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# Effects of Age, Mass, and Terrestrial Duration on Oxygen Store Development in Phocids and Otariids

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# Capstone of Rachael M. Stevenson

Submitted in Partial Fulfillment of the Requirements for the Degree of

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HALMOS COLLEGE OF NATURAL SCIENCES AND OCEANOGRAPHY

EFFECTS OF AGE, MASS, AND TERRESTRIAL DURATION ON  
OXYGEN STORE DEVELOPMENT IN PHOCIDS AND OTARIIDS

By

Rachael Stevenson

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Halmos College of Natural Sciences and Oceanography  
in partial fulfillment of the requirements for  
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## **Abstract**

Diving ontogeny among pinniped species was explored to determine how oxygen store development was affected by age, mass, and terrestrial duration between Phocidae and Otariidae. Distinct physiological and post-natal developmental differences exist between the two families which contribute to oxygen store and diving development. Blood oxygen data for six phocids and otariids and muscle oxygen data for two phocids and otariids were obtained. Body mass significantly ( $p < 0.01$ ) affected gray seal hematocrit, while oxygen stores in harbor seals were significantly ( $p < 0.05$ ) affected by age class. Age class and body mass significantly ( $p < 0.01$ ) affected hemoglobin and hematocrit in northern elephant seals and Australian fur seals. Body mass was the sole factor significantly ( $p < 0.01$ ) affecting blood oxygen store development in northern fur seals and Steller sea lions. Conversely, muscle myoglobin in northern elephant seals and Australian fur seals was significantly ( $p < 0.05$ ) impacted by both age class and body mass. Muscle oxygen development lagged behind blood oxygen development and did not significantly increase until after independent foraging began. This indicates muscle development occurs after blood development and requires hypoxia and aquatic activity to fully mature. This study recognized distinct differences in post-natal development between species, as it compared nursing, post-weaning, and maternal strategies in relation to diving development. This study emphasized the importance of considering diving ontogeny at a species-specific level and further supports research regarding oxygen store development between phocids and otariids.

**Keywords:** Phocidae, Otariidae, oxygen stores, hemoglobin, hematocrit, myoglobin, diving

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## Statement of Significance

Pinnipeds rely on a unique physiology of oxygen storage and utilization while diving and foraging (Kooyman et al. 1981; Lestyk et al. 2009). The mechanisms for oxygen (O<sub>2</sub>) storage are not fully developed at birth, but transition during the period from nursing to independent foraging. Therefore, pinniped pups must spend a portion of their lives terrestrially. This period comes in the form of a terrestrial post-weaning fast (PWF), which is crucial for rapid physiological maturity, or a prolonged lactation period that allows for slower maturity over a longer period of maternal dependence from pups to juveniles (Burns et al. 2005; Noren et al. 2005; Lestyk et al. 2009; Bennett et al. 2010). Among the pinnipeds, the family Phocidae, true seals, have exceptional diving capabilities. Phocids have large oxygen reserves, which result in prolonged diving activity (Kooyman et al. 1981). The family Otariidae, fur seals and sea lions, tend to make substantially shorter and shallower dives, indicating they have fewer oxygen reserves. Oxygen stores that must be developed for adequate diving and foraging include hemoglobin, hematocrit, and myoglobin (Scholander 1940; Butler and Jones 1997; Burns et al. 2007). The rate of oxygen development may be affected by inter-species differences in life history strategies, which in turn can affect the behavior and diving capacity of juvenile seals (Burns et al. 2007; Noren et al. 2008). These different strategies provide insight into the ontogeny and O<sub>2</sub> store development of pups throughout the weaning period and they may correlate to the terrestrial duration necessary for pups to gain sufficient oxygen stores for future diving and foraging (Burns et al. 2005, 2007; Noren et al. 2008).

This comprehensive review explores the variations in oxygen capacity of six phocid and otariid species from ontological development to resultant dive capacity. Raw data contributed by participating authors for the six species allowed for comparison of levels of oxygen-storing components, body mass, and age classes to help assess what influences the rate of oxygen store development and diving ability.

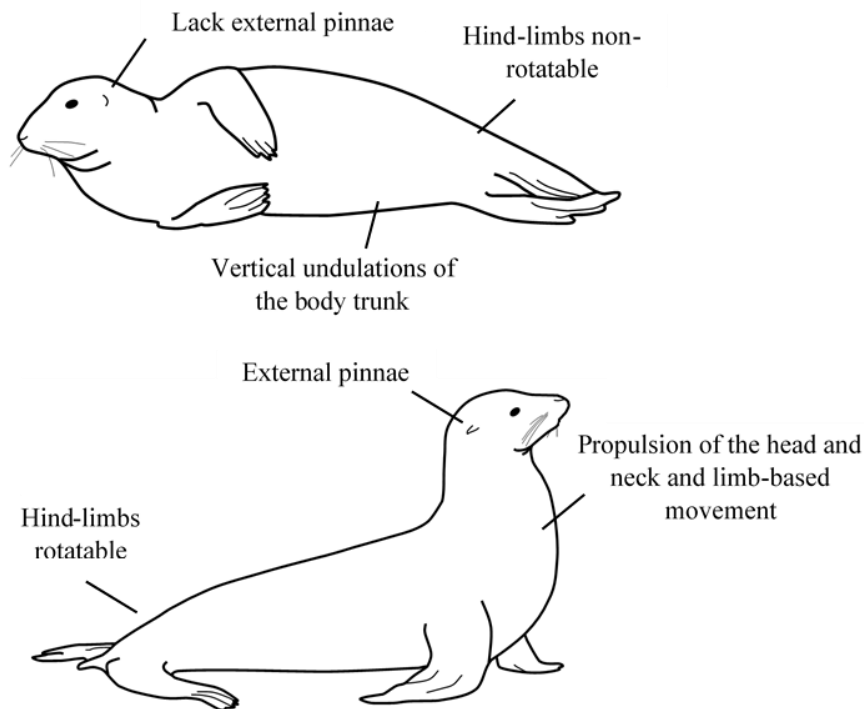
## Background

Pinnipedia is a sub-order of marine mammals, which includes seals, sea lions and fur seals, and walruses in three families, Phocidae, Otariidae, and Odobenidae, respectively (Boness and Bowen 1996). The pinnipeds are unique marine mammals in that they are both aquatic and terrestrial. At birth, pups are essentially terrestrial until they develop adequate diving and swimming skills and then they begin their amphibious lives (Bartholomew 1970; Kovacs and Lavigne 1986). As adults, pinnipeds spend a majority of their lives in the water, requiring land to haul out for resting, breeding, and molting. Phocids, true seals, have greater breath-holding abilities and greater oxygen storing capacity than otariids, which are the fur seals and sea lions (Kooyman et al. 1981). However, due to the longer lactation period of otariids compared to phocids, otariids are expected to be more physiologically mature at weaning (Costa 1991a; Kuhn et al. 2006). Several characteristics distinguish them from each other, including anatomy, diving and foraging strategies, and maternal attendance. These differences may affect the ontogeny of oxygen stores in their bodies and the resulting diving and foraging ability.

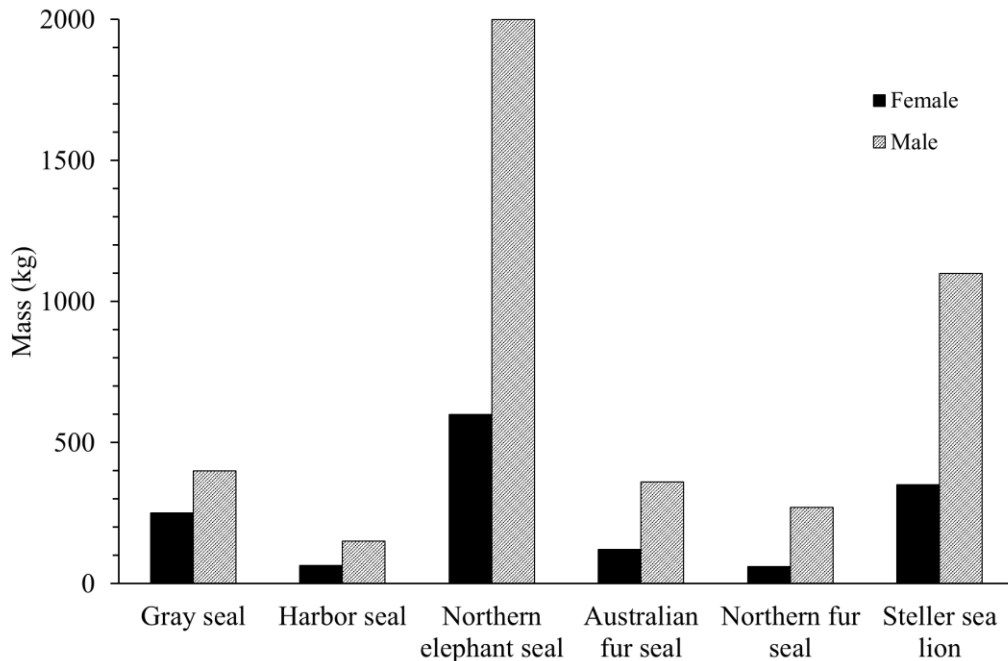
### *Anatomical Differences*

Phocids and otariids are unique from each other in their anatomy (Figure 1). Phocids lack external ears, or pinnae. Instead, they have small slits where their external ears would have been. Otariids have external pinnae but they are reduced in size to small flaps (Riedman 1990). Phocids tend to have shorter fore-flippers and are unable to rotate their hind-limbs forward. On land, they must hunch, bounce, and lunge their bodies forward while using their forelimbs for balance. Underwater, phocids become more agile. They use pelvic oscillations to propel through the water column and use their forelimbs for steering. Unlike phocids, otariids have rotatable hind-flippers that they can fold underneath their bodies. This allows otariids to virtually walk on land, while swinging their necks and heads side to side to create momentum. In the water column, otariids swim using pectoral oscillations while using their hind-limbs for maneuvering (Kooyman et al. 1981; Riedman 1990). Overall, there are substantial body mass differences amongst

species within the two families. Body size can be used as an indicator of oxygen storing capacity. If a seal has a larger body, they may also have a larger blood volume and muscle mass, both which contribute to the total amount of oxygen stored in the body. Most phocids lack sexual dimorphism, however, body size is highly variable across species in this family. Some species such as the northern elephant seal, are extremely sexually dimorphic, while other species have reversed dimorphism, where the female is larger than the male. The otariids are profoundly sexually dimorphic, with adult males weighing two to four times more than females (Gentry and Kooyman 1986; Riedman 1990). Figure 2 shows the variability of body size in the phocids and the sexual dimorphism that is characteristic of the otariids that were utilized in this study.



**Figure 1.** Major morphological differences between phocids (top) and otariids (bottom).

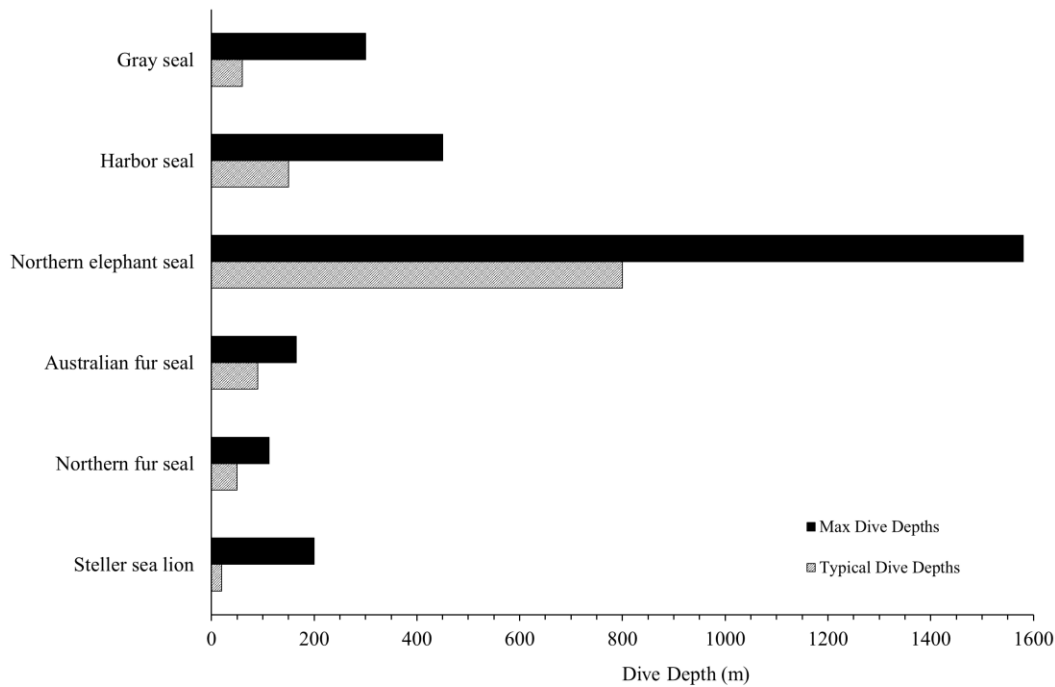


**Figure 2.** Average mass in kilograms (kg) of females and males of each study species. (Data excerpted from Bonner 1979b; Warneke 1979; Bonner 1981; Deutsch et al. 1990; Le Boeuf and Laws 1994; Fedak et al. 1994; Arnould and Hindell 1999, 2001; Kirkwood and Arnould 2008; Loughlin 2009; Gelatt et al. 2015; Bowen 2016; Lowry 2016; David A.S. Rosen, pers. comm.).

### *Diving Characteristics*

Other characteristics that sets phocids and otariids apart are their diving abilities and foraging strategies (Figure 3). Phocids are typically deep-divers. Multiple species routinely dive to 400-600 meters (m) for up to 30 minutes and some species can dive continuously to remarkable depths greater than 1200 meters (m) for longer than 50 minutes. While traveling, phocids may reach speeds of 12-20 knots, but regularly swim at approximately five knots or less (Riedman 1990). They spend a greater proportion of their time underwater with very little time at the surface, and therefore are classified as surfacers (Boyd 1997; Renouf 1991). The otariids spend substantially less time diving, diving for shorter periods and at shallower depths. Most otariids spend less than five minutes diving and typically only reach depths of approximately 100 m (Berta et al.

2006; Ponganis 2015). Otariids spend most of their time at the surface, tending to porpoise out of the water while cruising at speeds of 16 to 25-30 knots for fur seals and sea lions, respectively. They are classified as divers because they spend most of their time at the surface interspersed with short, shallow diving sessions (Riedman 1990; Renouf 1991). These differences in diving ability may be affected by the amount of available oxygen stores and, therefore, may affect foraging strategies among species.



**Figure 3.** Typical and maximum dive depths in meters (m) for each study species. (Data excerpted from Bonner 1979b; Gentry et al. 1986; Le Boeuf 1994; Merrick et al. 1994; Le Boeuf et al. 1996; Hindell and Pemberton 1997; Merrick and Loughlin 1997; Bowen et al. 1999; Arnould and Hindell 2001; Frost et al. 2001; Gjertz et al. 2001; Loughlin et al. 2002; Beck et al. 2003; Noren et al. 2005; Berta et al. 2006; Spence-Bailey et al. 2007; Kirkwood and Arnould 2008; Burns 2009; Gelatt et al. 2015; Lowry 2016).

## *Maternal Strategies*

Differing maternal strategies between phocids and otariids affect their lactation and weaning periods (Table 1). Phocids have an abbreviated nursing period, generally 4-50 days, when the female continuously nurses her pup and tends to forgo foraging (Oftedal et al. 1987; Noren et al. 2005). During this time, newborn pups, known as neonates, nurse intensively on protein and lipid-rich milk and quickly gain substantial weight as muscle and blubber (Bowen 1991). This rapid increase in body mass leads to increases in blood volume, which is directly related to increases in hemoglobin and hematocrit in the blood and as myoglobin in the skeletal muscle. Nursing tends to abruptly cease and the weaned pups will fast from four days to several weeks (Worthy and Lavigne 1983; Bowen et al. 1987; Reilly 1991). This period of fasting occurs on land and is known as the terrestrial post-weaning fast (PWF); it is defined as the period from the cessation of nursing to first independent foraging. During this time, pups typically spend a majority of their time on land or ice without feeding and may venture in the water, but do not forage. Altricial pups, or pups that are not well developed at birth, also use this time to shed their neonatal coat, known as a lanugo. Precocial pups, or pups that are well developed at birth, molt their lanugo *in utero* and are already born with a substantial blubber layer and therefore do not have to molt their coats (Lestyk et al. 2009). Throughout this period, all pups utilize the large reserves of lipids gained during nursing as an energy source until they can adequately forage (Bowen 1991; Noren et al. 2008). The length of the post-weaning fast is correlated with increasing levels of oxygen-storing hemoglobin, hematocrit, and myoglobin, although the mechanism of those increases remains uncertain (Oftedal et al. 1987; Thorson and Le Boeuf 1994; Burns et al. 2005; Noren et al. 2005). The rate of oxygen store development in pups may subsequently impact their diving capabilities and could have life-long ramifications on their foraging success.

Unlike phocids, otariids have extensive lactation periods. Typically, fur seals and sea lions exhibit a prolonged feeding-foraging cycle known as the female or maternal

attendance pattern, where the female alternates nursing on land and foraging out to sea while the pup fasts (Gentry 1970; Oftedal et al. 1987; Higgins et al. 1988). Adult female fur seals typically have long foraging cycles of 4-23 days while sea lions generally have shorter trips of 1-2 days (Arnould and Costa 2006). Pups gain weight slowly on milk that is approximately 50% less fatty than that of phocid seals, and thus do not exhibit the massive changes in body composition that are observed in phocids (Kovacs and Lavigne 1986; Riedman 1990; Burns et al. 2004). The ability of the females to replenish their energy reserves between bouts of nursing allows for longer-term investment in their pups (Oftedal et al. 1987). As time goes on, females progressively increase their time spent foraging and the pups are slowly weaned. Because of this, otariid pups have prolonged terrestrial periods, do not require a post-weaning fast, and their oxygen stores may develop more slowly than that of phocid pups. Although sea lions and fur seals have this extended nursing period, they can begin diving and swimming at an early age. For example, Steller sea lion pups begin swimming and diving around five months of age. This allows the blood and muscle oxygen stores to increase during nursing so that by the time pups begin to dive, their total body oxygen stores are 69% and by weaning, 80-90% that of the total body oxygen stores of adults (Merrick and Loughlin 1997; Burns et al. 2004; Richmond et al. 2006). Due to this different lactation strategy, otariids may be more physiologically mature at weaning and first independent foraging, and do not require a terrestrial PWF before they begin diving (Burns et al. 2004).

