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Myoglobin Distribution in the Locomotory Muscles of Cape Fur Seals (*Arctocephalus pusillus pusillus*)

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Abstract

Diving animals rely on oxygen stored in their blood, muscles, and lungs to maintain aerobic metabolism during routine dives. This is made possible primarily by an elevated mass-specific blood volume, hemoglobin concentration, and muscle myoglobin concentration relative to terrestrial animals. In our previous studies of harbor seals and five species of cetaceans, the distribution of myoglobin in the locomotory muscles (epaxial and hypaxial muscles along the spine) was not uniform and was elevated in areas that generated greater force during swimming. In this study, we examined the fine-scale distribution of myoglobin in transverse sections of the primary swimming, or locomotory, muscles (*pectoralis complex*) of six male and four female Cape fur seals (*Arctocephalus pusillus pusillus*). The mean myoglobin concentration for all muscle samples was 36.9 ± 5.8 mg g^{-1} (range of mean values = 28.4 to 51.1). There were no significant differences in the distribution of myoglobin within and among transverse sections; however, the mean concentrations in all sections were significantly higher ($p < 0.001$) in females (41.6 mg g^{-1} ± 6.1) than for males (33.8 mg g^{-1} ± 2.8). The results from this study and our previous research indicate sufficient myoglobin concentrations to support an ability to store oxygen in skeletal muscles, reflecting adaptations for aerobic diving.

Key Words: Cape fur seal, *Arctocephalus pusillus pusillus*, myoglobin, oxygen stores, pectoralis muscle

Introduction

To overcome drag during aquatic locomotion, fur seals and sea lions (Order Carnivora; Family Otariidae) have evolved fore flippers to improve thrust production and propulsive efficiency using lift-based pectoral oscillation (Fish, 1996, 2000). The primary propulsive muscles in this group of pinnipeds are in the *pectoralis complex*, a thick, fan-shaped group of *muscles* on the chest that is imperfectly separated into three parts: (1) ascending, (2) descending, and (3) superficial (hereafter referred to collectively as the *pectoralis*). Dorsal muscles along the thoracic vertebrae and attached to the scapulae contract and relax in coordination with these pectoralis muscles to lift and rotate the fore flippers during swimming, while the hind flippers and caudal muscles are used for maneuvering (Fish, 1996, 2000).

As with most aquatic mammals, birds, and reptiles, fur seals rely primarily on oxygen stored in their blood, muscles, and, to varying degrees, lungs to maintain aerobic metabolism during routine dives (Kooyman & Gentry, 1986; Kooyman, 1989; Butler & Jones, 1997). This is made possible by an elevated mass-specific blood volume, hemoglobin concentration, and muscle myoglobin concentration compared with terrestrial mammals (Reed et al., 1994; Davis, 2014). Fur seals and sea lions have total body oxygen stores (*ca.* 45 ml O_2 kg^{-1}) greater than terrestrial mammals, but only half of that found in true seals (Phocidae), which results in a shorter aerobic dive limit (ADL) than true seals (Davis, 2014).

Previous studies of myoglobin concentrations in the muscles of fur seals and sea lions have relied primarily on single samples that provided no information on regional differences in concentration (Reed et al., 1994; Kanatous et al., 1999; Spence-Baily et al., 2007; Shero et al., 2012). In our previous research, we examined the fine-scale distribution of myoglobin in the primary swimming muscles (the epaxial and hypaxial muscles along the spine) of harbor seals (*Phoca vitulina*) and five species of cetaceans (Indo-Pacific humpback dolphin [*Sousa chinensis*], dusky dolphin [*Lagenorhynchus obscurus*], striped dolphin [*Stenella coeruleoalba*], false killer whale [*Pseudorca crassidens*], and the Indo-Pacific bottlenose dolphin [*Tursiops aduncus*]) using three transverse sections—(1) anterior, (2) lower thoracic, and (3) caudal—that were sampled multiple times on a grid that uniformly covered each section (Polasek & Davis, 2001; Polasek et al., 2006). In these species, myoglobin is not homogeneously distributed in the locomotory muscles, and concentrations are highest in those areas that are known to produce greater force and, therefore, consume more oxygen during aerobic swimming. In this study, we measured the fine-scale distribution of myoglobin in the primary swimming muscles of Cape fur seals (*Arctocephalus pusillus pusillus*; CFSs), which use a different mode of locomotion (pectoral oscillation) compared with harbor seals (pelvic oscillation) and cetaceans (caudal oscillation). We hypothesized that myoglobin would be non-uniformly distributed in locomotory muscles, similar to locomotory muscles in harbor seals and cetaceans.

