Ontogeny of Oxygen Storage Capacity and Diving Ability in the Southern Sea Otter (*Enhydra lutris nereis*): Costs and Benefits of Large Lungs

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ABSTRACT

Small body size, large lungs, and dense pelage contribute to the unique challenges faced by diving sea otters (*Enhydra lutris*) when compared to other marine mammals. Here we determine the consequences of large lungs on the development of diving ability in southern sea otters (*Enhydra lutris nereis*) by examining the ontogeny of blood, muscle, and lung oxygen stores and calculating aerobic dive limits (cADL) for immature and mature age classes. Total oxygen storage capacity matures rapidly in sea otters, reaching adult levels by 2 mo postpartum. But this result is driven by exceptional lung capacity at birth, followed by a decrease in mass-specific lung volume with age. Blood and muscle oxygen stores remain well below adult values before weaning, with large pups exhibiting 74% and 54% of adult values, respectively. Slow muscle development limits the capacity of immature sea otters to dive against high positive buoyancy due to comparatively large lungs. Immature sea otters diving with total lung capacity (TLC) experience up to twice the mass-specific positive buoyancy as adults diving with TLC but can reduce these forces to comparable adult levels by using a smaller diving lung volume (DLV). The cADL of a juvenile with DLV is 3.62 min, while the cADL of an adult with TLC is 4.82 min. We find that the magnitude of positive buoyancy experienced by sea otters changes markedly with age and strongly influences the ontogeny of diving ability in this species.

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Introduction

Sea otters (*Enhydra lutris*) differ taxonomically, physiologically, and behaviorally from all other marine mammal species (Reynolds and Rommel 1999). They lack a blubber layer, which is used by nearly all marine mammal species for thermoregulation and energy storage (Kenyon 1969; Costa and Williams 1999). Instead, sea otters rely solely on their pelage for insulation. They maintain the densest fur of any mammal, which traps a substantial layer of air next to their skin and reduces heat loss to the marine environment (Williams et al. 1992; Fish et al. 2002). Due to their small body size and unique thermal strategy, sea otters exhibit one of the highest mass-specific metabolic rates of any marine mammal (Morrison et al. 1974; Costa and Kooyman 1982; Yeates et al. 2007; Thometz et al. 2014). Furthermore, sea otters have exceptional mass-specific lung capacities when compared to other marine and terrestrial mammals (Lenfant et al. 1970; Tarasoff and Kooyman 1973; Snyder 1983). The combination of air trapped within dense pelage and large lung capacity results in sea otters experiencing considerable positive buoyant forces (Cashman 2002; Fish et al. 2002).

Although a benefit when resting, grooming, manipulating prey, and caring for young at the water surface (Kenyon 1969; Costa and Kooyman 1982), high positive buoyancy can be a challenge for sea otters at depth. This makes foraging energetically expensive for sea otters in comparison to other marine mammals, which may be able to rely on neutral or negative buoyancy at depth, thereby reducing energetic costs associated with diving (Williams 1989; Crocker et al. 1997; Williams et al. 2000; Miller et al. 2004; Yeates et al. 2007; Aoki et al. 2011; Nousek-McGregor et al. 2014). In this regard, sea otters are more similar to diving bird species, which must dive with air both in the respiratory system and trapped in plumage (Wilson et al. 1992; Stephenson 1995; Ponganis and Kooyman 2000). For diving birds, there are inherent trade-offs between oxygen availability in the respiratory system, positive buoyant forces, and the energetic cost of diving (Lovvorn and Jones 1991; Wilson et al. 1992; de Leeuw 1996; Sato et al. 2002; Field et al. 2005). We hypothesize similar trade-offs for sea otters and that the consequences of large lungs and high positive buoyancy strongly influence the ontogeny of diving ability in this species.

