mb] in our studied species may indicate accelerated maturation. Alternatively, this result could be attributed to the small number of pups in our study and/or the inclusion of individuals up to 1-y-old within the pup age class. Regardless, the early development of muscle physiology as seen in the present study is likely beneficial for species that live and dive in unpredictable sea ice habitats (Laidre et al., 2002; Noren et al., 2015; Noren & Suydam, 2016).

Muscle fiber type and diameter seem to change little during development in the seals sampled. However, comparisons to other species are difficult due to limited information on the ontogenetic trends of marine mammal muscle fiber profiles. The longissimus dorsi muscle of northern elephant seal pups is reported to have a small number of fast-twitch fibers, but adult muscle only contains slow-twitch fibers, indicating

a developmental shift in fiber composition. Further, their muscle fiber diameters apparently increase with age (Moore et al., 2014). Weddell seals also have a predominance of slow-twitch fibers in locomotor muscles at all ages, but may show an increase in fast-twitch fibers with maturity (Kanatous et al., 2008). Both species are deep divers, and the prevalence of slow-twitch fibers at all ages likely supports prolonged use of aerobic metabolism to facilitate extraordinary breath-hold diving. As ringed, bearded, and spotted seals are not particularly deep-diving species, they likely do not require the same endurance capacities.

Environmental Change and Behavioral Flexibility

Ringed, bearded, and spotted seals rely on sea ice to varying degrees, making them vulnerable to warming Arctic conditions (Kovacs et al., 2011; Laidre et al., 2015; Reimer et al., 2019; Olnes et al., 2023). The combined effects of sea ice loss, climate driven alterations in prey abundance, and corresponding changes in seal distribution may require that individuals modify routine behavior with changing environmental conditions. Increased traveling distance has already been reported for ringed (Hamilton et al., 2015; Von Duyke et al., 2020) and bearded (Olnes et al., 2021) seals, and ringed seals have been documented making longer and deeper dives (Hamilton et al., 2015). Given the physiological parameters reported herein, adult ice seals appear more able to increase their diving and/or traveling effort, whereas pups are likely not as capable. Our data suggest that pups could be at a physiological disadvantage if loss or early breakup of sea ice forces premature entries into the water. The relationships between specific physiological parameters and behavioral flexibility in the context of a warming Arctic are necessarily complex but are best addressed with comparative data for different species and age classes such as those reported herein.

Conclusions

This work expands our understanding of the unique biology of ice-associated Arctic seals by defining the structure and biochemistry of skeletal muscle for three species across a range of age classes. The distinct muscle profiles of the three species were often related to routine diving behavior, while ontogenetic trends appeared to be linked to the timing of key life history events. We recommend further research into the muscle physiology of pups and subadult seals as additional data could provide a more nuanced understanding of the earliest developmental phases in these amphibious mammals. Further, a finer scale examination of the unique fiber types observed in bearded seals may clarify their functional significance and evolutionary basis. Ultimately, the data provided herein can be combined with other physiological and behavioral metrics to assess the sensitivity and/or resilience of Arctic seals to rapid and ongoing climate change.

Note: A supplemental table for this article is available in the "Supplemental Material" section of the *Aquatic Mammals* website: https://www.aquaticmammalsjournal.org/index.php?option=com_content&view=article&id=10&Itemid=147.

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Comparative Muscle Physiology of Ringed (*Pusa hispida*), Bearded (*Erignathus barbatus*), and Spotted (*Phoca largha*) Seals from the Bering and Chukchi Seas

Supplemental Table

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Table S1. Muscle physiology parameters measured for individual seals (myoglobin content ([Mb]), nonbicarbonate buffering capacity (β), fiber diameters, and percentage fast-twitch fibers) with associated metadata (individual, date collected, collection location, species, standard length [SL], age class, and sex).