Methods

Animals

Samples were collected in 1996 from six male and four female CFSs that were culled at Kleinsee (29° 34' 10" S, 16° 59' 48" E), Seal Island (False Bay; 34° 08' 18" S, 18° 35' 00" E), and Malgas Island (32° 57' 18" S, 17° 51' 42" E) in South Africa as part of ongoing work conducted by the Sea Fisheries Research Institute, Cape Town, South Africa, now Oceans & Coasts (Table 1). Mean body masses for males (adult and subadult) and females (adults) were 102.8 ± 27.5 kg and 53.0 ± 4.1 kg, respectively.

Tissue Samples

The entire pectoralis (left side of all animals except for AP5606) was excised and weighed, and three transverse sections (A, B, and C) were taken along the anterior-posterior axis of the muscle (Figure 1a). Each transverse section was precisely labeled for its location and orientation within the animal. Sections were then sampled at points on a grid using a stainless-steel borer, averaging 54 samples per section, with a range of 32 to 84 cores depending on muscle size (Figure 1b). All samples were taken within 6 h *postmortem* and frozen at -80° C until processed.

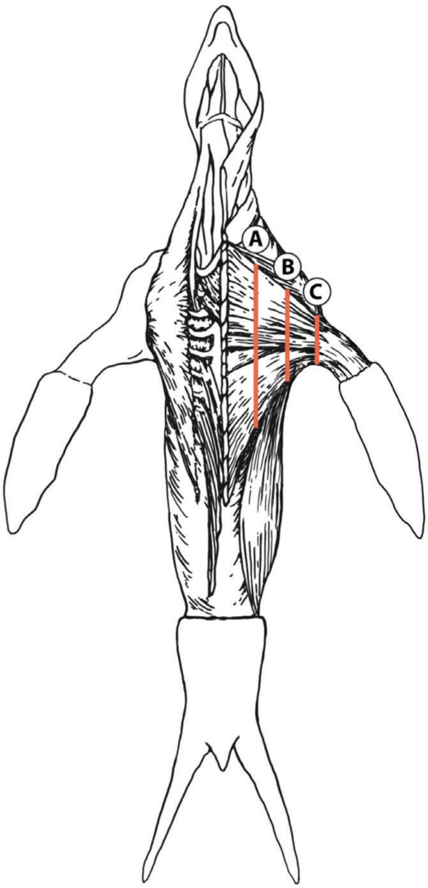
Myoglobin Determination

Myoglobin concentration was measured following methods from Reynafarje (1963). Muscle samples (200 to 300 mg) were cleaned of connective tissue, weighed, and homogenized in 15 ml 0.04 mol L^{-1} phosphate buffer at pH 6.6 using

Table 1. Sex, body mass, estimated age class (adult and subadult), standard (st) length, curvilinear (cuv) length, axillary (ax) girth, and pectoralis mass for Cape fur seals (*Arctocephalus pusillus pusillus*) sampled in this study. Animal location is denoted in parentheses after each animal number: Kleinsee (K), Seal Island (SI), and Malgas (M).

Animal	Sex	Mass (kg)	Est. age class	st length (cm)	cuv length (cm)	ax girth (cm)	Pectoralis (kg)
AP4605 (K)	M	116	Adult	180	194	111	2.9
AP4606 (K)	M	96	Subadult	169	181	106	3.0
AP4607 (K)	M	111	Adult	177	185	121	2.7
AP4608 (K)	M	145	Adult	193	208	128	2.8
AP4609 (K)	F	53	Adult	141	152	91	1.6
AP4610 (K)	M	67	Subadult	160	168	95	1.6
AP4972 (SI)	F	48	Adult	136	140	83	1.6
AP4973 (SI)	F	58	Adult	143	150	91	1.7
AP4974 (SI)	F	53	Adult	136	141	92	2.0
AP4977 (M)	M	82	Subadult	164	174	99	2.2

Figure 1a. Anatomical diagram showing the locations of the three cross sections of tissue (A, B, and C) taken from the pectoralis complex that comprises the ascending, descending, and superficial pectoralis muscles (Redrawn from Howell, 1929, by R. Deel). **b.** Cross-sectional view of muscle section A with location of points from which cores were taken. An average of 54 core samples were taken per section.