Some species of otariids exhibit nursing strategies that are more like phocids. The nursing and weaning duration varies in otariids from four months to three years (Stirling 1983; Gentry et al. 1986). Two different patterns are observed among fur seals, depending on latitude. Those that live in the tropics or sub-tropics, which include the majority of otariid species, have the classic extended lactation with nursing and foraging cycles, allowing pups to develop their diving and foraging skills before they are weaned (Gentry and Kooyman 1986; Horning and Trillmich 1997; Baker and Donohue 2000). In contrast, fur seals found in the subpolar regions, such as the northern fur seal and Antarctic fur seals, have shorter nursing and weaning periods (Peterson 1968; Gentry et al. 1986; Baker and Donohue 2000). Numerous studies have researched these differential



behavior patterns in the female attendance cycle and have found that these patterns hold true for high latitude species. The northern fur seal and the Antarctic fur seals wean their pups in approximately four months, while for fur seals and sea lions in temperate, tropic, and subtropical latitudes, the attendance cycles can last for more than two years, such as Australian fur seals (Costa and Gentry 1986; Oftedal et al. 1987; Costa and Trillmich 1988; Costa 1991a, 1991b; Boness and Bowen 1996; Donohue et al. 2002).

**Table 1.** Pupping season and duration of post-natal development.

Family	Pupping Season	Nursing Duration	PWF Duration	Maternal Attendance Pattern	Source
Phocidae					
<i>Gray seal</i>	Early Mar*	~ 18 days	10-28 days	-	1, 2, 3
<i>Harbor seal</i>	Feb-Sept	21-28 days	15-17 days	-	4, 5
<i>Northern elephant seal</i>	Dec-Mid-Mar	~28 days	2-2.5 months	-	6, 7, 8, 9, 10
Otariidae					
<i>Australian fur seal</i>	Nov-Dec	~1 year	-	6-7 days foraging, few days nursing	11, 12, 13, 14, 15
<i>Northern fur seal</i>	June-July	4 months	-	14 days foraging, 1-3 days nursing	16, 17, 18, 19
<i>Steller sea lion</i>	Early June-July	1-2 years	-	3 days foraging, up to 2 days nursing	20

**Note:**

\* Gray seal pupping season is dependent on population; peak is in early March

**Source:**

<sup>1</sup>Coulson and Hickling 1964; <sup>2</sup>Riedman 1990; <sup>3</sup>Reilly 1991; <sup>4</sup>Lawson and Renouf 1987; <sup>5</sup>Muelbert and Bowen 1993; <sup>6</sup>Le Boeuf et al. 1972; <sup>7</sup>Reiter et al. 1978; <sup>8</sup>Thorson 1993; <sup>9</sup>Le Boeuf and Laws 1994; <sup>10</sup>Thorson and Le Boeuf 1994; <sup>11</sup>Warneke 1979; <sup>12</sup>Warneke and Shaughnessy 1985; <sup>13</sup>Arnould and Hindell 2001; <sup>14</sup>Kirkwood et al. 2006; <sup>15</sup>Deacon and Arnould 2009; <sup>16</sup>Bartholomew and Hoel 1953; <sup>17</sup>Peterson 1968; <sup>18</sup>Gentry and Holt 1986; <sup>19</sup>Higgins et al. 1988; <sup>20</sup>Rea et al. 2000

## **Gray seal**

The gray seal (*Halichoerus grypus*) is widely distributed throughout the northwestern Atlantic Ocean and consists of three major populations: one in the Gulf of Finland, one throughout Iceland, Norway, and the British Isles, and one in the Gulf of St. Lawrence to Sable Island, Nova Scotia (Davies 1957; Bonner 1979a; Lesage and Hammill 2001; Hall and Thompson 2009; Bowen 2016; NOAA Marine Mammal Stock Assessment Report 2017). They are the most abundant pinniped on the Scotian Shelf (Bowen et al. 2003). They are robust phocids, with males reaching approximately three meters and 400 kilograms (kg) and females reaching two meters and 250 kg (Bonner 1981; Beck 2002; Bowen 2016). Gray seals dive along continental shelves, reaching depths greater than 250 m for a maximum of 22 minutes (Beck et al. 2003; Noren et al. 2005).

The time of pupping varies by population, with peak seasons ranging from February to December (Lesage and Hammill 2001). Gray seal females give birth on various substrates, including mainland, fast ice (ice attached to land) and pack ice (ice formed at sea) (Riedman 1990). At birth, pups are approximately 90 centimeters (cm) in length and weigh 10 kg (Worthy and Lavigne 1983; Iverson et al. 1993). They are also born with a white lanugo to keep them temporarily warm until their blubber layer forms after several weeks (Renouf 1991). Pups intensively nurse for about 18 days, tripling their body mass at a rate of 1.3-1.8 kg per day, and increasing their fat content by approximately 45% (Coulson and Hickling 1964; Iverson et al. 1993; Hall 1998; Noren et al. 2008; Bennett et al. 2010). Weaning is abrupt and marks the beginning of the post-weaning fast.

The PWF in gray seals ranges from 10-28 days (Reilly 1991; Hall 1998; Noren et al. 2008). Unlike other phocids whose post-weaning fast may depend on age, gray seals' body condition at weaning is the major factor that explains the PWF duration (Hall et al. 2001; Noren et al. 2008). After weaning, studies found that smaller and leaner pups had shorter fasts and began independently foraging earlier than larger, fatter pups (Noren et

al. 2003, 2008). Hemoglobin, hematocrit, and myoglobin increased by 28, 21, and 33%, respectively, from birth to the end of the post-weaning fast and were positively correlated with body mass (Noren et al. 2005).

### **Harbor seal**

The harbor seal (*Phoca vitulina*) is a highly precocial phocid distributed throughout the northern hemisphere in the Atlantic and Pacific oceans (Boulva and McLaren 1979; Bowen et al. 1992; Burns et al. 2005). It is one of the most abundant and widely distributed marine mammals in Prince William Sound, Alaska (Frost et al. 2001). Harbor seals are small to medium-sized, ranging from 1.2-2 m and weighing 65-170 kg at adulthood. They may dive between 10-150 m, although dives down to 450 m have been recorded, and most of their dives are less than ten minutes (Bonner 1979b; Bowen et al. 1999; Frost et al. 2001; Gjertz et al. 2001; Berta et al. 2006; Burns 2009; Lowry 2016).

Females give birth to their pups on large, sandy to gravel beaches where high tide can often decrease the amount of land available for resting. Because of this, nursing pups often enter the water shortly after birth (Knudston 1977; Boulva and McLaren 1979; Bowen et al. 1992; Muelbert and Bowen 1993; Jørgensen et al. 2001; Burns et al. 2005). At birth, pups weigh approximately 8-12 kg and have about 1.4 cm of blubber already developed, having shed their lanugo *in utero* (Bowen et al. 1992; Muelbert and Bowen 1993).

Neonates nurse for about 3-4 weeks and continuously enter the water with their mothers throughout the lactation period (Lawson and Renouf 1987; Muelbert and Bowen 1993). Compared to other phocids, harbor seal pups gain weight at a much slower rate of 0.6 kg per day (Boulva and McLaren 1979; Kovacs and Lavigne 1986). Weaning is abrupt and the pups fast for approximately 15-17 days (Muelbert and Bowen 1993).

Harbor seal pups appear to have mature oxygen stores at either birth or due to entering the water early on with their mothers. Burns et al. (2005) found that neonates had blood oxygen stores 57% that of adult values. However, muscle myoglobin stores were only 37% that of adults. This suggests that despite the harbor seal's highly precocial nature, they are not physiologically mature. Final muscle maturity may not depend solely

on physical activity and hypoxia, indicating that the post-weaning fast may be a critical period for total oxygen store development (Jørgensen et al. 2001; Burns et al. 2005; Prewitt et al. 2010).

### **Northern elephant seal**

The northern elephant seal (*Mirounga angustirostris*) is the largest phocid in the northern Hemisphere with major breeding colonies in central and southern California. Northern elephant seals (NES) are extremely sexually dimorphic, with adult males reaching three to four times the maximum mass of an adult female, approximately 2,000 kg and 600 kg, respectively (Deutsch et al. 1990; Fedak et al. 1994; Le Boeuf and Laws 1994). Northern elephant seals have one of the most widespread foraging ranges of all pinnipeds; adult seals make two migrations a year, one each after the breeding and molting seasons. They can travel great distances, as far north as the Aleutian Islands and the Gulf of Alaska and south to the southern Gulf of California and the Northwest Hawaiian Islands (Le Boeuf et al. 2000; Robinson et al. 2012; Hückstädt 2015).

NES can remain at sea, diving continuously, for eight to ten months at a time (Le Boeuf et al. 2000; Andrews and Enstipp 2016). These phocids are one of the deepest-diving pinnipeds, typically diving between 330-600 m with an average duration of 20-30 minutes, although individuals have been recorded at depths greater than 1,500 m and durations of two hours (Le Boeuf 1994; Le Boeuf et al. 1996).

Females typically give birth during the boreal winter from December to mid-March (Le Boeuf and Laws 1994). Neonates weigh approximately 30 kg (Le Boeuf et al. 1972). The female fasts while continuously nursing her pups for approximately 27 days before abruptly weaning her pup and returning to sea (Le Boeuf and Laws 1994; Noren et al 2003). During this time, pups triple their birth mass and increase their fat from 4-5% to approximately 50% (Rea and Costa 1992; Noren et al. 2008). Pups are born with low mass-specific levels of hemoglobin and myoglobin and must wait four to six weeks for their natal fur to molt before they can begin developing their diving and swimming abilities (Le Boeuf et al. 1972; Thorson 1993; Thorson and Le Boeuf 1994; Noren et al. 2005). Pups have a PWF that lasts up to two and a half months before they begin

independently foraging. Unlike some species, northern elephant seal pups enter the water within a few weeks of birth, learning to swim in tide pools or shallow waters (Le Boeuf et al. 1972; Reiter et al. 1978; Thorson and Le Boeuf 1994; Noren et al. 2003). Once pups leave the rookery and begin foraging independently, their diving ability improves quickly. Thorson and Le Boeuf (1994) found a 46.7% increase in mass-specific oxygen stores with concurrent increases in hemoglobin and myoglobin in the first ten weeks. By the end of the PWF, hemoglobin levels are mature and myoglobin levels are 76% that of adult values (Thorson 1993; Thorson and Le Boeuf 1994; Noren et al. 2005). Within a month of independence, pups' diving resembled that of adults, spending approximately 85% of their time submerged and diving on average to 206 m for ten minutes (Thorson and Le Boeuf 1994).

Northern elephant seal pups have one of the longest post-weaning fasts of any other phocid species. Because of their extreme diving capacity, pups may need longer to develop adequate oxygen stores to sustain their diving activity as juveniles and adults. Noren et al. (2005) compared the maturity of oxygen stores of NES pups to gray seal pups. At the onset of foraging, gray seal pups were less than half the age of NES pups and had already attained mature hemoglobin and myoglobin levels that were 68% of adult values. These differences in the rate of oxygen store maturity may correlate to the length of the PWF and thus relate to diving ability.

#### *Otariid Taxa*

#### **Australian fur seal**

Australian fur seals (*Arctocephalus pusillus doriferus*) are temperate otariids, endemic to ten breeding islands within the Bass Strait off the southeast coast of Australia (Warneke and Shaughnessy 1985; Pemberton and Kirkwood 1994; Arnould and Hindell 1999, 2001; Kirkwood et al. 2005; Spence-Bailey et al. 2007; Kirkwood and Arnould 2008). This species is the largest fur seals, with females weighing up to 120 kg and males reaching 218-360 kg (Warneke 1979; Warneke and Shaughnessy 1985; Arnould and Hindell 1999, 2001; Kirkwood and Arnould 2008). Australian fur seals are capable of

diving to depths greater than 300 m; however, they typically forage on the benthos along the continental shelf, with dives reaching 70-90 m depth for approximately three minutes (Hindell and Pemberton 1997; Arnould and Hindell 2001; Spence-Bailey et al. 2007; Kirkwood and Arnould 2008).

Females give birth to pups in November and December (austral summer) on rocky substrate (Arnould and Hindell 2001; Deacon and Arnould 2009). Pups weigh 7-8 kg at birth and nurse for approximately ten months. During this time, females alternate 6-7 days foraging at sea with a few days on shore nursing (Arnould and Hindell 2001; Kirkwood et al. 2006). Pups may enter the water a few weeks after birth but typically wait to begin diving until their natal fur has molted, 3-4 months after birth (Warneke and Shaughnessy 1985; Donohue et al. 2000). Pups typically wean within one year, although individual pups have been observed nursing into their second and third year (Warneke 1979; Warneke and Shaughnessy 1985; Hume et al. 2001; Spence-Bailey et al. 2007; Kirkwood and Arnould 2008; Deacon and Arnould 2009).

Spence-Bailey et al. (2007) examined the physiological development of diving in the Australian fur seal. By five months of age, pups had hemoglobin values that were already adult-like. In contrast, by nine months of age, myoglobin was only 21% of adult values. This suggests that although Australian fur seals can enter the water shortly after birth, pups are still not physiologically mature until they begin diving and foraging independently. This also indicates that otariid pups may not have as mature blood oxygen stores as many phocids do at birth.

### **Northern fur seal**

Northern fur seals (*Callorhinus ursinus*) are highly migratory members of the otariid family (Baker 2007). Breeding colonies are distributed throughout the Pribilof Islands and Bogoslof Island in the Bering Sea, and they range the North Pacific Ocean off Russia and Alaska to central California (Kenyon and Wilke 1953; Blix and Steen 1979). Adults are sexually dimorphic with males reaching approximately 270 kg and females about 40-50 kg (Gelatt et al. 2015; David A.S. Rosen, pers. comm.). This species forages in nearshore waters over continental shelves and pelagic waters, typically following

vertically migrating prey. They tend to make shallow dives ranging 50-60 m for less than three minutes; however, individuals have been recorded diving to approximately 200 m (Gentry et al. 1986; Gelatt et al. 2015).

Pups weigh approximately five to six kg at birth (Blix and Steen 1979). They are born with dense fur, have two to four millimeters (mm) thick layers of subcutaneous fat, and have metabolic rates that are similar to those of harbor seals (Miller and Irving 1975; Blix and Steen 1979). Lactating females spend anywhere from one to three days nursing on land and take intermittent foraging trips up to 14 days at a time. Throughout the nursing period, the foraging trips increase in duration with no change in the duration in nursing bouts (Gentry and Holt 1986; Higgins et al. 1988).

Pups are abruptly weaned at approximately four months of age (Bartholomew and Hoel 1953; Peterson 1968; Gentry and Holt 1986; Baker and Donohue 2000). Northern fur seals appear to have adapted to high latitude breeding, thus exhibiting a weaning strategy that resembles that of a phocid (Arnould et al. 1996; Donohue 1998; Baker and Donohue 2000). Once pups are weaned, they leave their natal islands and begin their first post-weaning migration (Baker 2007). Pups then lead a highly pelagic lifestyle and will not return to their natal islands until they are between 14 months to a few years of age (Kenyon and Wilke 1953; Bigg 1990; Baker 2007). Lea et al. (2010) found that during pups' first migration, the majority of dives were less than 20 m lasting approximately two minutes, although maximum depths averaged 112 m for five to six minutes. However, pups' diving ability increased linearly with age until 8-10 months. This was consistent with other research that has shown northern fur seal pups tend to feed close to the surface on vertically migrating prey (Baker 2007; Lea et al. 2010; Lee et al. 2014).

Shero et al. (2012) showed that northern fur seal pups had lower blood and muscle oxygen stores relative to adults by approximately 35 and 30%, respectively. However, pup myoglobin was relatively mature compared to other otariid species that were the same age and their physiological development appeared to be more phocid-like. It is likely that among the otariids, northern fur seal pups may be more physiologically mature at nursing and weaning to account for the short amount of developmental time they have before diving and foraging independently.

## Steller sea lion

Steller sea lions (*Eumetopias jubatus*) are the largest of all the otariids (Pendleton et al. 2006; Maniscalco 2014). They are polygynous and sexually dimorphic with males reaching up to 1,100 kg and females weighing up to 350 kg (Loughlin 2009). Steller sea lions (SSL) are distributed throughout the North Pacific Ocean, ranging from Japan to southeastern Alaska to central California (Pendleton et al. 2006; Loughlin 2009; Gelatt 2016). Adult Steller sea lions typically dive between 10-50 m with maximum depths greater than 200 m (Merrick et al. 1994; Merrick and Loughlin 1997; Loughlin et al. 2002).

The breeding season begins early to mid-May when males arrive at rookeries, or breeding colonies, and establish their territories. Female sea lions arrive shortly thereafter to give birth and copulate with bull males. Females give birth on rocky beaches from late May through early June to pups that weigh approximately 20 kg (Loughlin 2009). Pups nurse approximately nine days before females begin their first foraging cycle at sea, alternating about three days at sea and several hours to two days on shore nursing (Rea et al. 2000). Pups are born with small energy stores, designed to last for the short periods of time they spend fasting while the female is away at sea (Davis et al. 2006). They grow at a slow rate of approximately 0.3-0.12 kg per day (Burns et al. 2004; Richmond et al. 2006). Females begin to prolong their time spent at sea and slowly wean their pups at approximately one year of age, although there have been observations of pups suckling beyond two years of age.

Pups begin to enter the water approximately two to four weeks after birth, which could indicate that their diving ontogeny begins at an early age (Sandegren 1970; Merrick and Loughlin 1997; Pitcher et al. 2005; Pendleton et al. 2006). Burns et al. (2004) found hemoglobin and hematocrit increased rapidly within the first ten months; therefore, blood oxygen stores were similar to adult values by the time the pups reached one year of age. Myoglobin gradually improved through the first year but did not fully develop until after the second year, implying that like phocids, otariids require aquatic activity to fully develop their muscle oxygen stores (Burns et al. 2004; Richmond et al. 2006).



Diving is necessary for marine mammals to travel and forage for prey (Lestyk et al. 2009; Thomas and Ono 2015). During dives, pinnipeds experience prolonged periods of apnea, absence of breathing, and must rely on the concentrations of oxygen stored within their body tissues to sustain aerobic metabolism while diving (Kooyman et al. 1981; Noren et al. 2005). They must also conform to extreme pressure and temperature differences (Riedman 1990). Therefore, pinnipeds have several physiological and anatomical adaptations that allow them to optimize their O<sub>2</sub> reserves to contend with challenges of life at sea.

### **Physiological Responses**

Several physiological responses, known as the mammalian diving reflex, occur during diving to help reduce the rate of oxygen consumption. These responses include bradycardia, peripheral vasoconstriction, and metabolic rate reduction (Kooyman et al. 1981; Riedman 1990; Butler and Jones 1997; Burns et al. 2009; Lestyk et al. 2009; Prewitt et al. 2010). Among the pinnipeds, phocids tend to exhibit the most extreme responses, while otariids generally display moderate physiological responses to diving.