Individual	Date collected	Collection location	Species	SL (cm)	Age class	Sex	[Mb] (g 100 g wet tissue^{-1})	β (slykes)	Diameter (μm)		Fast-twitch fibers (%)
									Fast-twitch	Slow-twitch	
EB16PH014	4 June 2016	Point Hope	Bearded	171.0	Subadult	M	3.21	77.59	31.2	32.9	66.3
EB16PH016	4 June 2016	Point Hope	Bearded	213.5	Adult	F	4.79	77.97	57.2	49.8	59.5
EB16PH017	5 June 2016	Point Hope	Bearded	183.0	Subadult	F	4.00	73.93	36.7	36.8	72.5
EB16PH020	5 June 2016	Point Hope	Bearded	226.0	Adult	M	4.13	78.28	39.9	42.2	75.3
EB16PH021	5 June 2016	Point Hope	Bearded	178.0	Subadult	F	4.07	81.49	36.3	29.8	76.5
EB16PH022	5 June 2016	Point Hope	Bearded	144.7	Pup	F	3.11	74.29	42.9	44.6	55.4
EB16PH024	6 June 2016	Point Hope	Bearded	213.5	Adult	F	3.87	80.46	49.9	49.5	80.3
EB17PH001	3 June 2017	Point Hope	Bearded	223.0	Adult	F	4.91	73.85	29.2	26.7	59.7
EB17PH002	3 June 2017	Point Hope	Bearded	215.0	Adult	M	5.21	76.30	48.8	41.3	69.0
EB17PH003	1 June 2017	Point Hope	Bearded	227.0	Adult	M	4.45	77.66	--	--	--
EB17PH008	1 June 2017	Point Hope	Bearded	209.0	Adult	F	4.84	82.24	58.1	53.8	70.3
EB17PH009	2 June 2017	Point Hope	Bearded	209.5	Adult	F	4.49	75.44	48.4	40.0	70.5
EB17PH021	2 June 2017	Point Hope	Bearded	182.0	Subadult	M	4.30	71.90	41.3	35.9	64.7
EB17PH022	1 June 2017	Point Hope	Bearded	212.0	Adult	F	3.89	72.20	--	--	--
EB17PH030	3 June 2017	Point Hope	Bearded	135.0	Pup	M	3.52	64.98	30.0	26.8	63.0
EB17PH031	3 June 2017	Point Hope	Bearded	200.0	Subadult	M	4.91	75.07	47.8	40.6	70.6
EB17PH032	3 June 2017	Point Hope	Bearded	139.0	Pup	F	3.21	68.26	--	--	--
EB17PH033	3 June 2017	Point Hope	Bearded	225.0	Adult	M	4.31	72.57	46.4	44.6	74.3
EB17PH035	4 June 2017	Point Hope	Bearded	217.0	Adult	F	4.16	79.79	61.5	54.4	68.0
EB17PH036	5 June 2017	Point Hope	Bearded	223.0	Adult	F	4.78	83.26	--	--	--
EB17PH037	4 June 2017	Point Hope	Bearded	210.0	Adult	M	4.25	81.75	43.5	41.3	66.2
EB17PH038	4 June 2017	Point Hope	Bearded	213.0	Adult	M	5.07	83.62	46.6	36.5	71.7
EB17PH043	5 June 2017	Point Hope	Bearded	217.5	Adult	F	5.02	86.47	47.4	44.0	69.9
EB17PH044	2 June 2017	Point Hope	Bearded	184.0	Subadult	F	4.25	81.50	--	--	--
EB17PH045	5 June 2017	Point Hope	Bearded	170.0	Subadult	F	4.20	81.96	31.6	34.0	63.5
EB17PH046	5 June 2017	Point Hope	Bearded	219.5	Adult	M	4.96	82.01	49.0	42.5	67.1
EB17PH047	5 June 2017	Point Hope	Bearded	222.0	Adult	M	4.32	80.76	52.7	47.2	64.1
EB17PH066	6 June 2017	Point Hope	Bearded	210.0	Adult	F	4.86	81.43	51.1	41.1	63.7
EB18PH010	22 May 2018	Point Hope	Bearded	233.0	Adult	M	4.60	84.17	--	--	--
EB18PH011	22 May 2018	Point Hope	Bearded	184.0	Adult	F	4.78	81.30	--	--	--
EB18PH012	23 May 2018	Point Hope	Bearded	208.0	Adult	M	4.65	73.01	--	--	--