Pyrex tissue grinders in an ice bath. The homogenate was centrifuged at 28,000 g for 50 min at 4° C using a Sorvall RC5B refrigerated centrifuge. The supernatant was extracted and gently bubbled by insertion of the gas into the solution, with 99.9% carbon monoxide (CO) for 3 min to convert the Mb to carboxymyoglobin. Based on previous work by Polasek & Davis (2001), dithionite was not necessary for metmyoglobin reduction.

After conversion to carboxymyoglobin, the absorbance of the supernatant was measured at 538 and 568 nm to eliminate any influence from hemoglobin contamination using a Beckman DU-64 spectrophotometer with a 1-cm light path. A standard (equine Mb skeletal muscle powder; Sigma-Aldrich, St Louis, MO, USA) was run with each set of samples that were run in triplicate. The standard values were accepted if they were within 95% of expected values, and myoglobin replicate values were only accepted if they were within a 90% confidence interval. Myoglobin concentration was expressed in mg per gram of fresh tissue.

Statistical Analysis

Initial summaries of sex and transverse section contrasts suggested fundamentally lower myoglobin levels in males compared to females and a somewhat decreasing level of myoglobin as we moved from section A to C (Figure 2). We used a linear mixed-effects model with fixed effects of sex and transverse section and a random intercept component by individual to estimate differences in myoglobin levels using OpenBUGS in *R* (R Development Core Team, 2011).

Kriging, a Gaussian process regression for interpolation of data, was used to generate visual contours of myoglobin concentration for the three transverse sections of the *pectoralis* muscle from each fur seal. We used surface generation to visualize the subtle changes along each transverse section for both males and females. Data are expressed as mean myoglobin concentration ± standard deviation. Muscle size varied by body mass, resulting in an unequal spatial distribution and number of samples among Cape fur seals due to the gridded nature of the sampling points along each muscle, so predictive surface estimates tended to have larger variance near the edges. All statistical analyses were done in *R* (R Development Core Team, 2011).

Results

Animal total body mass (102.8 ± 27.5 kg; 53.0 ± 4.1 kg) and pectoralis mass (2.5 ± 0.5 kg; 1.7 ± 0.2 kg) were measured for males and females, respectively. Animals with larger body mass had larger pectoralis mass ($R^2 = 0.87$, $p = 0.001$); however, there was no correlation between body mass and myoglobin concentration ($R^2 = 0.324$, $p = 0.361$) nor pectoralis mass and myoglobin content ($R^2 = 0.619$, $p = 0.06$). The mean myoglobin concentration for male and female muscle samples was 33.8 ± 2.8 mg g^{-1} (range: 22 to 73 mg g^{-1}) and 41.6 ± 6.1 mg g^{-1} (range: 15 to 61 mg g^{-1}), respectively. The mean concentrations of myoglobin for subadult and adult male CFSs were within 3% and, therefore, were combined. The male pectoralis transverse sections A, B, and C mean myoglobin concentrations were 34.5 ± 2.4, 34.1 ± 2.4, and 32.7 ± 3.7 mg.g^{-1}, respectively (Table 2). The mean concentrations for female pectoralis transverse sections A, B, and C were 42.3 ± 7.3, 42.5 ± 6.5, and 39.9 ± 5.9 mg.g^{-1}, respectively. Overall,