#### *Bradycardia*

Bradycardia is the slowing of the heart rate (Irving 1964; Kooyman et al. 1981). During bradycardia, the heart rate drops significantly and then continues to slowly decrease (Butler 1982). Multiple experiments have shown that several seal species can adjust their heart rate in preparation for a dive. The studies have shown that their heart rates prior to diving were correlated with the subsequent dive durations (Fedak 1986; Hill et al. 1987; Elsner et al. 1989; Berta et al. 2006). Seals may reduce their cardiac output by 80-90% of the pre-dive rate while maintaining a constant stroke volume (Sinnott et al. 1978; Kooyman et al. 1981; Renouf 1991; Butler and Jones 1997). For example, harbor seals can decrease their heart rate to approximately four beats per minute (Scholander

1940; Hochachka 2000). Alternatively, studies have shown that SSL making short, shallow dives, can reduce their heart rate to 10-20 beats per minute (Ponganis et al. 1997; Hindle et al. 2010; McDonald and Ponganis 2014). However, SSL exhibiting deep dives were observed dropping their heart rate to less than ten beats per minute (McDonald and Ponganis 2014). By reducing the heart rate, blood output from the heart substantially reduces, which slows the flow of blood and consequently, oxygen, throughout the body (Panneton 2013).

### *Peripheral Vasoconstriction*

Peripheral vasoconstriction reduces blood flow to organs that can withstand hypoxia and maintains flow to those that cannot. Therefore, blood is shunted to critical organs, such as the brain, heart, and for pregnant females, the placenta. Other visceral organs, such as the liver and kidneys, are extremely tolerant of hypoxia and can undergo reduced blood flow (Riedman 1990; Berta et al. 2006). This reduces the amount of oxygen consumption during breath-holding because there are fewer interactions between oxygen and tissues (Kooyman et al. 1981; Riedman 1990; Renouf 1991).

### *Reduced Metabolic Rates*

Both bradycardia and vasoconstriction result in a reduced metabolic rate (Butler and Jones 1997). Studies of metabolic rates in pinnipeds have suggested that seals have resting metabolic rates 1.5-3 times greater than those of similar-sized terrestrial animals (Kooyman 1981; Riedman 1990). Kooyman et al. (1981) found that metabolism during diving was reduced to approximately 25% of resting metabolic rates in harbor seals. Evidence of a reduced metabolic rate during diving includes the decrease in core body temperature of seals even though peripheral vasoconstriction should increase the insulation of the core (Scholander et al. 1942; Kooyman et al. 1981). This suggests that there was a reduction in heat production (Kooyman et al. 1981). Pups have higher metabolic rates than adults due to their growth and development, which can affect the aerobic dive limit (Burns et al. 2005; Noren et al. 2005).

### *Aerobic Dive Limit*

The aerobic dive limit (ADL) is the longest dive that does not lead to an increase in blood lactate concentration during or after the dive (Kooyman 1985). The duration of the aerobic dive limit, which varies among species and individuals, depends on the amount of oxygen stores in the body and the level of activity (Kooyman 1985; Berta et al. 2006). To calculate the ADL, available oxygen stores are measured and then divided by the estimated metabolic rate, which is measured oxygen consumption per unit time (Berta et al. 2006). As long as a dive is performed within the ADL, lactate will not accumulate and metabolize, which means that as soon as depleted oxygen stores are restored, subsequent dives can be made. However, if marine mammals exceed their limits, lactate will accumulate and they will require a recovery period at the surface before they can make another dive (Kooyman 1985).

### **Anatomical Adaptations**

#### *Pressure*

Life at sea presents several challenges that marine mammals must overcome. For every ten meters of depth, pressure increases by one atmosphere (atm), which forces the lungs to collapse approximately two minutes into a dive at depths of approximately 40 m (Kooyman et al. 1972; Noren et al. 2005; Berta et al. 2006). Pinnipeds have flexible ribs used as cartilaginous reinforcements so that the alveoli and the lungs can collapse and prevent gas exchange (Riedman 1990; Noren et al. 2005; Ponganis 2015). Air remaining in the lungs is forced out of the alveoli into the bronchi and trachea. When this occurs, the thin walls of the alveoli are no longer in contact with the compressed air in the larger airways and gas exchange is prevented. This process ensures that little compressed gas is absorbed by the blood during deep dives, which protects marine mammals from experiencing decompression sickness (the bends) and nitrogen narcosis (Moon et al. 1995; Berta et al. 2006).

## *Temperature*

Marine mammals must also be adapted to minimize their heat loss in the water. Water has a thermal conductivity 25 times that of air, which causes a high rate of heat loss in mammals (Riedman 1990). To prevent significant heat loss, pinnipeds have substantial layers of blubber, well-developed fur, and use counter-current heat exchange at their extremities (Blix and Steen 1979). Most pinniped pups are born without adequate blubber stores and require a lanugo. The lanugo acts as a thermal layer that absorbs solar radiation but is a poor insulator when wet (Elsner et al. 1977; Riedman 1990). Because of this, pups are also equipped with a layer of brown adipose tissue used for non-shivering thermogenesis (Grav and Blix 1976; Blix and Steen 1979). Pups metabolize the brown adipose tissue without using energy through normal cellular respiration (Riedman 1990; Berta et al. 2006). Metabolizing the subcutaneous fat allows them to maintain their internal body temperature until the blubber layer forms (Blix and Steen 1979; Renouf 1991; Prewitt et al. 2010).

## **Oxygen Stores**

Pinnipeds are physiologically equipped with large, total body oxygen stores relative to their body mass by having a large blood volume and large concentrations of hemoglobin, hematocrit, and myoglobin (Kooyman et al. 1981; Oftedal et al. 1987; Lestyk et al. 2009). Hemoglobin, hematocrit, and blood volume constitutes blood oxygen stores while myoglobin and muscle mass constitute muscle oxygen stores, which is necessary for diving locomotion (Snyder 1983; Kooyman 1989; Noren et al. 2005). Blood provides the largest oxygen stores, ranging from 30-40 milliliters per kilogram ( $\text{ml kg}^{-1}$ ), while little oxygen is stored in the lungs (Kooyman et al. 1981; Ponganis et al. 2011; Thomas and Ono 2015). It is important to understand how these components contribute to the ontogeny of diving capabilities because pinniped pups are not born with adequate oxygen stores. Instead, these stores develop after birth.

### *Hemoglobin and Hematocrit*

Hemoglobin (Hb) is an oxygen-binding molecule of four polypeptide chains with iron-containing heme groups that allow four oxygen molecules to bind to them (Ponganis 2015). Generally, phocids have higher concentrations of Hb in their blood than otariids (Ponganis and Williams 2015). Marine mammals also tend to have large blood volumes. In relation to body mass, pinnipeds have a total blood volume 1.5-2 times more than comparable-sized terrestrial mammals (Riedman 1990). This results in an increase in the hematocrit (Hct), which is defined as the volume percentage of red blood cells in blood (Berta et al. 2006). Larger body masses and increased blood volume, which increase both Hb and Hct, can indicate the oxygen storage capacity of marine mammals.

A marine mammal's spleen is a site of red blood cell production and a substantial reservoir for oxygen storage. Pinnipeds have large spleen sizes, approximately 4.5% of their body weights (Berta et al. 2006; Ponganis 2015). Hochachka (2000) found pinnipeds with larger spleens typically had greater diving capabilities. Increased pressure during a dive compresses the spleen and forces red blood cells into the vast network of blood vessels comprising the circulatory system. This increase in red blood cells leads to an elevation in hemoglobin and hematocrit, which affects the concentration of oxygen stores (Qvist et al. 1986; Cabanac et al. 1997; Thornton et al. 2001; Panneton 2013). While research has shown that diving capacity of phocids was strongly correlated to spleen size, this result was not seen for otariids (Hochachka and Mottishaw 1998; Berta et al. 2006).

### *Myoglobin*

Myoglobin (Mb) is the dominant oxygen-binding protein within the muscle and is the major source of oxygen during a dive. During a dive, skeletal muscles require a substantial amount of oxygen (Riedman 1990). Therefore, it is critical for vast amounts of oxygen to be stored in the myoglobin. Diving mammals carry 10-30 times more Mb than terrestrial mammals (Scholander 1940; Castellini and Somero 1981; Kooyman 1989; Kanatous et al. 1999; Panneton 2013). Myoglobin develops more slowly than blood

oxygen stores and does not rapidly increase until the onset of foraging (Thorson and Le Boeuf 1994; Burns et al. 2007; Bennett et al. 2010).

### *Ontogeny of Diving*

Pinniped pups must transition from a terrestrial to an aquatic environment soon after birth. At birth, pups are terrestrial and do not have the physiological adaptations needed to successfully dive. They have a lower oxygen carrying capacity and tissue oxygen stores are approximately 50% less than adults (Noren et al. 2005; Burns et al. 2015). Pups are born with greater stores of hemoglobin than myoglobin (Geiseler et al. 2013). During the transition from pup to juvenile, both hemoglobin and myoglobin levels increase. Many studies have hypothesized that hypoxia is necessary to further develop oxygen stores (Burns et al. 2005, 2015; Lestyk et al. 2009; Prewitt et al. 2010). While *in utero*, mothers introduce their pups to hypoxia while diving (Elsner et al. 1969). Because pups have limited movement *in utero*, development of their muscle stores of myoglobin does not take place until after they are born. Therefore, pups have limited abilities to dive and forage compared to adults, which forces them to function close to their physiological limits (Kooyman et al. 1983; Thorson and Le Boeuf 1994; Bekkby and Bjørge 2000; Noren et al. 2005). The rate of physiological and behavioral maturity is associated with the time between birth and weaning, and may affect the age that pups begin foraging (Burns et al. 2005, 2007). Understanding how oxygen stores develop among various species may give insight into the complex relationship between pinniped physiology and diving ability.

## Objectives

The purpose of this study was to examine differences in oxygen store development and ontogeny of diving capacity in six phocid and otariid species. Different maternal strategies among species may affect the behavior of pups throughout the nursing and weaning period, which in turn may correlate to the amount of time it takes for pups to exhibit mature dives. The length of the terrestrial period is necessary for pups to gain sufficient O<sub>2</sub> stores to successfully forage on their own (Burns et al. 2007; Noren et al. 2008). The goal of the study was to 1) determine what factors or combination of factors (i.e. age class, body mass and terrestrial duration) affect the rate of O<sub>2</sub> store development among various phocid and otariid species, and 2) determine if there are differences among species and between the two families.

## Materials and Methods

Data for this review have been contributed by participating authors (Tables 2-8). All studies used comparable methodology for measuring hemoglobin, hematocrit, and myoglobin, which may be used as measures of blood and muscle oxygen stores. All associated authors standardized blood and muscle parameters for volume of tissue used. As this study did not physically work with the animals or tissues, no permits were required. Data from published studies and contributed research were collected with necessary permits (Somo et al. 2015; Thomas and Ono 2015). Data was contributed to a varying degree for each species; various other studies explored oxygen store development for these species and were not used because the data was either not contributed or the raw data was not available. However, those studies still provided the opportunity to compare with the results from this analysis.

**Table 2.** Sources of hematological data for analyses. Age (#) indicates age by days, weeks, or years.

Species	Age (#)	Age Class	Gender	Mass	Hemoglobin (Hb)	Hematocrit (Hct)	Myoglobin (Mb)	Source
Gray seal		X	X	X	X	X		1
Harbor seal	X	X	X	X	X	X		1, 2
Northern elephant seal	X	X	X	X	X	X	X	3, 4
Australian fur seal	X	X	X	X	X	X	X	5
Northern fur seal	X		X	X	X	X		6
Steller sea lion	X		X	X	X	X		6

**Source:** <sup>1</sup>Ailsa J. Hall, Sea Mammal Research Unit, University of St. Andrews, Scotland; <sup>2</sup>Thomas and Ono 2015; <sup>3</sup>Daniel E. Crocker, Sonoma State University, California; <sup>4</sup>Somo et al. 2015; <sup>5</sup>J.P.Y. Arnould, Deakin University, Australia; <sup>6</sup>David A.S. Rosen, University of British Columbia / Vancouver Aquarium, Canada



**Table 3.** Gray seal data from Ailsa J. Hall, Sea Mammal Research Unit, University of St. Andrews, Scotland. Values are means  $\pm$  standard deviations.

Age Class	N	Gender (F, M)	Mass (kg)	Hb (g dl <sup>-1</sup> )	Hct (%)
<i>Weaned</i>	30	(14, 16)	37.78 $\pm$ 5.56	19.31 $\pm$ 1.85	56.72 $\pm$ 6.57
<i>Juvenile</i>	12	(9, 3)	37.91 $\pm$ 13.92	19.78 $\pm$ 0.96	56.01 $\pm$ 5.03
<i>Adult</i>	13	(12, 1)	118.48 $\pm$ 31.03	19.57 $\pm$ 1.46	55.04 $\pm$ 4.15

**Table 4.** Harbor seal data from Ailsa J. Hall, Sea Mammal Research Unit (SMRU), St. Andrews University, Scotland and Thomas and Ono (2015), Marine Animal Rehabilitation and Conservation Program (MARC), University of New England, Maine. Values are means  $\pm$  standard deviations.

Location	Age Class	N	Gender (F, M, Unk)	Age (Days)	Mass (kg)	Hb (g dl <sup>-1</sup> )	Hct (%)
MARC	<i>Nursing</i>	31	(18, 12, 1)	16.87 $\pm$ 8.56	10.00 $\pm$ 2.21	18.98 $\pm$ 3.35	50.82 $\pm$ 8.52
	<i>Weaned</i>	21	(11, 9, 1)	51.69 $\pm$ 20.36	14.87 $\pm$ 4.22	14.82 $\pm$ 2.09	41.54 $\pm$ 5.63
	<i>Juvenile</i>	-	-	-	-	-	-
	<i>Adult</i>	-	-	-	-	-	-
			-				
SMRU	<i>Nursing</i>	-	-	-	-	-	-
	<i>Weaned</i>	2	(1, 0, 1)	-	14.40 $\pm$ 3.11	20.15 $\pm$ 2.05	59.20 $\pm$ 6.79
	<i>Juvenile</i>	6	(1, 0, 4)	-	38.80 $\pm$ 6.80	19.16 $\pm$ 2.03	54.42 $\pm$ 2.81
	<i>Adult</i>	13	(2, 0, 11)	-	82.01 $\pm$ 13.25	20.12 $\pm$ 2.38	56.51 $\pm$ 6.52

**Table 5.** Northern elephant seal data from Daniel E. Crocker, Sonoma State University, California.

Values are means  $\pm$  standard deviations.

Age Class	N	Gender (F, M)	Age (Years)	Mass (kg)	Hb (g dl <sup>-1</sup> )	Hct (%)	Mb (g 100 g <sup>-1</sup> )
<i>Juvenile</i>	12	(6, 6)	1.50 $\pm$ 0.18	174.50 $\pm$ 25.35	22.50 $\pm$ 1.44	52.83 $\pm$ 2.55	62.00 $\pm$ 0.80
<i>Adult</i>	28	(28, 0)	7.43 $\pm$ 3.14	376.89 $\pm$ 73.11	24.40 $\pm$ 1.73	56.86 $\pm$ 4.18	79.00 $\pm$ 1.17

**Table 6.** Weaned northern elephant seal pup data from Somo et al. (2015).

Individuals were subsequently sampled 0-, 2-, 5-, and 8-weeks during the post-weaning fast. Mb was measured in milligrams per gram wet mass (mg g<sup>-1</sup> wet mass). Values are means  $\pm$  standard deviations.

Age in Weeks	N	Mass (kg)	Hb (g dl <sup>-1</sup> )	Hct (%)	Mb (mg g <sup>-1</sup> wet mass)
0	19	135.26 $\pm$ 18.13	19.32 $\pm$ 1.71	44.99 $\pm$ 2.41	37.03 $\pm$ 12.36
2	19	120.37 $\pm$ 17.52	17.72 $\pm$ 1.54	45.83 $\pm$ 2.54	52.13 $\pm$ 14.26
5	19	106.00 $\pm$ 15.82	16.22 $\pm$ 1.87	48.14 $\pm$ 3.27	50.86 $\pm$ 18.11
8	15	95.87 $\pm$ 15.82	14.15 $\pm$ 5.38	45.11 $\pm$ 5.86	41.25 $\pm$ 17.97

**Table 7.** Nursing Australian fur seal pup data from J.P.Y. Arnould, Deakin University, Australia. Blood and muscle oxygen store parameters with different sample sizes are indicated with parentheses. Pups less than a month old are indicated by 0. Hemoglobin values were calculated for g dl<sup>-1</sup> by multiplying Hct (%) by approximate O<sub>2</sub> affinity of 1.34 (Kooyman 1989). Values are means ± standard deviations.

Age (Months)	N	Gender (F,M)	Mass (kg)	Hb (g dl <sup>-1</sup> )	Hct (%)	Mb (g Mb 100 g wet muscle <sup>-1</sup> )
0	34	(16, 18)	7.99 ± 1.90	17.24 ± 3.60 (27)	43.85 ± 6.99 (26)	0.45 ± 0.37 (10)
1	41	(21, 20)	12.17 ± 1.80	14.89 ± 3.02 (40)	38.64 ± 4.19 (37)	-
5	16	(8, 8)	21.02 ± 3.42	16.25 ± 0.84	46.44 ± 2.39	-
6	36	(16, 20)	21.15 ± 4.14	20.99 ± 4.81	45.70 ± 3.19	0.56 ± 0.20 (11)
8	18	(8, 10)	20.92 ± 4.03	22.63 ± 3.53 (15)	46.88 ± 3.30 (16)	0.89 ± 0.42 (4)
9	24	(8, 16)	23.79 ± 5.95	18.37 ± 2.04	48.09 ± 2.85	0.89 ± 0.32 (10)

**Table 8.** Captive female northern fur seal data from David A.S. Rosen, University of British Columbia / Vancouver Aquarium, Canada. Samples were measured multiple times over various years. Weaned pups were categorized as pups less than a year old, juveniles were categorized as individuals between a year old and sexual maturity, and adults were categorized as individuals that reached sexual maturity. Values are means  $\pm$  standard deviations.