A common index of diving ability is the aerobic dive limit (ADL; Kooyman 1989; Kooyman and Ponganis 1998; Ponganis 2011). ADL is defined by Kooyman et al. (1983) as the amount of time an animal may spend diving before blood lactate concentration increases above resting levels, indicating a shift to

anaerobic metabolism. Although individuals are capable of longer dives using anaerobic metabolism, such dives are energetically inefficient in comparison to aerobic dives, due to increased surface time necessary to process accumulated lactic acid (Kooyman et al. 1980, 1983). Therefore, ADL is a good metric of the time an animal is capable of foraging aerobically on a single dive. Although directly determined by measuring postdiving blood lactic levels (Kooyman et al. 1980, 1983), ADL can be calculated (cADL) by dividing total body oxygen stores (blood, muscle, lung) by oxygen demand (Kooyman 1989). In general, for immature marine divers, a combination of heightened oxygen demands and limited oxygen stores result in shorter cADLs when compared to adult conspecifics (Kooyman et al. 1983; Burns 1999; Richmond et al. 2006).

To determine age-specific diving capacities in sea otters as well as the costs and benefits of large lungs and high positive buoyancy, we examined the ontogeny of blood, muscle, and lung oxygen (O_2) stores in the southern sea otter across five age classes: neonates, small pups, large pups, juveniles, and adults (table 1). Key parameters included hemoglobin (Hb), hematocrit (Hct), red blood cell (RBC) count, mean corpuscular hemoglobin content (MCHC), plasma volume (PV), blood volume (BV), muscle mass percentage, myoglobin (Mb) content, lung mass (M_l), lung volume (V_l), and total body O_2 stores. Total body O_2 stores from this study were used in combination with age-specific metabolic rates of southern sea otters (Yeates et al. 2007; Thometz et al. 2014) to determine a cADL for each age class. Finally, we calculated positive buoyant forces experienced by sea otters in all age classes due to air in both the lungs and the pelage. We found that the magnitude of positive buoyant forces varies with age in the southern sea otter and influences the ontogeny of diving ability in this species.

Material and Methods

Blood, muscle, and lung samples from southern sea otters were obtained from 2010 to 2013 in collaboration with the Monterey Bay Aquarium (MBA) and the California Department of Fish and Wildlife (CDFW) Marine Wildlife Veterinary Care and Research Center (MWVCRC). The age of each study animal was estimated using a suite of morphological characteristics, including total body length, body mass, existence of natal pelage, existence of milk teeth, and tooth wear (Fisher 1941; Kenyon 1969; Garshelis 1984). For statistical comparisons, study animals were grouped into five different age classes consistent with age estimates and total body length measurements (table 1). Birth dates (± 1 wk) were known for a subset of study animals and were used to validate age classifications. All animal care use protocols were evaluated and approved by Institutional Animal Care and Use Committees at both the University of California at Santa Cruz and MBA.

Hematology

Hematological records of captive, wild, and wild-rehabilitated southern sea otters spanning a 13-yr period from 1997 to 2010 were obtained from MBA. Hematological parameters of interest included RBC count, Hb, Hct, and MCHC. Blood samples taken by MBA veterinary staff were sent to IDEXX Reference Laboratories (IDEXX Laboratories, Westbrook, ME) or ANTECH Diagnostics (Irvine, CA) for analysis and/or analyzed in-house at MBA using a VetScan HM5 (Abaxis, Union City, CA) to corroborate laboratory results. Sixty-seven sea otters (32 male, 35 female), ranging in age from 1 d to 11 yr old, were included in analyses. We excluded blood samples from sick or injured animals as identified by MBA veterinary staff, leaving 395 blood samples for this study. When multiple blood records existed for the same sea otter in a single age class, values were averaged, leaving 153 values of each hematological parameter for final analyses.