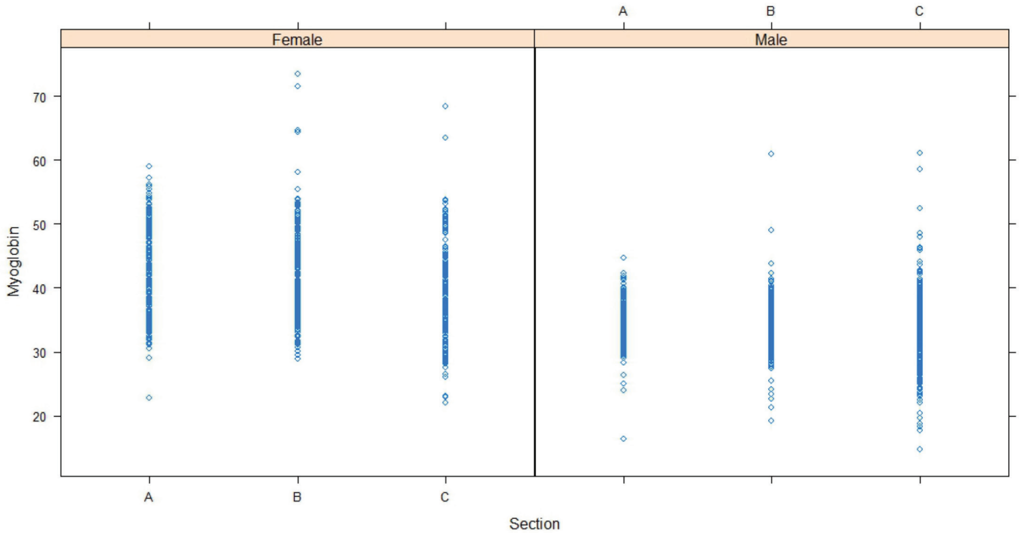


Figure 2. Summary of myoglobin concentration by sex (Female, Male) and transverse section (A, B, and C) for 1,522 samples of pectoralis muscle tissue taken from Cape fur seals (*Arctocephalus pusillus pusillus*)

Table 2. Mean (± SD) myoglobin concentrations (mg g^{-1}) for each transverse muscle section for the male and female Cape fur seals. Section A was closest to the midline and attachment to the sternum. Section C was closest to the muscle attachment on the shoulder ball joint. There were no significant differences among or within transverse sections.

		A	B	C
Males	AP4605	38.3 ± 2.3	37.7 ± 4.0	37.1 ± 4.8
	AP4606	37.1 ± 2.4	37.7 ± 4.9	38.1 ± 4.9
	AP4607	33.7 ± 2.3	32.4 ± 2.8	28.4 ± 5.3
	AP4608	35.0 ± 2.2	34.2 ± 3.1	35.3 ± 3.9
	AP4610	31.6 ± 1.9	31.3 ± 2.7	30.1 ± 4.1
	AP4977	32.8 ± 2.8	33.6 ± 2.3	33.2 ± 7.2
Male mean		34.8 ± 2.3	34.5 ± 3.3	33.7 ± 5.0
Females	AP4609	38.8 ± 3.8	41.1 ± 4.5	37.9 ± 5.5
	AP4972	34.4 ± 2.4	34.8 ± 2.6	33.8 ± 5.8
	AP4973	44.7 ± 2.8	44.2 ± 5.4	41.1 ± 4.4
	AP4974	51.1 ± 3.1	50.9 ± 4.4	47.3 ± 6.1
Female mean		42.3 ± 3.0	42.8 ± 4.2	40.0 ± 5.5

there were no significant differences in the distribution of myoglobin in the transverse sections (A, B, and C; Figure 3). However, the mean concentrations in all three sections were significantly higher ($p < 0.001$) in females (41.6 ± 6.1 mg g^{-1}) than for males (33.8 ± 2.8 mg g^{-1}), a difference supported by the coefficient estimate for sex in the mixed-effects model (Figure 4).

Discussion

The mean myoglobin concentration (36.9 mg g^{-1}) in CFSs was similar to that of northern fur seals (*Callorhinus ursinus*; 22.4 to 35.8 mg g^{-1}; Lenfant et al., 1970; Kanatous et al., 1999; Shero et al., 2012), Antarctic fur seals (*Arctocephalus gazella*; 24 to 31 mg g^{-1}; Reed et al., 1994), Australian fur seals (*Arctocephalus pusillus doriferus*; 41.6 mg g^{-1}; Spence-Bailey et al., 2007), and Steller sea lions (*Eumetopias jubatus*; 28.7 mg g^{-1}; Kanatous et al., 1999). We found that the mean concentration was significantly higher (21%) in females than in males. Similarly, a study of California sea lions also determined adult females had greater muscle myoglobin concentrations than adult males (Weise & Costa, 2007). Otariids (fur seals and sea lions) generally have myoglobin concentrations that are similar to the walrus (*Odobenus rosmarus*, Odobenidae) and coastal or shallow diving cetaceans such as the common bottlenose dolphin (*Tursiops truncatus*; 33 mg g^{-1}), but lower than in Phocids (true seals), especially deep diving species such as the northern elephant seal (*Mirounga angustirostris*; 65 mg g^{-1}) and in deep diving cetaceans such as sperm whales (*Physeter macrocephalus*; 70 mg g^{-1}) (see reviews: Mirceta et al., 2013; Davis, 2014). In marine mammals, oxygen bound to myoglobin in muscle contributes significantly (*ca*. 25 to 33%) to the total body oxygen store, and its concentration is directly correlated with the ADL and routine dive duration (Davis, 2014).