EB18PH013	22 May 2018	Point Hope	Bearded	213.0	Adult	F	4.53	82.78	--	--	--
EB18PH015	22 May 2018	Point Hope	Bearded	204.0	Adult	F	4.73	87.35	--	--	--
EB18PH016	23 May 2018	Point Hope	Bearded	189.0	Adult	M	4.50	87.07	--	--	--
EB18PH017	23 May 2018	Point Hope	Bearded	194.0	Subadult	F	4.29	89.58	--	--	--
EB18PH019	23 May 2018	Point Hope	Bearded	204.0	Adult	M	4.62	90.56	--	--	--
EB19PH035	30 May 2019	Point Hope	Bearded	201.0	Subadult	M	4.17	83.75	--	--	--
EB19PH037	30 May 2019	Point Hope	Bearded	220.3	Adult	F	3.81	82.12	--	--	--
EB19PH038	30 May 2019	Point Hope	Bearded	214.0	Adult	F	4.45	84.23	--	--	--
EB19PH042	30 May 2019	Point Hope	Bearded	134.2	Pup	F	3.29	69.44	--	--	--
EB1401	25 March 2016	Captive	Bearded	161.0	Subadult	M	4.30	67.48	43.7	38.4	70.2
PH16PH002	3 June 2016	Point Hope	Ringed	83.7	Pup	F	4.79	71.75	19.1	26.3	50.4
PH17GAM033	1 July 2017	Gambell	Ringed	62.5	Pup	M	3.21	50.06	16.1	18.1	60.2
PH17PH001	5 June 2017	Point Hope	Ringed	83.0	Pup	M	5.90	75.97	--	--	--
PH18SH013	24 Oct 2018	Shishmaref	Ringed	138.0	Adult	M	6.83	85.01	37.2	36.6	68.7
PH18SH021	14 Oct 2018	Shishmaref	Ringed	120.0	Adult	F	6.90	85.71	46.5	45.7	54.0
PH18SH022	27 Oct 2018	Shishmaref	Ringed	92.0	Pup	M	4.95	80.34	27.5	28.4	66.7
PH18SH034	5 Nov 2018	Shishmaref	Ringed	98.0	Pup	F	5.92	84.17	31.2	31.4	44.5
PH18SH035	5 Nov 2018	Shishmaref	Ringed	90.0	Pup	M	5.71	84.72	37.2	36.9	48.5
PH18SH051	5 Nov 2018	Shishmaref	Ringed	120.0	Adult	M	6.28	82.40	38.7	37.3	54.1
PH18SH052	4 Nov 2018	Shishmaref	Ringed	95.0	Pup	M	5.77	75.83	27.1	28.4	51.6
PH18SH053	4 Nov 2018	Shishmaref	Ringed	112.0	Subadult	F	6.57	84.36	40.0	35.1	56.0
PL16BRW001	14 Aug 2016	Barrow	Spotted	112.0	Pup	M	5.17	74.50	35.0	38.4	64.5
PL17GAM007	1 July 2017	Gambell	Spotted	65.5	Pup	F	2.55	62.51	29.3	36.6	46.8
PL18SH019	14 Sept 2018	Shishmaref	Spotted	157.0	Adult	F	5.49	75.79	42.6	36.5	54.7
PL18SH023	13 Sept 2018	Shishmaref	Spotted	141.0	Adult	F	4.90	78.57	28.8	25.3	61.1
PL18SH032	12 Sept 2018	Shishmaref	Spotted	171.0	Adult	F	5.58	76.34	26.7	25.3	52.3
PL18SH040	12 Sept 2018	Shishmaref	Spotted	135.0	Subadult	M	6.01	80.36	--	--	--
PL18SH049	11 Sept 2018	Shishmaref	Spotted	127.0	Subadult	M	5.61	87.36	25.8	24.1	55.7
PL18SH056	23 Oct 2018	Shishmaref	Spotted	133.0	Subadult	F	4.68	85.39	--	--	--
PL18SH062	19 Sept 2018	Shishmaref	Spotted	111.0	Pup	F	3.87	68.47	--	--	--
PL18SH064	9 Oct 2018	Shishmaref	Spotted	149.0	Subadult	F	5.43	77.52	35.8	35.9	50.8
PL18SH065	7 Oct 2018	Shishmaref	Spotted	147.0	Subadult	M	5.26	80.24	37.4	30.0	53.0
PL18SH076	25 Sept 2018	Shishmaref	Spotted	155.0	Adult	M	6.10	77.88	28.1	26.7	52.3