Plasma and Blood Volume

PV and BV were determined using the Evans blue dye technique (Foldager and Blomqvist 1991; El-Sayed et al. 1995). Ten immature sea otters (5 male, 5 female), ranging in age from 11 d to 1 yr old, and three adults (0 male, 3 female) were measured at MBA. Animals were sedated with fentanyl citrate and midazolam hydrochloride using standard protocols (Monson et al. 2001). All intravenous injections and blood collection occurred via the common jugular vein. An initial blood sample of approximately 3 mL was taken before injection of Evans blue dye (Sigma Diagnostics, St. Louis, MO). Next, a predetermined amount (0.5 g kg^{-1}) of Evans blue dye was injected into the bloodstream, and the syringe was flushed with blood and saline repeatedly to ensure complete delivery

Table 1: Age class descriptions for sea otters included in this study

Age class	Age range	Body length range (cm)	Body mass (kg)	Body length (cm)
Neonate	>1 mo	45–69	2.55 ± .18 (16)	59.03 ± 1.79 (16)
Small pup	1–2 mo	70–84	4.43 ± .30 (9)	75.48 ± 2.12 (9)
Large pup	3–5 mo	85–99	8.50 ± .62 (11)	93.51 ± 1.39 (11)
Juvenile	6 mo–1.5 yr	100–110	12.24 ± .99 (10)	105.34 ± .84 (10)
Adult	>1.5 yr	>110	19.89 ± 1.01 (29)	118.86 ± 3.83 (29)

Note. Average body mass and body length of study animals (not including animals from Monterey Bay Aquarium hematological records) are displayed (means ± SE), and sample size (n) is given in parentheses.

of the solution. To ensure accuracy in the recorded amount of Evans blue dye injected into each animal, syringes were weighed (XS2002S scale, Mettler-Toledo) empty, Evans blue dye was drawn into each syringe to a predetermined level (0.5 g kg^{-1}), and syringes were reweighed. Thus, the entire volume of Evans blue dye injected into each animal was known. Three serial blood samples (2–3 mL) were taken at 7- to 10-min intervals after the initial injection. Once all samples were collected, naltrexone hydrochloride was administered to reverse the effects of the fentanyl, and the sea otters were returned to holding pools.

Blood was collected into lithium-heparinized vacutainers. Blood from the initial (pre–Evans blue) blood sample was drawn into two Hct capillary tubes by capillary action and spun at 10,400 rpm for 3.5 min to determine Hct in duplicate. All vacutainers were spun for 15 min at 3,500 rpm to separate RBCs from plasma. The supernatant was pipetted into 2-mL cryovials and stored at −80°C until analysis. The photometric absorbance of plasma samples was measured at 624 and 740 nm (Epoch microplate spectrophotometer, BioTek Instruments) to account for potential hemolysis and precipitate (Foldager and Blomqvist 1991). Standard dilution curves were created and used to determine Evans blue dye concentrations in serial plasma samples. All plasma samples were logarithmically transformed and used to determine the instantaneous dilution volume at time of injection following standard methods (Foldager and Blomqvist 1991; El-Sayed et al. 1995; Costa et al. 1998). BV was calculated using measured PV and Hct following the equation

$$\mathrm{BV} = \mathrm{PV} \times (1 - \mathrm{Hct})^{-1}.$$

Myoglobin

Muscle samples from 47 sea otters (31 male, 16 female), ranging in age from neonate to aged adult (1.3–31.4 kg), were obtained from CDFW MWVCRC. Muscle samples were taken during routine necropsies of fresh and fresh-frozen carcasses. Two major locomotor muscles, the *longissimus dorsi* and *gracilis*, were sampled. Each sample was immediately wrapped in aluminum foil and frozen until analysis. Mb content was determined following the methods of Reynafarje (1963) and Castellini and Somero (1981), as described by Noren et al. (2001). Mountain lion (*Puma concolor*) muscle and harbor porpoise (*Phocoena phocoena*) muscle of known Mb content were used as assay controls bounding upper and lower Mb concentrations ([Mb]).

Muscle Mass

Complete dissections of 19 fresh and fresh-frozen sea otter carcasses (11 male, 8 female; 1.7–28.0 kg) were completed at CDFW MWVCRC. Total body mass was determined using either an infant scale (model 30, Acme Medical Scale, San Leandro, CA) or a hanging scale (model I-20W, OHAUS, Florham Park, NJ). Morphological measurements were taken before each dissection. Carcasses were carefully pelted, internal organs examined, specific organs (heart, lungs, liver, and kidneys) weighed, and all organs removed. All muscle was removed and weighed in a standardized fashion, and percent muscle mass was calculated by dividing muscle mass by total body mass.