The female CFSs had a higher myoglobin concentration than the males, indicating a higher capacity to bind oxygen. The animals in the study were mature females and subadult or adult males based on reproductive organs collected and classified from each animal (data collected by Oceans & Coasts, Department of Environmental Affairs, Cape Town, South Africa). Higher oxygen-binding

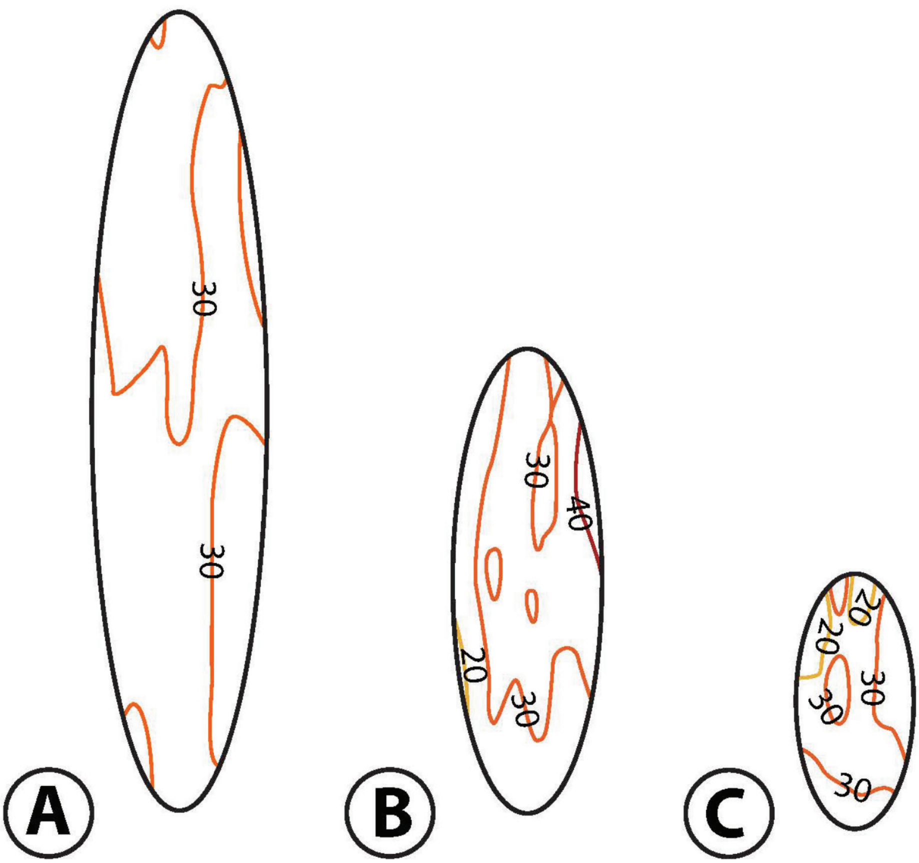


Figure 3. An example of contours of myoglobin concentration (mg g^{-1} wet tissues) in sections A, B, and C of the pectoralis complex (AP4977); the orientation of the contours is from the flipper looking toward the sternum.