<b>Age Class</b>	<b>N</b>	<b>Mass (kg)</b>	<b>Hb (g dl<sup>-1</sup>)</b>	<b>Hct (%)</b>
Northern fur seal	6	12.91 $\pm$ 1.82	17.55 $\pm$ 1.08	51.43 $\pm$ 3.37
<i>Weaned</i>				
<i>Juvenile</i>	6	14.46 $\pm$ 2.74	17.74 $\pm$ 1.15	50.51 $\pm$ 3.17
<i>Adult</i>	6	25.03 $\pm$ 4.40	17.90 $\pm$ 1.18	51.05 $\pm$ 3.44
Steller sea lion				
<i>Nursing</i>	12	35.84 $\pm$ 14.55	13.60 $\pm$ 2.20	39.10 $\pm$ 6.26
<i>Juvenile</i>	12	109.91 $\pm$ 20.69	14.45 $\pm$ 2.34	41.93 $\pm$ 6.78
<i>Adult</i>	12	164.74 $\pm$ 22.78	15.24 $\pm$ 1.42	44.03 $\pm$ 1.43

## *Field Methods*

All studies from which data were obtained for this review utilized similar techniques and measurements to gather data on age and body mass. Age and body mass are major components that may influence physical and physiological maturity of pups. Samples were either taken once (cross sampling a population) or multiple times; if individuals were measured multiple times, they were marked for identification by either tagging or dyeing small patches of fur throughout the study period (Somo et al. 2015). To determine body mass, seals were captured and weighed. Once captured, they were either manually restrained or anesthetized through the extradural vein with a sedative, such as diazepam (Hall 1998; Burns et al. 2005, 2007; Noren et al. 2005, 2008; Spence-Bailey et al. 2007; Lestyk et al. 2009; Bennett et al. 2010; Somo et al. 2015; Thomas and Ono 2015).

In the aforementioned studies, individuals were separated into distinct age categories based primarily on body mass and observations of mother-pup pairs. Additional information to assign age class included pup appearance (i.e. pelage, or coat), female attendance, canine teeth eruption, and pup stomach contents (the presence or absence of milk). Yearlings and adults were generally determined based on standard length and mass (Bowen et al. 1987; Hall 1998; Burns et al. 2005, 2007, 2009; Richmond et al. 2006; Lestyk et al. 2009; Thomas and Ono 2015). This study utilized data from individuals in four age categories: 1) nursing pups, 2) weaned pups, 3) juveniles, and 4) adults. In some situations, contributed samples were not classified into age classes (i.e. northern fur seals and Steller sea lions). In this study, nursing pups were classified as pups who were within their nursing periods, weaned pups as those who were beyond their nursing period but within a year old, and juveniles as individuals who were post-weaned and over a year old. For the purposes of this study, juveniles were considered as such until they reached reproductive maturity, at which point they were considered adults.

All studies used comparable methods to collect hematological samples. To collect hemoglobin and hematocrit measurements, blood samples were generally taken from the extradural vein into vacutainer tubes and followed by intravenous injections of Evan's blue dye at various concentrations. Samples were immediately placed on ice if they were

soon to be analyzed or stored frozen until analyzed (Hall 1998; Burns et al. 2004, 2005, 2007, 2009; Noren et al. 2005; Spence-Bailey et al. 2007; Somo et al. 2015; Thomas and Ono 2015).

For myoglobin, muscle biopsies (approximately 0.05-0.2 grams) were taken using biopsy punches. For phocids, samples were taken from the primary locomotor muscle, *longissimus dorsi* (Burns et al. 2004, 2005; Noren et al. 2005; Somo et al. 2015). For otariids, biopsies were collected from various muscles including the pectoralis, which is the primary swimming muscle, the *latissimus dorsi*, which is a muscle used for terrestrial locomotion, or the *humerothrapezius* (Richmond et al. 2006; Spence-Bailey et al. 2007). All samples were stored on ice or frozen until analyzed.

### *Laboratory Analysis*

All studies used similar methodology to extract hematological values. Hemoglobin concentration in grams per deciliter ( $\text{g dl}^{-1}$ ) was determined from the blood samples with cyanmethemoglobin techniques using methods defined by total hemoglobin kits (i.e. Sigma Kit 525A or Sigma Kit 625A) (Hall 1998; Burns et al. 2005, 2007; Noren et al. 2005; Richmond et al. 2006; Spence-Bailey et al. 2007; Somo et al. 2015; Thomas and Ono 2015). Hematocrit (percent red blood cells by volume) was determined using micro-centrifugation (Hall 1998; Burns et al. 2005, 2007; Noren et al. 2005; Richmond et al. 2006; Spence-Bailey et al. 2007; Somo et al. 2015; Thomas and Ono 2015). Myoglobin was determined using methods described by Reynafarje (1963) (Burns et al. 2004, 2005; Noren et al. 2005; Richmond et al. 2006; Spence-Bailey et al. 2007; Thomas and Ono 2015; Somo et al. 2015).

### *Data Description*

The following hematological data were contributed to varying degrees for each species: hemoglobin, hematocrit, and myoglobin. Blood and muscle oxygen stores are calculated based on a variety of parameters and assumptions, including arterial and venous blood contribution, blood volume, and proportion of muscle mass (Ponganis et al.

2011; Ponganis and Williams 2015). This study did not include these calculations due to recent inconsistencies in assumptions of oxygen usage during dives. Research has shown, for example, that NES become hypoxic much earlier in dives than initially believed. Differences in the rates of oxygen consumption have been reported in several species, including California sea lions, harbor seals, and northern elephant seals (Meir et al. 2009, 2013; Daniel E. Crocker, pers. comm.). This means that normal assumptions for calculating the estimated proportion of oxygen between arterial and venous blood may not be accurate for all species. For the purposes of this study, only hemoglobin, hematocrit, and myoglobin were included. Therefore, in this paper, blood oxygen stores only consider hemoglobin and hematocrit measurements, and muscle oxygen stores only consider myoglobin unless otherwise stated.

### *Statistical Analysis*

Statistical analyses were conducted on a variety of raw data from contributing sources. These data were collected and analyzed using the comparable methods previously described. The datasets for individual species were analyzed separately and then tested across species. All comparable measurements among species were analyzed using multi-variate statistical analyses, with a significance level of  $p < 0.05$ . This study used a combination of parametric and non-parametric tests using R-statistical software (Version 1.0.153) to determine how age class, body mass, and terrestrial period (nursing and PWF) were related to hemoglobin, hematocrit, and myoglobin among all six species. Data were tested for normality and homogeneity of variances using Shapiro and Bartlett tests. Simple and multivariate analysis of variance (ANOVA and MANOVA) and analysis of covariance (ANVOCA) were used to determine the relationships of oxygen store concentrations (hemoglobin, hematocrit, and myoglobin) among species due to age, body mass, and terrestrial durations. In situations where multivariate analyses could not be done due to variability of data (i.e. variation in collection methods), post-hoc comparisons tested significant differences among factors for species.

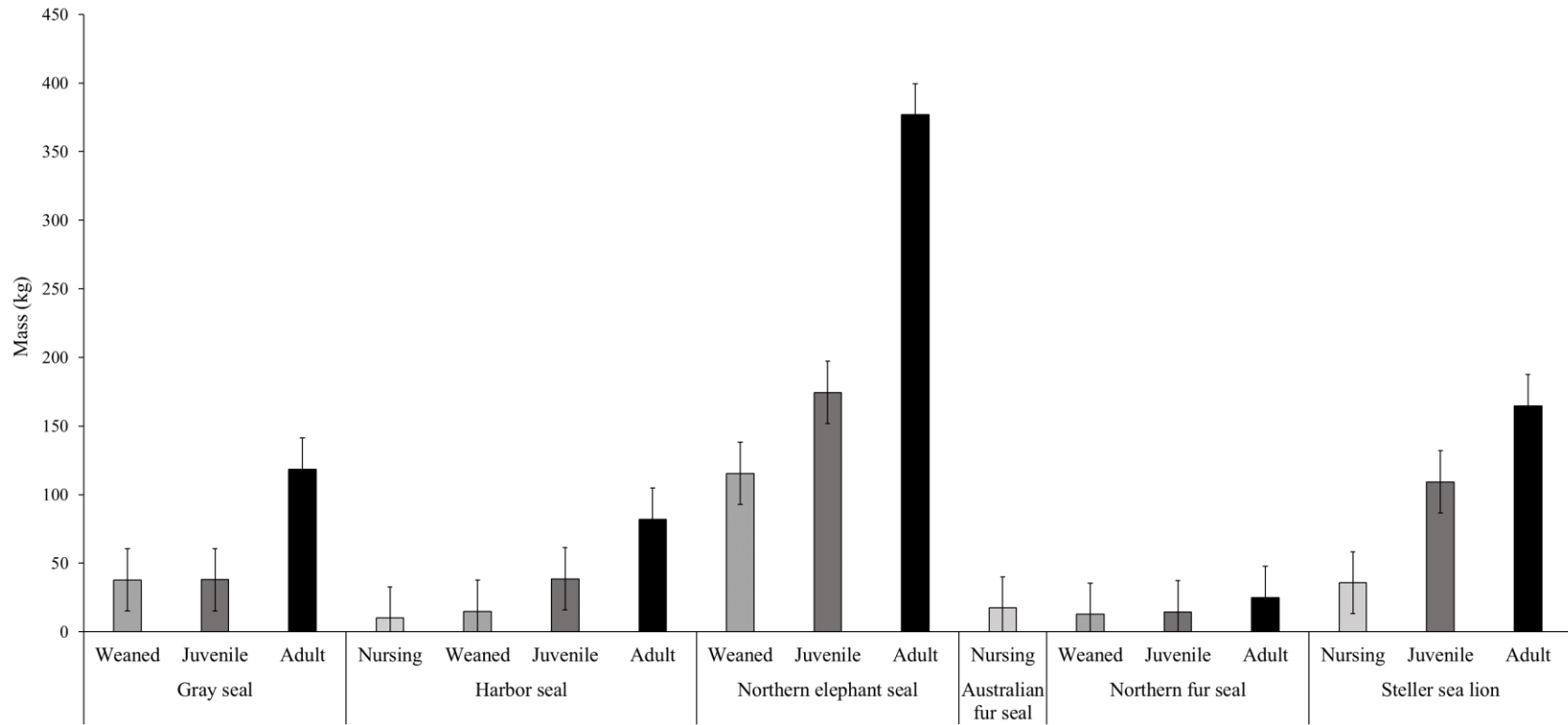
## Results

Phocids and otariids distinctly differ in their physiological development; therefore, the results are specific to each species. The following variables were available for all species: age, mass, hemoglobin, and hematocrit. Myoglobin was only available for northern elephant seals and Australian fur seal pups.

Among the otariids, NFS and SSL were comparable in that individuals were captive animals from the Vancouver Aquarium that were measured multiple times throughout their lives. The Australian fur seals were distinct in that only pups were analyzed throughout their first nine months of life.

In all species, mass significantly increased with age ( $p < 0.01$ ,  $R^2 = 0.93$ , Figure 4). Generally, the effects of mass combined with species significantly altered hemoglobin and hematocrit, accounting for 50% of the variation in these parameters ( $p < 0.01$ ,  $R^2 = 0.50$ , Table 9). Age and mass had significant effects on the differences in oxygen stores between families and among species ( $p < 0.01$ ). Therefore, the oxygen store components were examined per species to determine these effects.





**Figure 4.** Comparison of mass in kilograms (kg) amongst age class for each study species. Standard deviations are indicated by error bars.

**Table 9.** Blood oxygen stores amongst age class in each study species. Values are means  $\pm$  standard deviations.

Species	Age Class	N	Mass (kg)	Hb (g dl <sup>-1</sup> )	Hct (%)
Gray seal	<i>Weaned</i>	30	37.78 $\pm$ 5.56	19.31 $\pm$ 1.85	56.72 $\pm$ 6.57
	<i>Juvenile</i>	12	37.91 $\pm$ 13.92	19.78 $\pm$ 0.96	56.01 $\pm$ 5.03
	<i>Adult</i>	13	118.48 $\pm$ 31.03	19.57 $\pm$ 1.46	55.04 $\pm$ 4.15
Harbor seal	<i>Nursing</i>	17	9.44 $\pm$ 1.70	20.14 $\pm$ 2.63	53.81 $\pm$ 7.30
	<i>Weaned</i>	15	14.64 $\pm$ 2.99	16.14 $\pm$ 2.32	45.56 $\pm$ 7.16
	<i>Juvenile</i>	6	38.57 $\pm$ 6.12	19.60 $\pm$ 2.10	54.05 $\pm$ 2.68
	<i>Adult</i>	13	82.02 $\pm$ 13.25	20.12 $\pm$ 2.38	56.51 $\pm$ 6.52
Northern elephant seal	<i>Weaned</i>	19	113.27 $\pm$ 21.49	17.14 $\pm$ 3.19	46.36 $\pm$ 3.79
	<i>Juvenile</i>	12	174.50 $\pm$ 25.35	22.50 $\pm$ 1.44	52.83 $\pm$ 2.55
	<i>Adult</i>	28	376.89 $\pm$ 73.11	24.40 $\pm$ 1.73	56.86 $\pm$ 4.18
Australian fur seal	<i>Nursing</i>	169	16.70 $\pm$ 6.93	18.09 $\pm$ 4.28	44.27 $\pm$ 5.36
Northern fur seal	<i>Weaned</i>	6	12.91 $\pm$ 1.82	17.55 $\pm$ 1.08	51.43 $\pm$ 3.37
	<i>Juvenile</i>	6	14.46 $\pm$ 2.74	17.74 $\pm$ 1.15	50.51 $\pm$ 3.17
	<i>Adult</i>	6	25.03 $\pm$ 4.40	17.90 $\pm$ 1.18	51.05 $\pm$ 3.44
Steller sea lion	<i>Nursing</i>	12	35.84 $\pm$ 14.55	13.60 $\pm$ 2.20	39.10 $\pm$ 6.26
	<i>Juvenile</i>	12	109.91 $\pm$ 20.69	14.45 $\pm$ 2.34	41.93 $\pm$ 6.78
	<i>Adult</i>	12	164.74 $\pm$ 22.78	15.24 $\pm$ 1.42	44.03 $\pm$ 1.43

### *Gray seal*

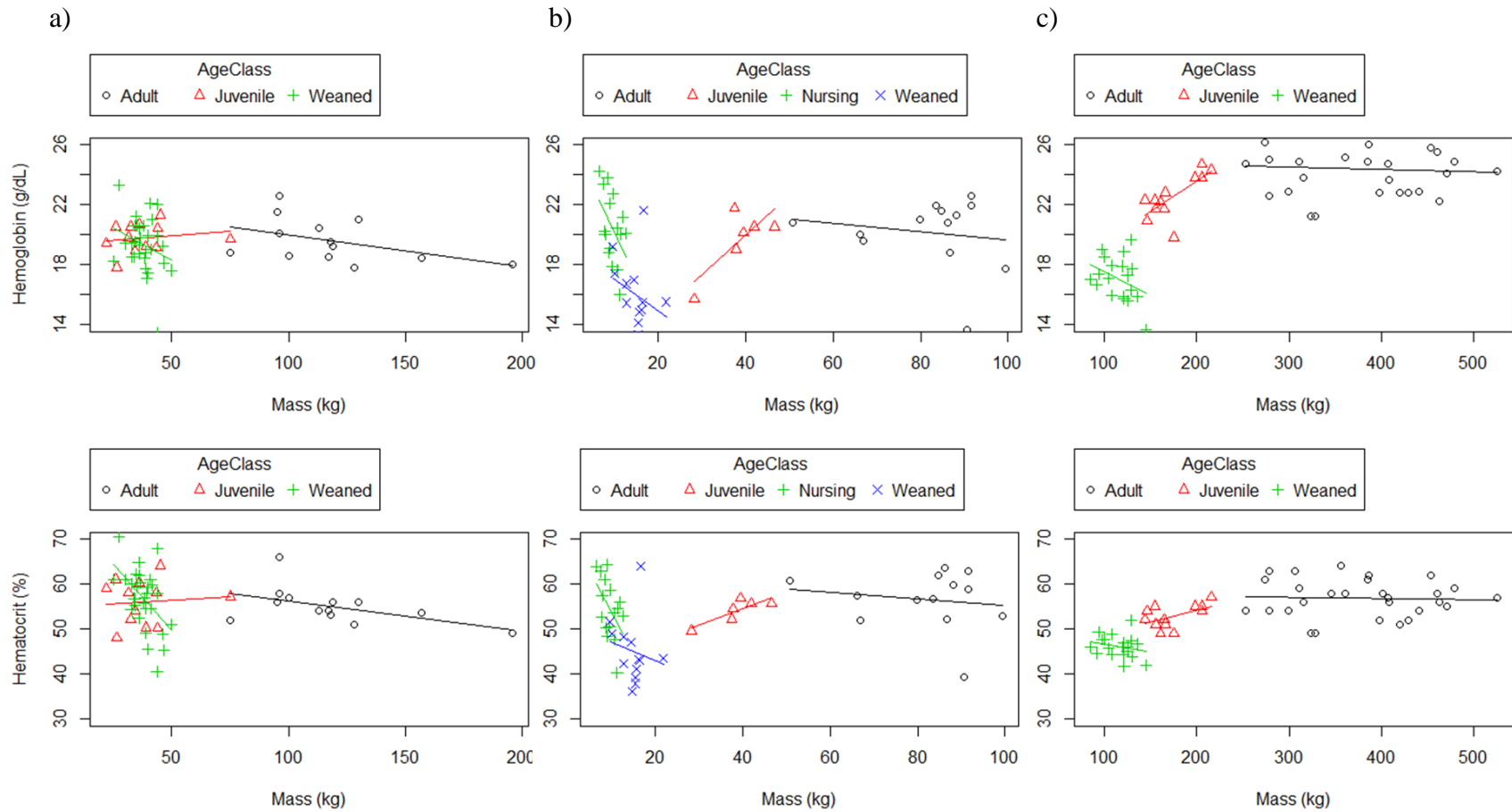
Mass significantly ( $p < 0.01$ ) increased with age between weaned pups and adults and between juveniles and adults. Age had no effect on Hb or Hct; however, body mass had a significant effect on Hct values, wherein lower mass resulted in significantly decreased Hct levels (Figure 5a).

### *Harbor seal*

Mass significantly ( $p < 0.05$ ) increased among all four age classes. Unlike the gray seal, age significantly ( $p < 0.01$ ) altered Hb and Hct concentrations, varying among age classes. Hb and Hct decreased in value from nursing to weaned pups, then increased between weaned pups and juveniles and weaned pups and adults. Mass had no effect on Hb or Hct (Figure 5b).