Lung Mass and Lung Volume

Sea otter lung mass was determined across age classes by excising and weighing lungs with trachea attached from 20 fresh and fresh-frozen carcasses (12 male, 8 female) at CDFW MWVCRC. As it has not been determined whether sea otters dive with completely full lungs, the mean mass of adult sea otter lungs ($n = 6$) was used in combination with two published values of adult sea otter lung volume—diving lung volume (DLV) = 207 mL kg^{-1} (Ponganis et al. 2003; Ponganis 2011) and total lung capacity (TLC) = 345 mL kg^{-1} (Lenfant et al. 1970)—to determine mean lung capacity per gram lung tissue. These values were used in combination with age-specific lung masses to calculate DLV and TLC for all age classes.

Total Oxygen Storage Capacity

Total O_2 storage capacity was determined by summing blood, muscle, and lung O_2 stores (Lenfant et al. 1970; Kooyman 1989). Blood O_2 stores were determined following the methods of Kooyman (1989), as explained by Ponganis (2011), and were calculated by summing arterial and venous O_2 stores:

$$\text{arterial } \mathrm{O_2} = \frac{1}{3} \times (\mathrm{BV}) \times (0.95 - 0.20 \text{ saturation}) \times (\mathrm{O_2} \text{ capacity}),$$

$$\text{venous } \mathrm{O_2} = \frac{2}{3} \times (\text{arterial } \mathrm{O_2} \text{ content} - 5\% \text{ by volume}),$$

where BV is in milliliters and 1/3 and 2/3 are estimated proportions of arterial and venous blood, respectively (Lenfant et al. 1970), and 1.34 mL O_2 g^{-1} Hb at 100% saturation (Kooyman 1989). Muscle O_2 storage capacity was calculated using the equation

$$\text{muscle } \mathrm{O_2} = [\mathrm{Mb}] \times (1.34 \text{ mL } \mathrm{O_2}\ g^{-1} \text{ Mb}) \times (\text{body mass} \times p),$$

where 1.34 mL O_2 g^{-1} Mb is the O_2 binding capacity of Mb (Kooyman 1989) and p is the proportion of muscle mass. Lung O_2 storage capacity was calculated using the equation

$$\text{lung } \mathrm{O_2} = V_l \times 0.15\ \mathrm{Fo_2},$$

where V_l is lung volume and 0.15 Fo_2 is the assumed O_2 extraction efficiency of lungs (Kooyman 1989) and was calculated two ways, assuming DLV and assuming TLC, which gave a range of potential lung volumes that sea otters may use while diving.

Calculated Aerobic Dive Limit

Aerobic dive limits were calculated based on an equation from Kooyman et al. (1983):

$$\text{cADL} = \text{total } O_2 \text{ stores (muscle } O_2 + \text{blood } O_2 + \text{lung } O_2) \times (\text{diving MR})^{-1},$$

where age class–specific diving metabolic rates (mL O_2 min^{-1} kg^{-1}) reported in Thometz et al. (2014) and Yeates et al. (2007) were used to calculate ADLs for immature and adult sea otters, respectively. The diving metabolic rates from Thometz et al. (2014) may be conservative estimates of the diving metabolic rates of wild immature sea otters, as animals were postabsorptive when measured (Costa and Kooyman 1984) and sea otters in the wild are likely seldom postabsorptive (Kenyon 1969; Riedman and Estes 1990; Tinker et al. 2008; Thometz et al. 2014). As a result, cADL values presented here may overestimate diving limits for immature sea otters. In contrast, the adult diving metabolic rate from Yeates et al. (2007) was determined using two adult male sea otters that were postprandial when measured. The metabolic rate from Yeates et al. (2007) was not scaled to body mass for the adult cADL calculation due to nearly identical empirically measured mass-specific basal metabolic rates for male and female southern sea otters in the literature (Williams 1989; Yeates et al. 2007).