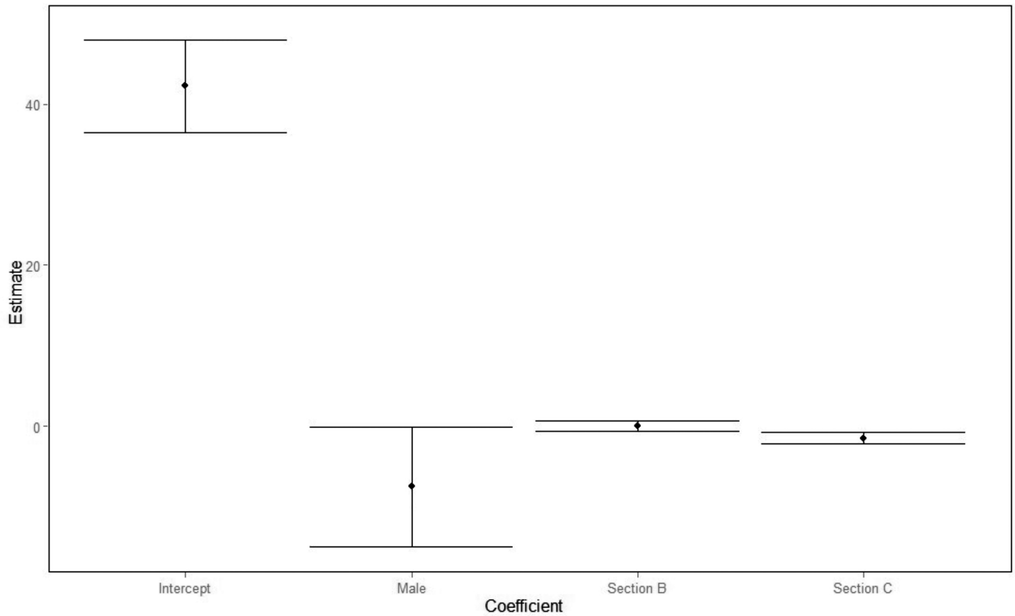


Figure 4. Coefficient estimates (with 95% CI) from a linear mixed-effects model of myoglobin concentration by sex, transverse pectoralis section, and a random intercept component by individual

capacity in the females is supported by sexual differences in the duration of foraging trips. Adult female CFSs have longer foraging trips than males (Kirkman, 2010). Weise & Costa (2007) speculate that sexual dimorphism may contribute to the higher myoglobin levels in female California sea lions. The females are smaller than males and have longer dive durations which likely increases apnea and may drive up myoglobin levels. Weise & Costa also postulate that females compensate for their smaller size with increased myoglobin concentrations to increase mass specific oxygen stores, which result in an increased ADL. Female Australian fur seals also have longer foraging trips with a mean dive duration of 2.9 min and a maximum dive duration of 8.9 min (Arnould & Hindell, 2001), whereas males have a mean dive duration of 2.5 min and a maximum dive duration of 6.8 min (Hindell & Pemberton, 1997), further supporting the findings in this study. In contrast, New Zealand fur seal males dive longer than females with average dive lengths of 3.6 ± 2.5 min and 2.7 ± 1.3 min, respectively (Page et al., 2005). The differing dive patterns are likely due to resource partitioning among Australian and New Zealand fur seals as sympatric species (Page et al., 2005). Although Page et al. (2005) only include one adult male, the significantly higher myoglobin concentration in female CFSs is likely developed for foraging success when pups are waiting to be fed onshore and females have to swim long distances between bouts of foraging and nursing.

In our previous research on harbor seals, we found variations in myoglobin concentration, primarily along the length of the epaxial muscles (caudal > mid lumbar > cranial) (Polasek et al., 2006). In addition, the myoglobin concentration in cetaceans was significantly higher (11%) in the interior of the epaxial and hypaxial muscles lying closest to the vertebrae rather than in the exterior of the muscle, and the myoglobin concentration was significantly higher in the caudal region closest to the flukes (Polasek & Davis, 2001). Although there was some variation in distribution of myoglobin within a male or female's individual pectorals section, or between sections within an individual, there were no consistent and significant differences in the myoglobin concentration patterns of distribution in the CFS pectoralis. This suggests that the entire pectoralis musculature complex in the chest contributes equally to force generation during swimming using pectoral oscillation. In contrast, the epaxial and hypaxial muscles that lie along the spine from the head to the hind flippers or flukes in phocids and cetaceans, respectively, do not contribute equally to thrust during swimming (Videler & Kamermans, 1985). This may result, in part, from the greater lengths of the epaxial and hypaxial muscles compared with the shorter pectoralis muscle. The results from this study did not find a difference in myoglobin concentration within the pectoralis muscle, a very different conclusion than our previous research on other marine mammals. This study did find that myoglobin concentration varied with gender; this is likely due to species-specific adaptations for aerobic diving.

Acknowledgments

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