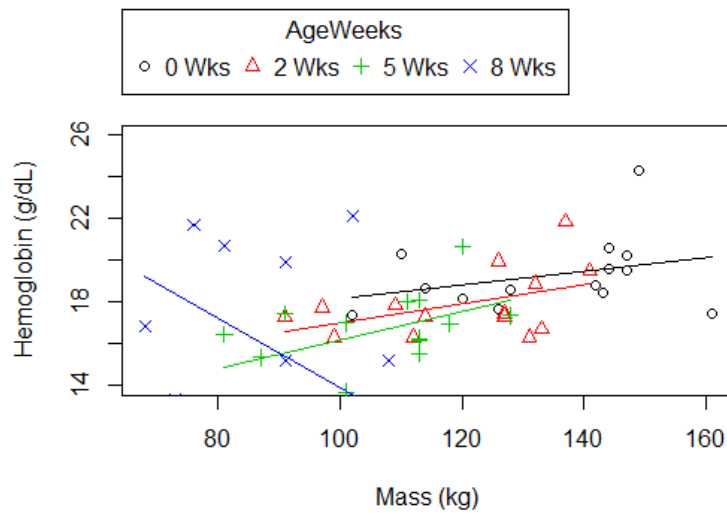
### *Northern elephant seal*

Mass also significantly ( $p < 0.01$ ) increased with age among weaned pups, juveniles, and adults. Both age class and mass resulted in significantly ( $p < 0.01$ ) increased Hb and Hct among all age classes (Figure 5c). The weaned NES pup dataset from Somo et al. (2015) measured Hb and Hct multiple times over the course of the weaning period at intervals of 0-, 2-, 5-, and 8-weeks. This dataset was analyzed separately for effects within pups' fasting period. Significant ( $p < 0.01$ ) decreases in mass were detected throughout the weaning period. Hb significantly ( $p < 0.05$ ) decreased with age between 0 to 2, 0 to 5, 0 to 8, and 5 to 8 weeks age classes in weaned pups. There was no measurable effect on Hct. Additionally, Hb significantly ( $p < 0.01$ ) decreased with mass, but there was no effect on Hct (Figure 6).

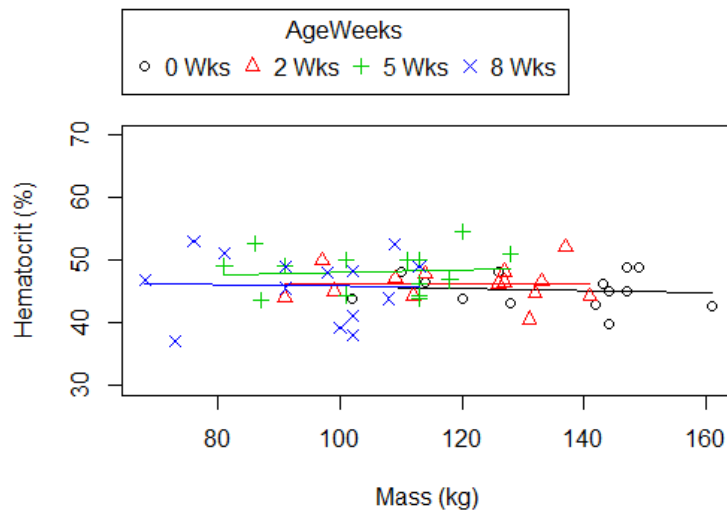


**Figure 5.** Effects of age class and mass in kilograms (kg) on hemoglobin (top) and hematocrit (bottom) in a) gray, b) harbor, and c) northern elephant seals.

a)



b)

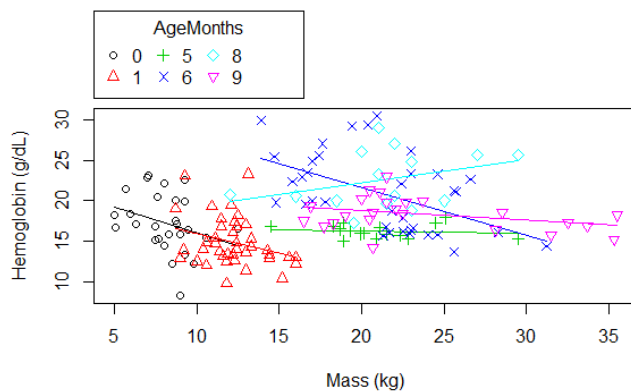


**Figure 6.** Effects of age (in weeks) and mass in kilograms (kg) on a) hemoglobin and b) hematocrit during the post-weaning fast in weaned northern elephant seal pups.

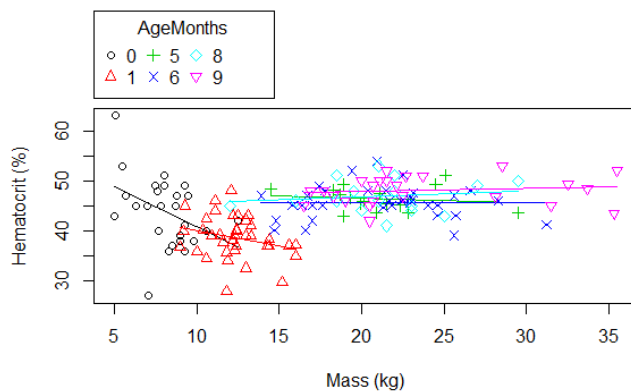
## *Australian fur seal*

Australian fur seal pups were measured nine months after birth for comparison of oxygen store development throughout their nursing period. Pups were measured at less than a month (0), one, five, six, eight, and nine months of age. Mass significantly ( $p < 0.01$ ) increased with age (in months). A post-hoc Steel test revealed significant differences between the first and second months after birth and every subsequent month sampled thereafter. Significant ( $p < 0.01$ ) increases of Hb and Hct at various stages throughout the nine-month period were due to both age and mass (Figure 7).

a)



b)



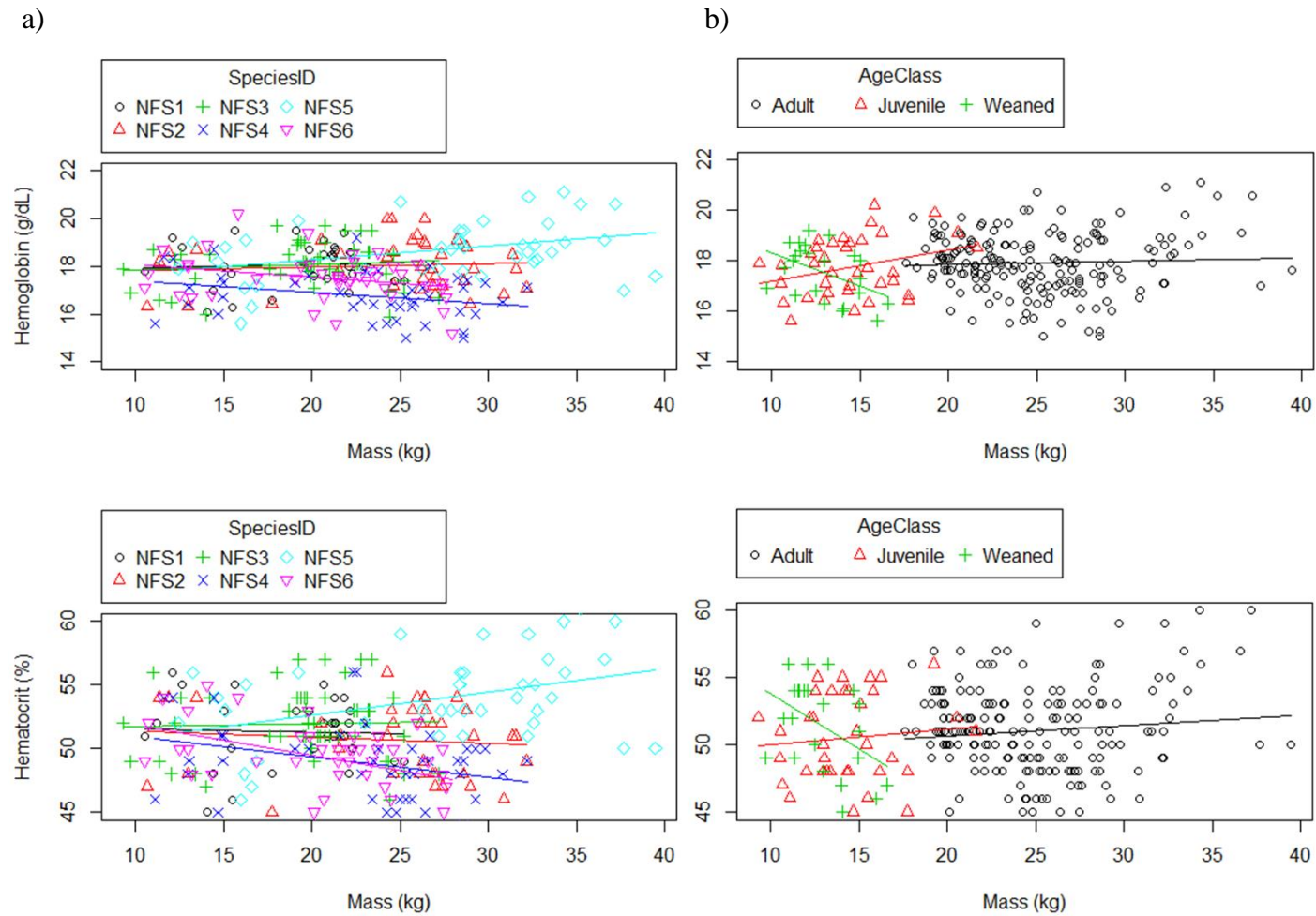
**Figure 7.** Effects of age (in months) and mass in kilograms (kg) on a) hemoglobin and b) hematocrit in nursing Australian fur seal pups.

### *Northern fur seal*

The northern fur seal dataset provided oxygen stores of six individuals who were measured multiple times from weaning to adulthood, so individual variation could be analyzed in the current study. Mass significantly ( $p < 0.01$ ) increased with age for all six individuals throughout the years. A best-fit model selection using the Akaike Information Criterion (AIC) score indicated that mass combined with individual variation significantly ( $p < 0.01$ ) impacted blood oxygen stores in northern fur seals (Figure 8).

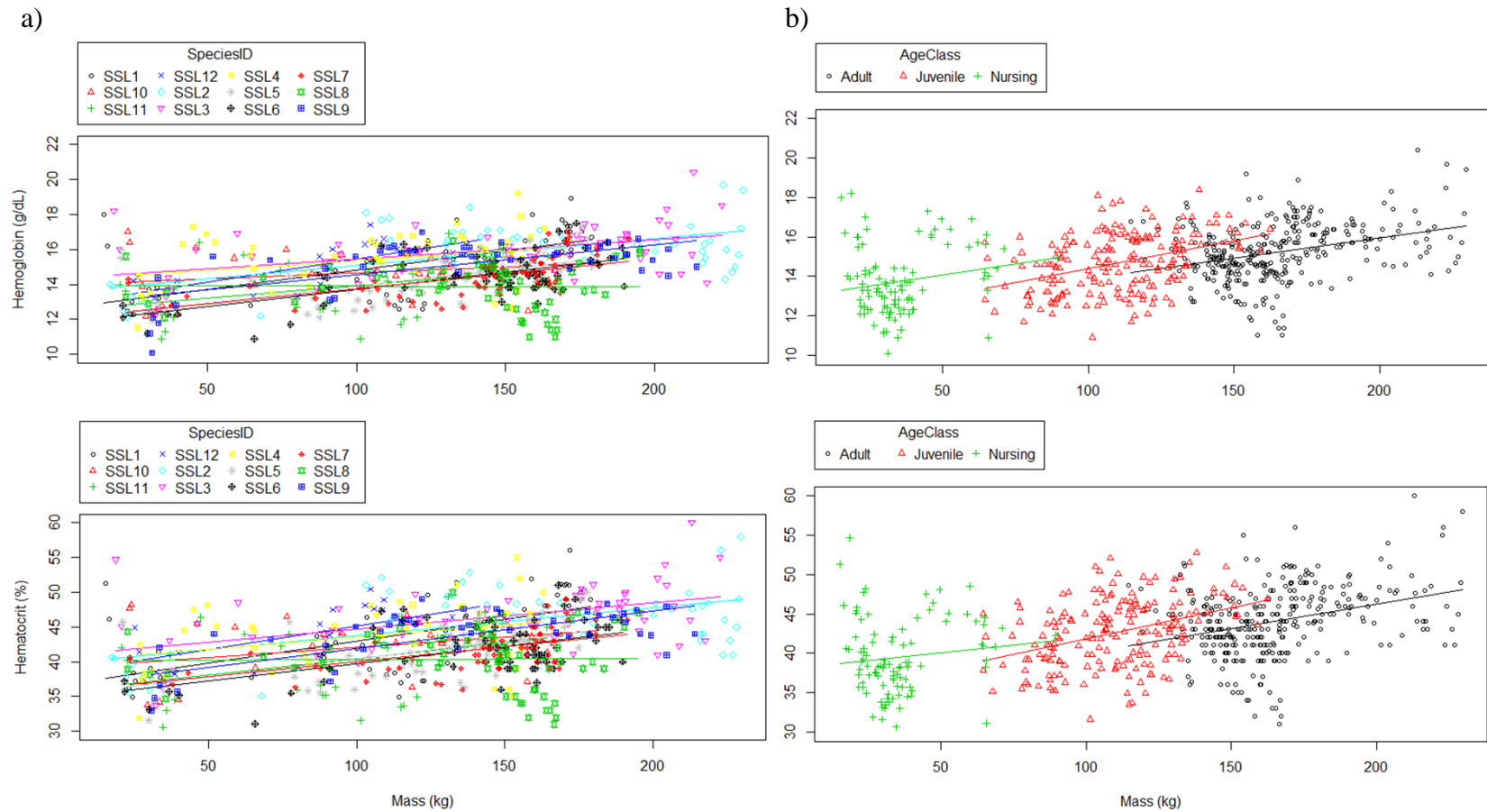
### *Steller sea lion*

Similar to the NFS dataset, the dataset from Steller sea lions provided oxygen stores of 12 individuals who were measured multiple times from less than a year old to adulthood, allowing for an analysis of individual variation in the current study. Mass significantly ( $p < 0.01$ ) increased with age for all 12 individuals. Likewise, a best-fit model using AIC indicated that mass and individual variation significantly ( $p < 0.01$ ) impacted hemoglobin and hematocrit levels in SSL (Figure 9).



**Figure 8.** Effects of mass in kilograms (kg) on hemoglobin (top) and hematocrit (bottom) due to a) individual variation and b) age class in female northern fur seals.





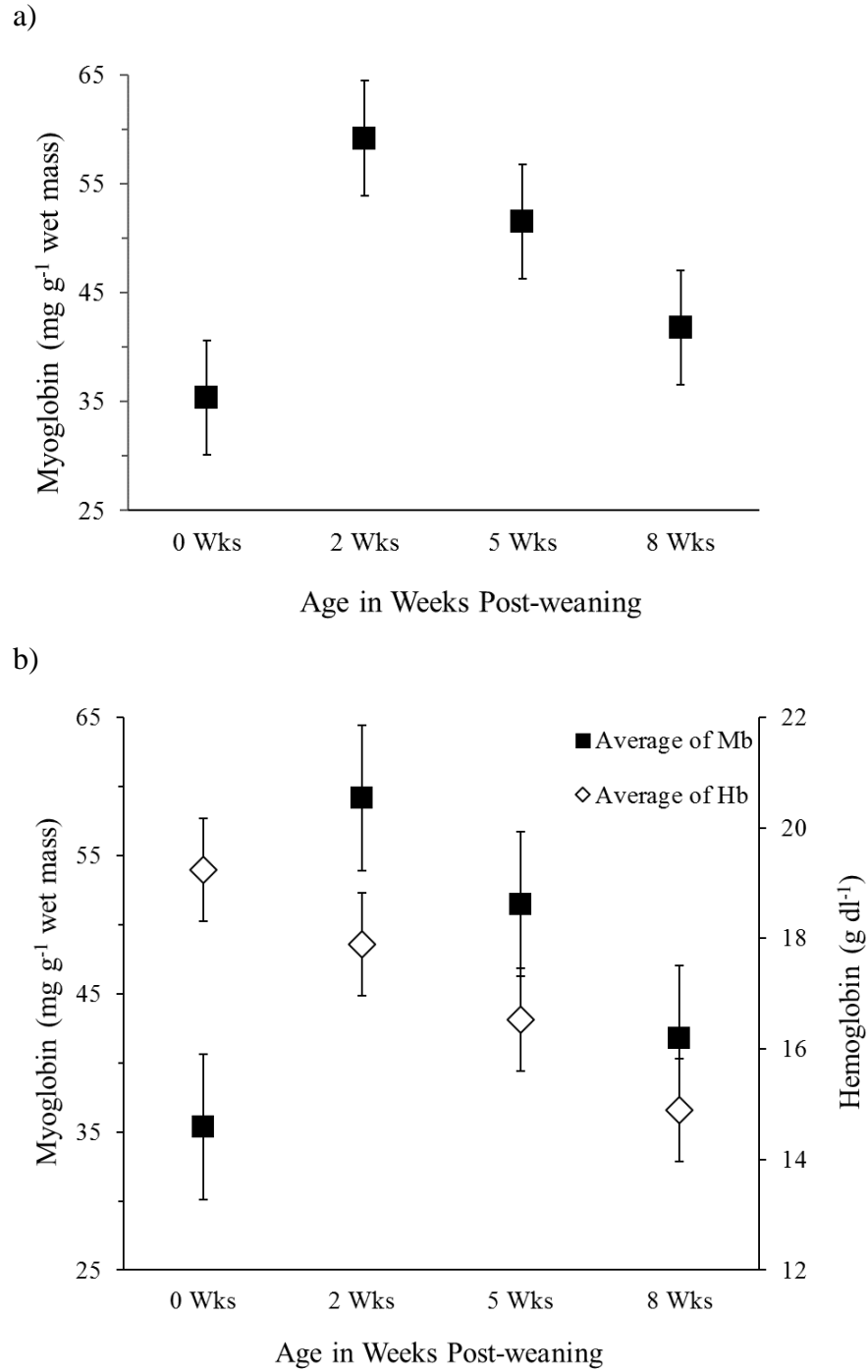
**Figure 9.** Effects of mass in kilograms (kg) on hemoglobin (top) and hematocrit (bottom) due to a) individual variation and b) age class in female Steller sea lions.