Statistical Analyses

Sample size limited our ability to examine differences between males and females in nearly all parameters; however, within age class, sex differences in RBC, Hb, Hct, and MCHC were examined using unpaired *t*-tests assuming unequal variance. Inter-age class differences for RBC, Hb, Hct, MCHC, PV, BV, *longissimus dorsi* [Mb], *gracilis* [Mb], average [Mb], muscle mass percentage, lung mass, and the total O_2 storage capacity of blood, muscle, and lungs were determined using one-way ANOVAs with Tukey's post hoc comparisons. Differences in [Mb] between the *longissimus dorsi* and *gracilis* muscles at each age class were analyzed using paired *t*-tests. Linear regression analyses were used to describe changes in PV, BV, muscle mass, and lung mass, as a function of total body mass. All statistical analyses were completed using JMP 10 statistical software program (SAS Institute, Cary, NC). Means are reported ± standard error. Results were considered significant if $P < 0.05$.

Results

Hematology

There were no differences between the sexes within any age class for any hematological variable. Therefore, data were combined in all subsequent analyses. Sea otter RBC ($F_{4,148} = 57.31$, $P < 0.0001$), Hb ($F_{4,148} = 54.76$, $P < 0.0001$), and Hct ($F_{4,148} = 49.04$, $P < 0.0001$) all increased significantly throughout ontogeny (table 2). Juvenile and adult sea otters were not statistically different in any blood parameter (RBC: $P = 0.93$; Hb: $P = 0.75$; Hct: $P = 0.78$), but large pups showed significantly lower RBC, Hb, and Hct than both juveniles and adults ($P < 0.05$ for all comparisons). This suggests that sea otter blood parameters mature at or around the average age of weaning (6 mo). Neonates and small pups were not significantly different from one another in any blood parameter ($P > 0.05$ for all comparisons) and displayed approximately 67%, 64%, and 64% of adult values for RBC, Hb, and Hct, respectively. Large pups differed greatly from all other age classes (table 2) with regard to RBC, Hb, and Hct, suggesting that sea otters undergo rapid hematological development around 3–5 mo postpartum. There was no difference in MCHC between any age classes ($F_{4,148} = 0.89$, $P = 0.47$). Our hematological values for adult sea otters agree closely with those published by Williams and Pulley (1983) for adult southern sea otters.

Plasma and Blood Volume

Total PV and total BV increased significantly with body mass. Total PV increased linearly following the equation

$$\text{PV (mL)} = 74.67 \times M_b + 64.25$$

($n = 13$, $r^2 = 0.96$, $P < 0.0001$), where M_b is total body mass. Total BV increased linearly following the equation

$$\text{BV (mL)} = 170.03 \times M_b - 105.98$$

($n = 13$, $r^2 = 0.98$, $P < 0.0001$). Mass-specific PV (table 2) differed slightly between age classes ($F_{4,8} = 3.93$, $P = 0.05$), with the only significant difference occurring between neonates and juveniles ($P = 0.04$). Mass-specific PV decreased as a function of body mass following the equation

$$\text{PV (mL kg}^{-1}) = -1.19 \times M_b + 98.85$$

($n = 13$, $r^2 = 0.41$, $P = 0.04$). Mass-specific BV did not differ by age class ($F_{4,8} = 2.13$, $P = 0.17$), but it marginally increased as a function of body mass following the equation

$$\text{BV (mL kg}^{-1}) = 0.98 \times M_b + 145.44$$

($n = 13$, $r^2 = 0.24$, $P = 0.08$). We report that adult sea otter mass-specific BV is 173.48 ± 9.85 mL kg^{-1}, which is nearly two times higher than the previously published value (91 mL kg^{-1}; Lenfant et al. 1970). However, our values are comparable to more recent estimates of adult BV and PV as determined for northern sea otters (*Enhydra lutris kenyoni*) using the same technique described here (J. Burns, personal communication).