## *Myoglobin*

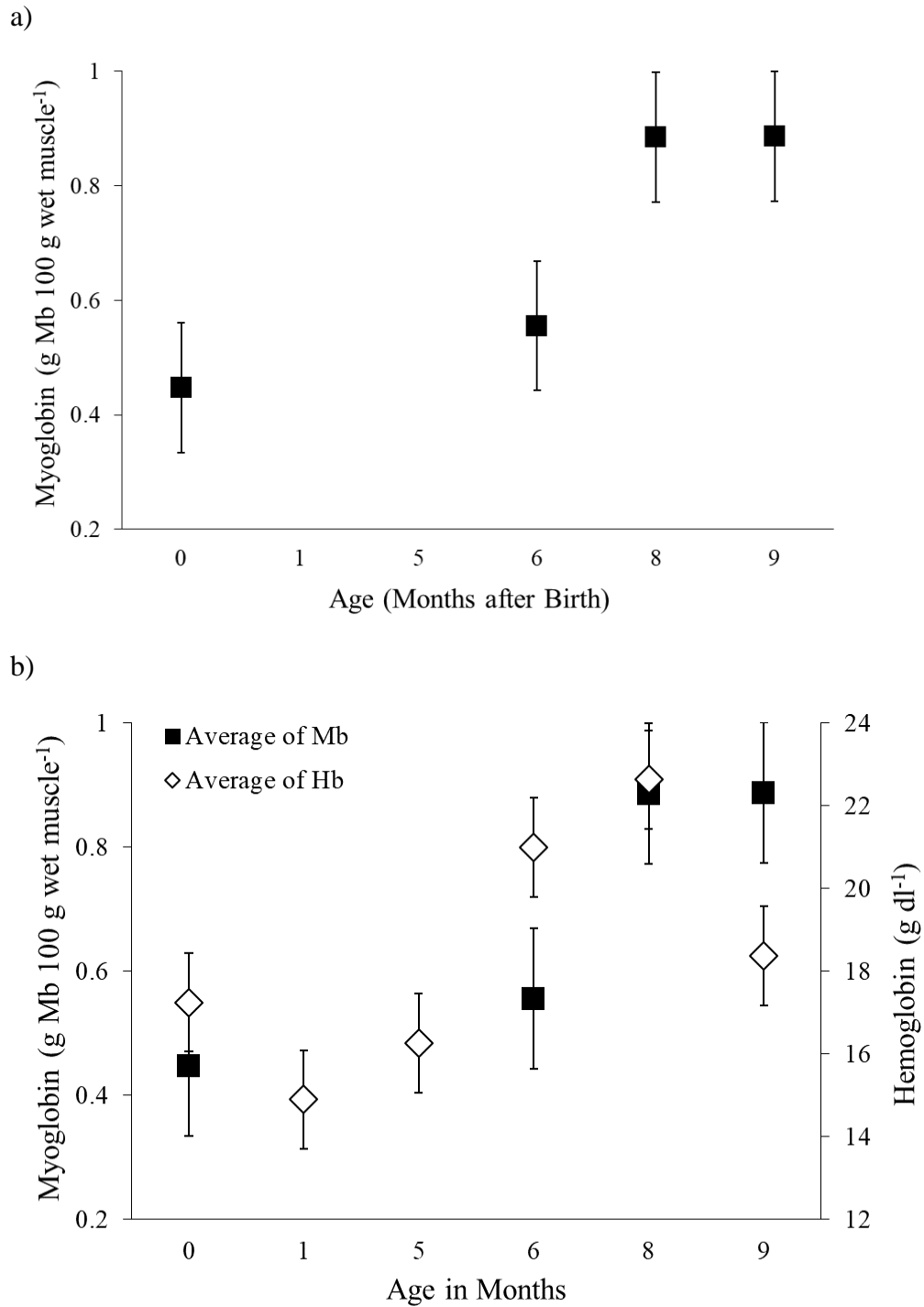
This study concatenated data from multiple previous studies to compare myoglobin development in a phocid and an otariid pup, the northern elephant seal and the Australian fur seal (AUFS). Myoglobin data for weaned pups, juvenile, and adult NES were available, while myoglobin data for AUFS was only available for nursing pups. Because myoglobin measurements for each species were sampled at different points of development (i.e. nursing and post-weaning), the data were analyzed separately by species and the results were then compared.

Myoglobin data were obtained for northern elephant seal pups during an eight-week PWF, and for juveniles and adults. Mb significantly ( $p < 0.05$ ) changed throughout the post-weaning fast, with increases between 0 to 2, 0 to 5, and 0 to 8 weeks and decreases after two weeks (Figure 10). Additionally, myoglobin significantly ( $p < 0.01$ ) increased among weaned pups, juveniles, and adults. In both datasets, myoglobin was significantly affected by age and mass ( $p < 0.05$ ).

Age and mass both had significant ( $p < 0.05$ ) effects on the development of myoglobin in AUFS pups. No myoglobin measurements were available for one month and five month old pups. However, significant ( $p < 0.05$ ) increases in myoglobin were detected in pups that were less than a month old (0), six months, and nine months of age while there were no significant changes between pups that were eight months old versus nine months old (Figure 11).



**Figure 10.** Comparison of a) myoglobin development and b) myoglobin and hemoglobin development in weaned northern elephant seal pups.



**Figure 11.** Comparison of a) myoglobin development and b) myoglobin and hemoglobin development in nursing Australian fur seal pups.

## Discussion

Significant physiological and developmental differences exist between families Phocidae and Otariidae. Generally, body mass was a significant factor in differentiating oxygen store capacity among phocid and otariid species. These results are consistent with other studies addressing the relationship between body size and diving behavior in phocids. (Schreer and Kovacs 1997). A larger body size is associated with a greater oxygen store capacity and therefore may affect diving ability; seals with larger body masses are likely to have larger blood volumes and more muscle mass than smaller seals and therefore, are likely to have increased diving capacities. Between the two families, phocids are generally larger, have lower mass-specific metabolic rates, and contain higher concentrations of oxygen in their tissues (Lenfant et al. 1970; Berta et al. 2006).

Northern and southern elephant seals are the most extreme divers as well as the largest members of the family (Le Boeuf and Laws 1994; Berta et al. 2006). Northern elephant seals regularly dive up to 600 m with dives greater than 1,500 m (Le Boeuf 1994, 1996). Southern elephant seal males have been recorded regularly making dives in excess of 2,000 m (McIntyre et al. 2010). The northern elephant seals were significantly larger in all age classes than all other pinniped species used in this study (Figure 4). Accordingly, NES are significantly superior divers among the six species in this study.

Intermediate-sized phocids display diving behavior relative to their body size, with the smallest phocids tending to exhibit the shallowest dives. However, there are exceptions. For example, the smallest phocid in this study, the harbor seal, can dive down to 450 m for approximately 30 minutes (Bowen et al. 1999; Gjertz et al. 2001; Berta et al. 2006). Alternatively, bearded seals are larger phocids, weighing between 250-400 kg, and usually dive in coastal waters to depths between 130-200 m for less than ten minutes (Gjertz et al. 2001; Berta et al. 2006).

Compared to adult phocids, adult otariids tend to be smaller and show sexual dimorphism between males and females. Because of this, species within this family typically follow two patterns: 1) smaller otariids make shorter and shallower dives, and 2) males tend to dive deeper than females (Schreer and Kovacs 1997; Berta et al. 2006). A notable difference in body size exists between sea lions and fur seals. Sea lion adults

generally weigh three times that of adult fur seals. This implies that diving differences between sea lions and fur seals has some dependency on mass. Among the otariids in this study, the Steller sea lion is the largest of the family, with adults reaching up to 350 kg in females and 1,100 in males (Loughlin 2009). Among all adult fur seals, the Australian fur seal is the largest fur seal, reaching a max of 360 kg and the northern fur seal is one of the smallest fur seals, weighing between 60-120 kg (Warneke 1979; Arnould and Hindell 1999; Kirkwood and Arnould 2008; Gelatt et al. 2015; David A.S. Rosen, pers. comm.). Therefore, among the three species of otariids in the current study, there are variable effects of mass on the total body oxygen storage that likely influence diving abilities between sea lions and fur seals adults and males and females.

There are several physiological differences between phocids and otariids that affect their diving behavior. Phocids are adapted to sustain long and deep dives for foraging, whereas otariids are built with more hydrodynamic bodies that reflect their short and shallow diving behavior (Berta et al. 2006). Additionally, phocids tend to be larger, have lower mass-specific metabolic rates, larger oxygen store capacities, and elevated blood oxygen stores compared to otariids (Lenfant et al. 1970; Costa 1993; Berta et al. 2006). For example, phocids can store approximately 60 milliliters of oxygen per kg ( $\text{ml O}_2 \text{ kg}^{-1}$ ) of body mass compared to 40  $\text{ml O}_2 \text{ kg}^{-1}$  in otariids (Kooyman 1985; Costa 1993). Having larger body mass is associated with low mass-specific metabolic rates while lower body mass is associated with elevated mass-specific metabolic rates (Costa 1993). Lower metabolic rates allow phocids to make deeper dives for longer periods of time without resorting to anaerobic metabolism (Costa 1993; Berta et al. 2006). All of these factors contribute to phocids' abilities to sustain deep, prolonged dives.

There are two diving strategies that describe the type of diving behavior exhibited by both families: energy conserving and energy dissipative (Hochachka and Mottishaw 1998; Berta et al. 2006). Phocids' diving is "energy conservative". While diving, they exhibit the most extreme bradycardia and vasoconstriction and swim at slower speeds which allow them to minimize their metabolic costs.

Otariids exhibit a diving strategy described as "energy dissipative" (Hochachka and Mottishaw 1998; Berta et al. 2006). Diving occurs during short time periods at relatively shallow depths. Otariids tend to trade-off time spent foraging for quicker

swimming speeds and their dive behavior tends to follow the vertical distribution of prey in the water column rather than their physiological limits (Gentry 2001; Costa et al. 2001; Berta et al. 2006). This indicates that although body mass can affect oxygen storage capacity, other physiological factors play a role in oxygen store development and diving ability.

This study confirmed the primary differences between the two families occurred during the transition from maternal dependence to independence. Three maternal strategies are utilized by the pinnipeds: aquatic nursing, foraging cycle, and fasting (Bonner 1984; Gentry and Kooyman 1986; Oftedal et al. 1987; Costa 1991a; Boness and Bowen 1996; Schulz and Bowen 2005). The only pinniped that utilizes the aquatic nursing strategy is the walrus, where pups are born on ice floes but nurse with their mothers at sea (Boness and Bowen 1996). The remaining two strategies are used by the phocids and the otariids.

Phocids generally use the fasting strategy, exhibiting intense and short lactation periods, in which pups must rapidly gain substantial weight and adequate oxygen stores over a limited time of 4-50 days. After nursing concludes, pups are abandoned and must rely on the blubber stores gained during lactation to sustain them during the PWF. Amongst the species examined in this study, the gray seal and the northern elephant seal followed this strategy. Gray seal pups are born weighing approximately 10 kg and triple their mass over the course of about 18 days (Coulson and Hickling 1964; Iverson et al. 1993). The weaned pups in this study weighed  $37.78 \pm 5.56$  kg, indicating this three-fold increase in body mass from birth and nursing. Gray seal pups have a plastic PWF, ranging from 10-28 days, in which the duration of their fast depends on their body condition at the time of weaning (Hall 1998; Noren et al. 2005, 2008). This correlates with the females' health and body condition at the time pups are born (Hall 1998). Due to this plasticity, pups can end their fast earlier or later depending on their body condition.

The northern elephant seal also exhibited this strategy. Pups weigh approximately 30 kg at birth, three times the mass of gray seal neonates, and nurse for 27 days before they are weaned (Le Boeuf et al. 1972; Le Boeuf and Laws 1994; Noren et al. 2003). Similarly, during nursing, they triple their birth mass before their plastic post-weaning fast of 2-2.5 months begins (Rea and Costa 1992; Noren et al. 2008). The NES pups used

in this study were sampled as they began their fast. At this time, the pups weighed  $135.6 \pm 18.13$  kg. Throughout the PWF, the pups lost substantial amounts of mass. By the eighth week of the fast, pups averaged  $95.87 \pm 15.82$  kg, having lost approximately 40 kg. This strategy of fasting allows periods of rapid mass growth and loss in a short amount of time and requires vast quantities of energy output. For example, during nursing, phocids gain 0.8-7.1 kg per day compared to 0.06-0.38 kg per day in otariids (Boness and Bowen 1996). This forces phocid pups to rapidly develop their oxygen storing capacity and diving ability for early-in-life survival.

Alternatively, otariids utilize the foraging cycle strategy. Females alternate nursing their pups and departing to forage, leaving the pups to fast on land for short time spans. Pups tend to depend on their mothers for a prolonged time and, therefore, gain weight and adequate diving abilities slowly. This strategy allows some species' pups to nurse for up to three years. In this study, the Australian fur seal and Steller sea lion are examples of the foraging cycle strategy.

Australian fur seal pups are 7-8 kg at birth and nurse for about ten months, during which time the female rotates between 6-7 days foraging at sea with intermittent nursing periods of a few days (Arnould and Hindell 2001; Kirkwood et al. 2006). This pattern continues on average for one year, at which point the pup completes its weaning. During this study, AUFS pups that were less than a month old weighed  $7.99 \pm 1.90$  kg. By the end of the nine-month study period, they weighed  $23.79 \pm 5.95$  kg, indicating they gained on average 0.06 kg per day. Steller sea lions also nurse their pups in cycles, alternating three days at sea and up to two days nursing on land (Rea et al. 2000). These pups gained weight at approximately 0.3-0.12 kg per day (Burns et al. 2004; Richmond et al. 2006). The captive Steller sea lion pups less than one-half year-old in this study weighed  $30.54 \pm 7.7$  kg. Towards the end of the first year, the pups weighed  $63.33 \pm 9.9$  kg, nearly doubling their mass by adding approximately 0.18 kg per day. In both cases, these otariids gained weight slowly over a prolonged nursing period, bypassing the requirement of a PWF and necessity of rapid physiological development.

It was previously believed that all phocids followed the fasting strategy, while all otariids followed the foraging cycle strategy; however, these strategies are not specific to family, rather they are species-specific. There are exceptions among the species of both



families that may be in part due to the physiological nature of the species or due to other variables, for example latitudinal differences. Two species used in the current study, the harbor seal and the northern fur seal, are examples of exceptions.

Harbor seals have exhibited maternal characteristics consistent with an intermediate strategy that includes both phocid and otariid characteristics (Boness and Bowen 1996). Lactating females partake in small foraging trips lasting about seven hours during mid-to-late lactation; during this time, they are often accompanied by their pups (Bowen et al. 1999). Another characteristic that differentiates harbor seals from the typical fasting strategy is the amount of fat stores in females at the beginning of parturition. Females have approximately 21 kg of fat, which is substantially less than most phocid females and more like that of otariids (Bowen et al. 1992; Boness and Bowen 1996). Harbor seals have extremely short lactation periods of 15-17 days, which is characteristic of phocids (Muelbert and Bowen 1993). Harbor seals are smaller members of family Phocidae, which may explain why they have less amounts of body fat stores than other phocids. Additionally, harbor seals birth highly precocial pups already born with substantial layers of blubber, so it may not be necessary for reproducing females to accrue vast stores of fat and energy to sustain their pups (Boulva and McLaren 1979; Bowen et al. 1992; Bowen et al. 1999; Burns et al. 2005).

In contrast, northern fur seals are small, subpolar otariids that have adapted to more phocid-like maternal strategies, likely due to their high latitude breeding. Pups are born weighing 5-6 kg with dense fur, several millimeters of subcutaneous fat, and metabolic rates that are comparable to those of harbor seals (Miller and Irving 1975; Blix and Steen 1979). Females exhibit foraging cycles, alternating 1-3 days nursing on land with foraging trips lasting as long as two weeks; however, pups are weaned abruptly at approximately four months and must become independent (Bartholomew and Hoel 1953; Peterson 1968; Gentry and Holt 1986; Baker and Donohue 2000). Additionally, during the short lactation period, northern fur seal pups store much more fat compared to other otariid species, which sustains the newly independent pups as they begin a pelagic post-weaning lifestyle that may last up to 21 months (Gentry and Holt 1986; Baker and Donohue 2000; Baker 2007; Lea et al. 2010; Liwanag 2010; Rosen et al. 2012). This

higher latitude breeding has likely caused the northern fur seal to adapt to a strategy that is intermediate between the phocid and otariid maternal strategies.

Different diving and foraging styles and maternal strategies can affect how oxygen storage develops and is utilized throughout pinnipeds' lives. However, each species has unique, underlying physiological mechanisms that drive oxygen storage capacity. The distribution of the total amount of oxygen stores available within the body tend to fall into two categories that correlate with diving ability. Otariids, who dive to shallower depths for shorter durations tend to have a larger proportion of oxygen stored in their lungs (Ponganis et al. 2011). For example, the northern fur seal has a total O<sub>2</sub> store of approximately 42 ml O<sub>2</sub> kg<sup>-1</sup>, with lung, blood, and muscle constituting 24, 43, and 33% of concentrations, respectively. Alternatively, northern elephant seals have a total O<sub>2</sub> store capacity of approximately 94 ml O<sub>2</sub> kg<sup>-1</sup>. Of this capacity, 3% constitutes the contribution from the lungs, while the blood and muscle contributes 71 and 26%, respectively (Lenfant et al. 1970; Simpson et al. 1970; Thorson and Le Boeuf 1994; Ponganis et al. 2011; Ponganis 2015; Ponganis and Williams 2015). Deeper divers do not have to rely on uptake of oxygen within the stores in their lungs, which helps mediate lung collapse and bradycardia by decreasing the need for gas exchange at depth (Ponganis et al. 2011; Ponganis and Williams 2015).

Blood oxygen stores are the largest stores of available O<sub>2</sub> in most marine mammals. Determining blood oxygen stores includes the following parameters: blood volume, hemoglobin concentration, and the amount of oxygen acquired from the hemoglobin. Among these parameters, pinnipeds have elevated values; for example, gray seals and northern elephant seals have blood volumes of 213 and 216 ml kg<sup>-1</sup> and average hemoglobin concentrations of approximately 20 and 25 grams per deciliter (g dl<sup>-1</sup>), respectively (Ponganis and Williams 2015). It is important to compare hemoglobin with blood volume because having higher relative blood volume allows for more red blood cells per unit volume of blood and has been correlated with diving ability (Berta et al. 2006). Among the pinnipeds, phocids tend to have more elevated values than the otariids. Comparatively, in this study, the average hemoglobin concentration was  $19.48 \pm 1.59$  and  $21.64 \pm 3.64$  g dl<sup>-1</sup> for gray seals and northern elephant seals, respectively. There were no differences between age classes for the gray seal. In contrast, juvenile and adult elephant

seals had substantially higher concentrations than the weaned NES pups, at  $22.5 \pm 1.44$  and  $24.4 \pm 1.73$  g dl<sup>-1</sup>. This exemplifies that although phocids and otariids tend to follow patterns within their families, there are still interspecific differences that occur.

Two other major factors influence blood oxygen stores: hematocrit and spleen size. Together, hemoglobin and blood volume increase the hematocrit, which is the percent packed red blood cell volume. Consequently, marine mammals also tend to have elevated levels of hematocrit (Davis 2014). Among the blood oxygen stores, hematocrit has been shown to vary with age, stress, and animal handling. For example, increased levels are induced by factors such as handling or abrupt weaning and abandonment because stress causes splenic contractions, which increases the amount of red blood cells circulating within the body (Castellini et al. 1996; Hall 1998; Jørgensen et al. 2001; Thomas and Ono 2015). Thus, it is important to consider external factors that may be influencing values when comparing oxygen stores among species. The spleen may also affect the amount of oxygen circulating throughout the body. The spleen is a reservoir for red blood cells and the site of red blood cell production, and is believed to account for 43% of increase in blood volume (Bryden and Lim 1969; Qvist et al. 1986; Ponganis et al. 1993; Thorson 1993). During a dive, seals' spleens contract, releasing stored red blood cells, which would increase the amount of red blood cells, and, therefore, oxygen in the circulatory system (Ponganis et al. 1993; Castellini et al. 1992, 1996; Thomas and Ono 2015).

Blood oxygen stores are correlated with the length of the nursing and post-weaning durations in phocids. Based on this, Burns et al. (2007) hypothesized that species with shorter terrestrial periods must have more mature oxygen stores to compensate for less time on land for physiological development. The current study utilized only hemoglobin and hematocrit as measurements of blood oxygen stores and myoglobin as muscle oxygen stores. However, it is important to note sources cited in this study used additional parameters to calculate blood and muscle oxygen stores other than hemoglobin, hematocrit, and myoglobin. Therefore, there is a distinction between oxygen stores in the current study and cited literature (Thorson 1993; Burns et al. 2004, 2005; Noren et al. 2005; Richmond et al. 2006; Spence-Bailey et al. 2007; Somo et al. 2015).

Overall among the phocids, the northern elephant seals had the highest mean Hb, followed by harbor seals and gray seals. Harbor seals were the only phocid in this study for which data were available on nursing pups. The nursing pups had relatively elevated values of Hb and Hct compared to other age classes. Oxygen stores tend to be elevated in pinniped neonates because pups require high oxygen affinity while in the uterus in order to acquire enough oxygen (Burns et al. 2005). This was evident in the harbor seals, whose blood oxygen values of nursing pups were elevated compared to the other age classes. These values declined in weaned pups but increased by the time pups were juveniles. It is believed this decrease is due to dilution of blood cell production, which cannot keep up with the rapid body growth and subsequent rapid increases of plasma volume of phocid pups while they are nursing (Thomas and Ono 2015).

In studies of wild individuals, Burns et al. (2005) found harbor seal pups were weaned with blood oxygen values similar to those of adults. In contrast, northern elephant seal pups, which have an extended post-weaning fast had values that were much smaller than those of adults (Thorson and Le Boeuf 1994; Burns et al. 2005). The results of the current study were consistent with those findings. Weaned harbor seal pups in this study had Hb values that were 80% that of adults at the time of weaning, while NES pups had Hb values 70% that of adults. It is important to note that most of the harbor seal pups used in this study were captive individuals who were brought into a rehabilitation center, thus were in variable health and body conditions (Thomas and Ono 2015). This may have affected the lower blood oxygen values observed in the nursing and weaned pups. Similarly, weaned gray seal pups, who also have shorter PWF than NES pups, had blood oxygen stores that were mature, i.e. similar to adult values. This supports the idea that pups with shorter terrestrial durations, who begin independently foraging at an earlier age, are weaned with blood oxygen stores that are more mature than pups with longer terrestrial periods.

This pattern was also evident in the otariids as most otariids are weaned with adult-like oxygen stores (Burns et al. 2004). Among the three otariid species, the northern fur seals have the shortest terrestrial duration at four months. At the time of weaning, pups had Hb values at  $17.55 \pm 1.08 \text{ g dl}^{-1}$ . This was elevated compared to concentrations in weaned Steller sea lion pups at one year old, which was  $13.60 \pm 2.20 \text{ g dl}^{-1}$ . However,

it was slightly lower compared to Australian fur seal pups that were nine months old, with values over  $18 \text{ g dl}^{-1}$ . Once again, it is important to note that both NFS and SSL individuals were captive, so there may have been constraints on their natural life cycles and diving abilities. Australian fur seals are the largest of the fur seals, and these pups were wild individuals compared to NFS and SSL, so this may have affected this outcome. However, northern fur seal pups had Hb values that were no different from adult values. It is evident that pups have to quickly become physiologically equipped early in life because they are abruptly weaned and must immediately begin their first foraging migration (Baker 2007).

Comparatively, there are two developmental patterns observed within each family (Burns et al. 2004). For phocid pups, the post-weaning fast is an integral part of their physiological development and correlates to the maturity of their oxygen stores at weaning and diving ability. Pups rapidly develop adequate oxygen stores to sustain early independent diving and foraging. In contrast, otariids have much longer lactation periods that allow them to slowly mature physiologically so that by the time they are weaned, pups have oxygen stores that are adult-like. There is longer-term investment in physiological development that prevents otariids from requiring a terrestrial post-weaning fast before they begin diving. However, this study showed there were species-specific differences in development that should be considered, as discussed below.

### *Gray seal*

In this study, age significantly affected mass between weaned pups and adults and between juveniles and adults. At birth, gray seals weigh approximately 10 kg. During the 18-day nursing period, pups gain weight at a rate of approximately 1.3-1.8 kg per day and begin their PWF weighing an average of 37.9 kg (Worthy and Lavigne 1983; Iverson et al. 1993). The weaned pups in the current study averaged  $37.78 \pm 5.56 \text{ kg}$ , which was consistent with previous studies (Hall 1998; Hall et al. 2001; Noren et al. 2005, 2008). However, juvenile body mass was lower than the previous studies; probably due to biological factors, such as time of year, age in relation to that specific age class, etc. (Noren et al. 2005). The exact age of these animals is unknown. Weaned pups are up to

one year of age, while juveniles are between a year old and sexual maturity. Therefore, there can be substantial differences in weight unless all of the studies measured individuals of the same known age. Therefore, it is likely that these mass differences are due to age at the time of measurement.

Adults weighed up to three times more than juveniles, averaging  $118.48 \pm 31.03$  kg. These values were also substantially lower than previously published values (Noren et al. 2005). This may also be due to a number of biological factors, including age relative to sexual reproduction or time of year, including breeding, fasting, and individual foraging efforts. Another factor that should be considered is the ratio of female-to-male individuals. Twelve females and a single male were sampled. The male in this study weighed 157.20 kg, which was 40 kg larger than the average mass of the females. Although this was a marginal difference, gray seals are sexually dimorphic by the time they reach sexual maturity, at which point males are capable of weighing up to three times more than females.

In contrast, age class did not affect hemoglobin or hematocrit per gram body mass values between weaned pups, juveniles, and adults. These results were inconsistent with previous studies that longitudinally sampled pups for blood and muscle oxygen stores (Noren et al. 2005, 2008; Hall et al. 2001). Noren et al. (2005) found significant differences among age classes in both Hb and Hct. However, the individuals in that study were measured at the following age categories: neonates (three days postpartum), pups sampled at 0-, 12-, and 24-days post-weaning, yearlings, and adults (greater than six years). This current review used broader age categories and may not have shown those increases at a detailed level. Additionally, because individuals were measured solely by age class, it is not clear at which point in time those individuals were sampled. For example, it is unknown whether the weaned pups in this study were undergoing their post-weaning fast or if they were between post-weaning and their first year of life.

For this study, mass was the significant factor affecting blood oxygen stores. These results supports multiple studies that have determined that body mass and composition at the time of weaning determine the length of the post-weaning fast in gray seals. This species shows great plasticity in the PWF duration, ranging from 10-28 days. Research has found pups that are smaller and leaner at weaning tend to have shorter

terrestrial durations and begin foraging at an earlier age than pups that are larger and heavier (Hall 1998; Hall et al. 2001; Noren et al. 2005, 2008). A possible explanation for plasticity during a PWF involves phases of food deprivation that occurs when an animal is fasting (Noren et al. 2008). There are three phases, characterized by different types of fuel used for energy. During phase I, animals are using reserves of glycogen as their main source of energy. In phase II, the body begins to oxidize fat stores for energy. Phase III, terminal starvation, is the point at which 30-50% protein is being catabolized (Noren et al. 2008). It is crucial for fasting pups to begin foraging before they reach phase III, which can result in death by starvation. Therefore, although smaller and leaner pups may not yet have blood oxygen stores that are mature for foraging, they must end their PWF to avoid using too much of their energy reserves. It was beyond the scope of this study to determine exact PWF durations for this species. However, the current study highlighted the significant effect of mass on blood oxygen stores, which supports the conclusion that phocid pups use post-weaning fasts to acquire oxygen stores adequate for diving. This explanation has helped define the plasticity of the PWF in gray seals and may also apply to other species of phocids.

### *Harbor seal*

Harbor seal age class was the significant factor affecting blood oxygen store development. Harbor seal pups are highly precocial at birth. Pups shed their lanugo *in utero* and are born with blubber reserves (Bowen et al. 1992; Muelbert and Bowen 1993). These pups are also large relative to maternal size compared to other phocid seals (Kovacs and Lavigne 1986; Bowen 1991; Bowen et al. 1992; Muelbert and Bowen 1993). During the 21-28 day nursing period, pups continuously enter the water with their mothers, which may result in mature oxygen stores soon after birth (Lawson and Renouf 1987; Muelbert and Bowen 1993; Burns et al. 2005). Among the phocids utilized in this study, there was only nursing data for harbor seals. Nursing pups had more elevated Hb levels at  $20.14 \pm 2.63 \text{ g dl}^{-1}$  compared to published values of nursing gray and northern elephant seal pups (Thorson 1993; Noren et al. 2005).

In this study, weaned pups and juveniles had hemoglobin values that were 80 and 97% that of adult values. The results of the current study are consistent with Burns et al. (2005), who found neonates, weaned pups, and juveniles had mature oxygen stores that were 57, 75, and 90% that of adults. This supports the conclusion that harbor seal development is accelerated compared with other phocids. Harbor seals are weaned with adult-like oxygen stores, unlike NES pups, who require a longer terrestrial period to develop oxygen stores and are weaned with relatively immature stores.

This accelerated development is likely due to the short terrestrial period and early onset of diving behavior of harbor seal pups. Throughout lactation, pups gradually spend more time in the water and increased their dive depths and durations (Jørgensen et al. 2001). During this time, blood oxygen stores do not significantly decrease throughout nursing, which is consistent with this study's findings. The only significant increases in hemoglobin occurred between weaned pups and juveniles and between weaned pups and adults, with no difference between nursing and weaned pups. These data were similar to findings from Burns et al. (2005). Blood oxygen stores (on a mass-specific basis) varied with age and were only significantly different between yearlings and adults and nursing pups. However, despite the highly precocial nature of harbor seal pups, they are still not physiologically mature at the time of weaning.

#### *Northern elephant seal*

For northern elephant seals, age class significantly affected mass, Hb, and Hct. Mass significantly changed among weaned pups, juveniles, and adults. NES pups weigh approximately 30 kg at birth (Le Boeuf et al. 1972), which is three times that of all other species' pups in the current study. During the 28-day nursing period, pups triple their mass to greater than 90 kg and increase their body fat up to 50% (Rea and Costa 1992; Iverson et al. 1993; Noren et al. 2008). The weaned pups utilized in this study began the PWF weighing  $135.26 \pm 18.13$  kg. On average, the weaned pups consistently lost 12 kg every 2-3 weeks. By the end of the 8-week sampling period, pups lost nearly 30% of their pre-weaning body weight.



NES pups show plasticity and can shorten and extend the duration of their PWF from 2-2.5 months, although the range is not as variable as gray seal pups. Also similar to gray seals, body composition at weaning contributes to the length of the fast because it determines how much fat and protein can be utilized throughout the post-weaning fast (Noren et al. 2003; Somo et al. 2015). Noren et al. (2003) found that the length of the post-weaning fast increased with body mass, where pups that were heavier and fatter at the beginning of weaning fasted upwards of two months, while smaller and leaner pups ended their fast earlier. However, there is a trade-off associated with fasting plasticity. Pups that end their fast prematurely may not be physiologically mature to be adequate divers. For example, studies have shown that leaner NES pups who fasted for 5-6 weeks ended their PWF with approximately 69% of adult mass-specific oxygen stores compared to fatter pups who fasted for 10-11 weeks, which had approximately 78% that of adults (Thorson 1993; Noren et al. 2003, 2008). Although this study did not compare differences between PWF durations, the results are still consistent with body composition being a major factor in developing oxygen stores during the fast.

This study found Hb was significantly affected by age and mass throughout the weaning period, while there was not an effect on Hct. Hb concentrations were elevated at 0-weeks weaning at  $19.32 \pm 1.71 \text{ g dl}^{-1}$ , then continuously decreased to  $14.14 \pm 5.38 \text{ g dl}^{-1}$  by the eighth week of weaning. Overall, there were significant increases in Hb and Hct among weaned pups, juveniles, and adults. This is consistent with Thorson (1993) who found that blood oxygen stores increased 44-55% from the end of the PWF to adulthood. Although this study showed decreases in blood oxygen stores throughout the weaning period, Thorson (1993) found that Hb significantly increased during the PWF. This could be due to differences in how pups were sampled, as has been shown in a previous study (Somo et al. 2015). In some studies used for this analysis, seals were sampled once across a population for different age classes. Alternatively, other studies used in this analysis sampled the same individuals multiple times over a specific time period.

Noren et al. (2005) speculated phocids with shorter terrestrial durations obtain mature levels of blood and muscle oxygen stores earlier in life. For example, at the end of the PWF at about 90 days of age, elephant seal pups have adult-like levels of Hb when they begin independently foraging. In comparison, gray seals are less than half of the age

of elephant seal pups (<45 days) when they end their post-weaning fast and begin independently foraging (Noren et al. 2005). At this time, gray seal pups have similarly mature oxygen stores as elephant seal pups, who still have at minimum, one month left of their fast. NES pups have been observed during weaning, taking experimental swimming and diving trips within the first few weeks of their fast (Reiter et al. 1978; Thorson and Le Boeuf 1994). During this time, dive duration increases from 1.7 to 5.9 minutes with a maximum of 15 minutes (Thorson 1993). Northern elephant seal pups may require a long terrestrial period to develop the amount of oxygen stores adequate for their diving abilities. Once they begin foraging, they adopt the prolonged and continuous diving that is seen in adults; for example, one of the individuals Thorson (1993) studied during its first trips out to sea after weaning, increased its dive depth from 16 m to greater than 200 m (Thorson 1993). This indicates that there was adequate oxygen developed during weaning, allowing pups to begin their extreme diving early on.

#### *Australian fur seal*

In this study, mass and age affected Hb and Hct levels throughout the nine-month nursing period. At less than one month old, neonatal AUFS pups had levels of Hb at  $17.24 \pm 3.60 \text{ g dl}^{-1}$ , which is similar to other pinniped neonates. These values decreased after the first month of birth, then significantly increased throughout the rest of nursing. Hct followed this same trend and stabilized around the five-month point.

Physiological development appears to be slower in otariid species that have longer lactations. Compared to the phocids, which had mature blood oxygen levels at the time of weaning between 1-2 months, the Australian fur seal pups did not acquire more adult-like levels of hemoglobin and hematocrit until approximately 5-6 months of age. This coincides with the period at which AUFS pups molt their natal coat (3-4 months) and begin spending more time swimming and diving (Warneke and Shaughnessy 1985; Donohue et al. 2000). The current study did not have access to raw adult AUFS data. However, published analyses on adults from Spence-Bailey et al. (2007) showed that Hb and Hct were significantly less in pups than in adults. By nine months of age, Australian fur seal pups only had 71% mass-specific total body oxygen stores of adults.

Although physiological development is slower than phocids, otariids develop diving and foraging skills throughout their nursing period. Average dive depths and duration increased in pups that were five and seven months old at approximately  $4.4 \pm 0.3$  m and  $0.35 \pm 0.03$  minutes and  $8.1 \pm 0.5$  m and  $0.55 \pm 0.05$  minutes, respectively. Max depths for pups five and seven months old exceeded 30 and 60 m, respectively, although this was still significantly lower than adult females, who were recorded at approximately 80 m. There was no significant change in pups' mean dive durations (Spence-Bailey et al. 2007). Although this evidence shows that pups are rapidly gaining diving and foraging experience, their dive depths and durations are substantially less than adults. Adult females on average spent less than three minutes diving and at depths of approximately 60 m (Arnould and Hindell 2001; Spence-Bailey et al. 2007). Thus, even though pups take longer to develop, they are more physiologically equipped at the point of weaning and do not require the PWF that phocid pups do (Burns et al. 2004).

#### *Northern fur seal*

The northern fur seals and Steller sea lions from which data were utilized in this study were all captive females at the Vancouver Aquarium (David A.S. Rosen, pers. comm.). Six individual fur seals were measured over several years, which allowed for the opportunity to look at oxygen store development at a finer, individual scale. Age significantly affected mass in all six females, with the weaned pups averaging approximately 13 kg while adults reached on average 25 kg. It is important to note that the adult average masses were 25-40 kg, which is substantially smaller than values of wild northern fur seal females, which weigh 40-50 kg (Gelatt et al. 2015; David A.S. Rosen, pers. comm.). It is unknown exactly why there is such a difference between the mass of the wild and captive individuals, although there is speculation that a combination of factors including captivity and lack of active reproduction influence this (David A.S. Rosen, pers. comm.).

Increases in mass positively affected the development of blood oxygen stores in NFS, although there was individual variation driving these changes (Figure 8a). There were no significant differences among age classes, with both Hb and Hct remaining

stable at approximately 17 g dl<sup>-1</sup> and 51%, respectively, among weaned pups, juveniles, and adults. Shero et al. (2012), comparing blood and muscle oxygen stores between wild pups (less than a month old) and adults, found a positive relationship between age and Hb and Hct. Pups had hemoglobin and hematocrit values approximately 12 g dl<sup>-1</sup> and 41% compared to adults who had 16 g dl<sup>-1</sup> and 45%, respectively. The northern fur seals utilized in the current study were captive individuals, therefore, they may not exhibit the same diving and foraging behavior as wild individuals, which may have affected the results from this current study.

Northern fur seals are unique otariids in that they abruptly wean their pups after four months of intense nursing (Bartholomew and Hoel 1953; Peterson 1968; Gentry and Holt 1986; Baker and Donohue 2000). They also store larger amounts of lipid than other species in the otariid family. These are adaptations to high latitude breeding (Arnould et al. 1996; Donohue 1998; Baker and Donohue 2000). Baker and Donohue (2000) examined diving behavior of nearly weaned pups. Most dives were shallow, between 2.7-3.3 m and all under one minute long, with maximum depths reaching 10-18 m under three minutes. For this species, time spent in the water throughout the nursing period was based on the development of the adult pelage. They also observed that once pups reached 100 days old, they spent an average 35% of their time in the water before their post-weaning migration. Once pups are abruptly weaned, they immediately begin their first post-weaning migration at which point their diving abilities begin to dramatically improve as they spend more time submerged at sea and take longer dives (Baker 2007).

### *Steller sea lion*

Like the northern fur seals, the Steller sea lion data came from captive females at the Vancouver Aquarium. There were 12 individuals measured multiple times across several years. Mass significantly increased among nursing pups, juveniles, and adults. Nursing pups' mass increased by 67% to juveniles and juveniles increased their mass by 33% by adulthood. Like NFS, the adult females weighed substantially less for wild individuals. Wild female Steller sea lions can reach approximately 350 kg (Loughlin

2009), but the captive females averaged 165 kg. Lack of active reproduction in a captive environment may explain this difference.

Typically, in the wild, neonates are born averaging about 20 kg (Davis et al. 2006). During nursing, they grow slowly, gaining less than 0.5 kg per day (Burns et al. 2004; Richmond et al. 2006). In this study, pups early during the nursing period weighed approximately 23 kg and reached approximately 62 kg before they were one year old. These pups gained approximately 0.11 kg per day. Steller sea lion pups usually undergo short fasting periods in which they substantially lose weight when their mothers leave for foraging trips (Rea et al. 2000). However, since the pups used in the current study were captive, they did not undergo the same short-term physiological changes.

SSL blood oxygen stores increased significantly due to mass and were affected by individual variation. Hemoglobin values in nursing pups were low at  $13.60 \pm 2.20 \text{ g dl}^{-1}$  and continued to increase to  $14.45 \pm 2.34$  and  $15.42 \pm 1.42 \text{ g dl}^{-1}$  in juveniles and adults, respectively. Similar increases in Hct were noted among the three age classes. Nursing pups had Hct levels of approximately 39%, which increased to nearly 42% and 44% in juveniles and adults. These values are consistent with findings that suggest that once pups are weaned, they have blood oxygen stores that are 80-90% that of adults (Burns et al. 2004). These values are low compared to that of studies that utilized wild animals. Richmond et al. (2006) compared changes in mass-specific blood oxygen stores in yearlings, juveniles, and adults. Both juveniles and adults had substantially higher blood oxygen stores than yearlings. It is possible that these discrepancies are also due to captivity.

### *Myoglobin*

This study provided the opportunity to compare myoglobin development in a phocid and an otariid pup, the northern elephant seal and the Australian fur seal. Muscle oxygen store capacity includes myoglobin content and muscle mass (Kooyman 1989; Noren et al. 2005). Myoglobin development is important in pinnipeds because it is the second largest storage of oxygen, accounting for approximately 30-35% capacity in

phocids and otariids. Consequently, it is important to understand how Mb changes throughout the terrestrial period.

In the weaned NES pups, Mb significantly increased between the cessation of nursing and 2 weeks and then began to decrease from 5-8 weeks post-weaning (Figure 10). Somo et al. (2015) suggested that, as in blood oxygen stores, elevated levels of Mb early on during the post-weaning fast may have been caused by exposure to hypoxia *in utero*. However, during the prolonged PWF, there was not a substantial increase in Mb until the eighth week of weaning. This indicates that although the PWF may benefit blood oxygen concentrations, it does not necessarily promote the development of muscle oxygen stores (Somo et al. 2015). This study demonstrated significant increases in Mb among weaned pups, juveniles, and adults, which is consistent with other studies. Thorson (1993) found nursing pups had  $2.9 \pm 0.8$  grams per 100 grams ( $\text{g } 100 \text{ g}^{-1}$ ) of myoglobin. By the end of the PWF, this had increased to  $5.1 \pm 1.2 \text{ g } 100 \text{ g}^{-1}$  and then increased further to over  $6 \text{ g } 100 \text{ g}^{-1}$  in adults. In this study, juveniles had significantly lower Mb concentrations than adults,  $6.16 \pm 0.80$  and  $7.9 \pm 1.17 \text{ g } 100 \text{ g}^{-1}$ , respectively. This is consistent with Thorson (1993) who found increases of 28-31% of muscle oxygen storage capacity in pups at the end of the PWF and adults.

There were also significant changes in Mb throughout the nursing period in Australian fur seal pups. Substantial increases were noted in pups that were less than one month old to pups that were six months and nine months old, while there were no measurable differences in pups that were between eight and nine months of age (Figure 11). Australian fur seal pups do not undergo a PWF like phocids do. After about 3-4 months, when their natal fur is shed, pups begin to develop their diving and swimming skills in shallow pools (Warneke and Shaughnessy 1985; Hume et al. 2001; Spence-Bailey et al. 2007; Deacon and Arnould 2009). Spence-Bailey et al. (2007) found that pups spent <8% of their time in the water before they molted. Afterwards, they spent approximately 27% of their time in the water diving. This allows pups to begin developing their muscle oxygen stores early on. This is evident in the significant increase in Mb content from pups <1 month old and six months old. Additionally, the significant increase between pups six and nine months old is likely due to increased diving. However, just as the PWF in northern elephant seals did not significantly affect

myoglobin content past eight weeks, there was no change in myoglobin content after AUFS pups reached eight months old.

Although this study only compared AUFS pups, other studies examined juveniles and adults. Pups' myoglobin concentrations were significantly lower than that of published adult female values, at  $4.16 \pm 0.70$  grams Mb per 100 grams wet muscle<sup>-1</sup> (g Mb 100 g wet muscle<sup>-1</sup>) (Spence-Bailey et al. 2007). By the time pups were nine months old, they had only acquired approximately 21% that of adult female values. This is substantially lower compared to NES pups, who once weaned, have values that are 68% that of adult values (Thorson 1993; Thorson and Le Boeuf 1994; Noren et al. 2005). This is consistent with other studies that have compared myoglobin development in phocids and otariids. Phocids' muscle oxygen stores increase after weaning and generally reach adult values by the end of the first year while concentrations in otariids gradually increase during their prolonged nursing and do not reach adult values until after the second year (Burns et al. 2004, 2005; Richmond et al. 2006).

Noren et al. (2005) compared development among neonates, weaned pups, yearlings, and adult gray seals. Gray seals store approximately 27% of their total oxygen storage capacity in the muscle (Noren et al. 2005). Neonates had the smallest amount of Mb at  $1.7 \pm 0.1$  g 100 g muscle<sup>-1</sup>. Throughout the PWF, mean Mb increased by approximately 29% although the increase was not considered significant. However, it was noted the sample size may have been too small for statistical significance. There were significant differences found among pups, yearlings, and adults. By the time pups are weaned, they acquire myoglobin levels approximately 68% that of mass-specific adult values. In comparison, northern elephant seal pup of the same age were still undergoing their PWF, and, therefore, had less mature values than gray seal pups. However, once NES pup were weaned, they had mature levels 76% that of adults, indicating they are more physiologically developed at the time of weaning (Thorson 1993; Thorson and Le Boeuf 1994; Noren et al. 2005).

However, there is a substantial difference in Mb concentrations between gray and NES juveniles and adults. Gray seal yearlings and adults in the Noren et al. (2005) study had the highest values at  $3.2 \pm 0.3$  and  $4.0 \pm 0.3$  g 100 g muscle<sup>-1</sup>. The NES juveniles and adults in this study had Mb concentrations of  $6.16 \pm 0.80$  and  $7.9 \pm 1.17$  g 100 g muscle<sup>-1</sup>,

respectively, indicating more muscle oxygen storage capacity. Phocids with shorter nursing and PWF durations should be more physiologically equipped with large amounts of blood and muscle oxygen stores earlier in life than those that have longer terrestrial periods (Noren et al. 2005). However, those who have greater diving capabilities may require more time to develop adequate stores to sustain their diving activities, such as the northern elephant seals. NES have one of the longest terrestrial periods of pinnipeds. Because they need to develop oxygen stores that can sustain their extreme diving patterns, pups may need a longer time for their oxygen stores to sufficiently mature.

Gray and northern elephant seals are altricial at birth compared to harbor seal pups. Harbor seals are highly precocial at birth, with pups shedding their lanugo and developing a blubber layer *in utero* and able to swim and dive shortly after birth (Knudston 1977; Boulva and McLaren 1979; Lawson and Renouf 1987; Bowen et al. 1992; Muelbert and Bowen 1993; Jørgensen et al. 2001; Burns et al. 2005). Burns et al. (2005) found significant differences in muscle oxygen store development among age classes in harbor seals. Neonates had the smallest amount of muscle oxygen stores at  $4.0 \pm .6 \text{ ml O}_2 \text{ kg}^{-1}$ . These values increased to  $5.9 \pm .5$  and  $7.0 \pm .7 \text{ ml O}_2 \text{ kg}^{-1}$  in nursing and weaned pups, respectively, although these increases were not considered significant. This development appears to be consistent with that of the gray seals and northern elephant seals. Harbor seals quickly develop some minimal limit of muscle oxygen storing capacity to sustain their early diving and swimming (Burns et al. 2005).

There were differences in muscle oxygen stores between pup and juvenile and adult harbor seals. Juveniles and adults had significantly higher values than pups at  $16.3 \pm 1.6$  and  $14.7 \pm 1.1 \text{ ml O}_2 \text{ kg}^{-1}$ , respectively. Although pups are highly precocial at birth, Burns et al. (2005) found neonates had myoglobin concentrations that were 37% of adults. During nursing and post-weaning, these values slightly increased so that by the time of weaning, total muscle oxygen stores were 64% that of adults. Gray seals showed similar increases in muscle oxygen stores throughout their terrestrial period, with newborns having values of  $4.2 \pm 0.1 \text{ ml O}_2 \text{ kg}^{-1}$  and weaned pups reaching over  $6 \text{ ml O}_2 \text{ kg}^{-1}$  (Noren et al. 2005). Similar to harbor seals, gray seal juveniles and adults had  $12.9 \pm 1.3$  and  $15.9 \pm 1.3 \text{ ml O}_2 \text{ kg}^{-1}$ , respectively, of muscle oxygen stores. These two phocids have similar diving abilities; gray seals dive along continental shelves around 250 m



while harbor seals can reach 150 m with maximum depths of 450 m (Gjertz et al. 2001; Beck et al. 2003; Noren et al. 2005; Berta et al. 2006; Burns 2009).

Research on muscle myoglobin data is sparse in otariids; there were not data for northern fur seals but there were published data on Steller sea lions. Richmond et al. (2006) found significant increases in myoglobin development due to age. Pups were sampled at 1-, 5-, and 9-months old during the lactation period as well as juveniles and adults. Nursing pups had the smallest muscle oxygen storage capacity at  $2.3 \pm 0.1$  and  $5.5 \pm 0.3$  ml O<sub>2</sub> kg<sup>-1</sup> for one month old and five month old pups. These values did not significantly increase until pups were nine months old, to  $9.9 \pm 0.6$  ml O<sub>2</sub> kg<sup>-1</sup>. Pups begin increasing and developing their diving and swimming abilities at 5-6 months after birth, which corresponds to the significant increase in muscle development between pups that are five and nine months old (Merrick and Loughlin 1997; Loughlin et al. 2002; Raum-Suryan et al. 2004; Burns et al. 2004; Richmond et al. 2006).

From this point forward, muscle oxygen storage significantly increased from pups and juveniles to  $15.2 \pm 1.1$  ml O<sub>2</sub> kg<sup>-1</sup>. There were significant differences between adult female and male Steller sea lions. Females averaged  $14.0$  ml O<sub>2</sub> kg<sup>-1</sup>, which was not measurably different from juveniles. However, males had significantly higher muscle oxygen storage capacity at  $23.9 \pm 1.8$  ml O<sub>2</sub> kg<sup>-1</sup>. Steller sea lions are the largest of all the otariids and exhibit extreme sexual dimorphism (Pendleton et al. 2006; Loughlin 2009; Maniscalco 2014). Pitcher et al. (2005) found significant differences between sexes in diving abilities in juveniles. There has not been much research that has focused on differences in diving behavior between adult males and females. However, there is a correlation between body size and diving capacity (Shreer and Kovacs 1997). For example, the otariids in this study had blood oxygen storing capacity that increased with mass. Therefore, due to the substantial differences in body size, male Steller sea lions may have greater diving abilities than females.

In all pinnipeds utilized in the current study and others, muscle myoglobin did not significantly increase until pups begin independently foraging and reached adolescence. Research has demonstrated a lag between muscle oxygen store development and blood oxygen store development (Thorson and Le Boeuf 1994; Burns et al. 2005, 2007; Noren et al. 2005). It has been suggested that blood and muscle oxygen stores cannot be

developed simultaneously (Burns et al. 2005). Furthermore, the development of muscle myoglobin increases with increased muscular activity (Folkow et al. 2010; Geiseler et al. 2013).

Blood constitutes the largest oxygen stores, with phocids and otariids containing over 60% and 40% of their total body oxygen stores in the blood (Figure 19; Kooyman et al. 1981; Kooyman 1985; Ponganis et al. 2011). The development of hemoglobin may be prioritized to sustain the metabolic demands of vital tissues such as the heart, lungs, and brain (Burns et al. 2005). In this study, hemoglobin and hematocrit levels were relatively mature compared to myoglobin concentrations. This is consistent with various other studies (Thorson 1993; Thorson and Le Boeuf 1994; Burns et al. 2004, 2005, 2007, 2009; Noren et al. 2005, 2008; Somo et al. 2015; Thomas and Ono 2015). For example, Burns et al. (2005) suggested blood oxygen store development may support early diving and swimming until the muscle oxygen stores can develop in harbor seals, which lends to their highly precocial nature.

Several studies hypothesized that blood oxygen stores mature earlier to provide oxygen needed for aerobic metabolism until myoglobin can develop (Burns et al. 2004, 2007, 2009; Lestyk et al. 2009; Prewitt et al. 2010; Geiseler et al. 2013; Burns et al. 2015). While pups are *in utero*, they are consistently exposed to hypoxia while their mothers are diving, which may elicit the prenatal development of blood oxygen stores (Elsner et al. 1969). This stimulates an increase in red blood cell production (Thomas and Ono 2015). Additionally, there must be a high oxygen affinity of fetal blood in order to receive sufficient oxygen from the mother across the placenta (Burns et al. 2005). Myoglobin has a higher affinity for oxygen than hemoglobin. If there were high concentrations of Mb in a pup while *in utero*, myoglobin would deplete oxygen from the blood, which would explain why it is advantageous for pups to have a lag in their muscle oxygen store development (Geiseler et al. 2013).

Myoglobin development depends on the type and function of muscle (Prewitt et al. 2010). For example, cardiac muscle is more developed at birth and weaning than skeletal muscle (Burns et al. 2005). Additionally, muscle mass is proportioned differently throughout the body between pups and adults. Pups have greater proportions of their muscle mass in the postural muscles such as the shoulder and neck, which are utilized for

nursing and movement on land, while adults had the most mass and Mb concentration in the major swimming muscles (Lestyk et al. 2009). Finally, pups' muscles cannot sustain aerobic metabolism in part because of their high mass-specific metabolic rates, which requires more enzyme activity and energy (Lestyk et al. 2009; Prewitt et al. 2010; Burns et al. 2015). These are all factors that affect the development of myoglobin in pups before they begin independently foraging.

Although there is some myoglobin development throughout the nursing and weaning period, concentrations do not begin to reach adult values until after independent foraging (Thorson and Le Boeuf 1994; Burns and Castellini 1996; Burns et al. 2005). This has implications for both phocids and otariids. The PWF in phocids is thought to be a period of physiological development so that pups can sustain some sort of aerobic diving. Otariids are able to swim and dive at earlier ages throughout their nursing period; however their Mb levels are not mature. For example, in this study, the weaned northern elephant seal pups had Mb levels that were approximately 59% that of adult values, whereas juveniles had values that were 81% that of adult values. Similarly, the nursing AUFS pups in this study had Mb levels that were less than 25% that of published adult values. This suggests that prenatal and terrestrial development can only do so much for muscle maturation. Further aquatic activity and hypoxia may be necessary for pups' muscle oxygen stores to mature, which does not occur until they begin regularly diving (Burns et al. 2009).

## Conclusion

The results of this study showed distinct physiological differences in oxygen store development exist between phocids and otariids. The diversity of age class related to post-natal development (terrestrial duration and maternal strategies) and associated body mass contributed to significant effects in development between the two families and among species.

- Overall, phocids had significantly higher amounts of hemoglobin and hematocrit than otariids in this study. These greater oxygen-storing capacities support previous observations that phocids have superior diving abilities. However, there were species-specific differences in oxygen store development, dependent on age, mass, and terrestrial duration.
- Gray seals' blood oxygen stores were significantly affected by mass, consistent with studies comparing body mass and composition and post-weaning fast durations (Hall et al. 2001; Noren et al. 2008).
- The highly precocial harbor seal pups had adult-like levels of blood oxygen stores, likely due to their accelerated development (Burns et al. 2005).
- Northern elephant seals' oxygen store development was significantly affected by age and mass, likely due to their extreme terrestrial period (nursing and post-weaning fast). NES had significantly higher amounts of hemoglobin and hematocrit than any other species in this study, which is indicative of their extreme diving abilities.
- Otariids' oxygen stores were consistently affected by mass and had slower physiological and terrestrial development (i.e. nursing). AUFS pups did not gain mature hemoglobin and hematocrit levels until they were 5-6 months of age.
- In contrast, there were no significant differences in blood oxygen stores due to age in the northern fur seals. This is likely because NFS have adapted phocid-like nursing and weaning strategies to contend with high latitude breeding (Baker and Donohue 2000).

- Steller sea lions had significant increases in hemoglobin and hematocrit but did not obtain mature stores until weaning at approximately one year.
- In northern elephant seal pups, Mb significantly increased between the first two weeks of the post-weaning fast and decreased thereafter, likely due to the substantial mass loss during their extensive fast. Mb also significantly increased in Australian fur seal pups that were six and eight months old, which coincides with the time in which pups begin learning and improving their diving and foraging skills (Spence-Bailey et al. 2007; Deacon and Arnould 2009). This study also supported other studies that have shown muscle oxygen store development lags behind blood oxygen store development (Thorson and Le Boeuf 1994; Burns et al. 2005, 2007; Noren et al. 2005).

This study compared oxygen store development in six distinct phocids and otariids and is the first to make in-depth comparisons among these species. Although there are general trends between the two families, distinct differences exist in maternal strategies, terrestrial durations, and overall factors, thereby affecting blood oxygen store development (i.e. age class, mass, individual variation). The results presented here emphasized the importance of considering pups' physiological development at a species-specific level, which is important for future research in understanding the ontogeny of individual species.

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