Myoglobin

Muscle [Mb] in both the *longissimus dorsi* ($F_{4,42} = 97.85$, $P < 0.001$) and *gracilis* ($F_{4,42} = 63.86$, $P < 0.001$) increased significantly with age; however, there was no significant difference in [Mb] between the *longissimus dorsi* and *gracilis* muscles ($t_{46} = -1.76$, $P = 0.08$) within age classes (fig. 1). Therefore, [Mb] values for both muscles within individuals were averaged

Table 2: Hematological variables, plasma volume, and blood volume for sea otters across five age classes

Age class	RBC ($10^6/\mu$L)	Hb (g dL^{-1})	Hct (%)	MCHC (g dL^{-1})	PV (mL kg^{-1})	BV (mL kg^{-1})
Neonate	3.49 ± .08^{A} (30)	12.01 ± .22^{A} (30)	35.93 ± .89^{A} (30)	33.60 ± .41^{A} (30)	100.87 ± 5.49^{A} (3)	152.83 ± 8.31^{A} (3)
Small pup	3.42 ± .10^{A} (29)	12.25 ± .36^{A} (29)	35.86 ± 1.05^{A} (29)	34.21 ± .27^{A} (29)	92.58 ± 10.39^{AB} (2)	144.22 ± 7.22^{A} (2)
Large pup	4.58 ± .09^{B} (45)	16.03 ± .39^{B} (45)	48.01 ± 1.21^{B} (45)	33.49 ± .24^{A} (45)	77.81 ± 1.91^{AB} (2)	151.55 ± 6.30^{A} (2)
Juvenile	4.99 ± .11^{C} (40)	18.04 ± .40^{C} (40)	53.54 ± 1.26^{C} (40)	33.74 ± .21^{A} (40)	72.60 ± .57^{B} (3)	153.69 ± 1.88^{A} (3)
Adult	5.15 ± .06^{C} (9)	19.01 ± .23^{C} (9)	56.49 ± 1.36^{C} (9)	33.81 ± .48^{A} (9)	84.07 ± 7.35^{AB} (3)	173.47 ± 9.85^{A} (3)

Note. Data presented are means ± SE. Superscripts display connecting letters report from Tukey's post hoc pairwise comparisons. Sample size (n) is given in parentheses. RBC = red blood cell; Hb = hemoglobin; Hct = hematocrit; MCHC = mean corpuscular hemoglobin content; PV = plasma volume; BV = blood volume.

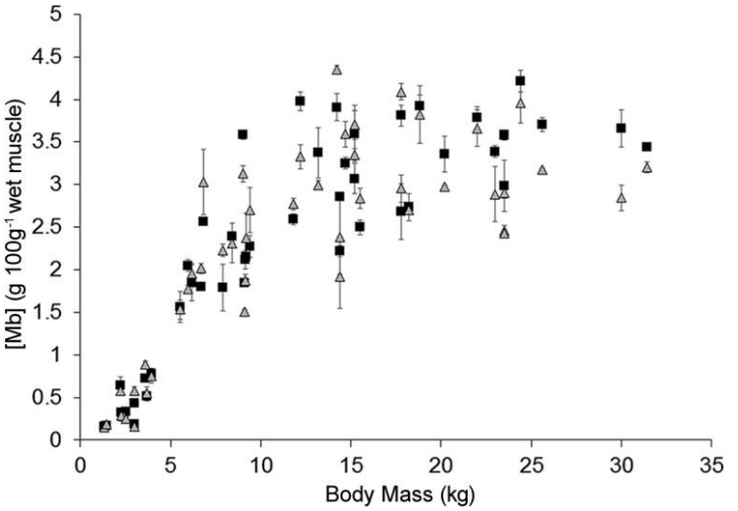

Figure 1. Muscle myoglobin concentration ([Mb]) of two major locomotor muscles, the *longissimus dorsi* (squares) and *gracilis* (triangles), in relation to body mass. Samples were run in triplicate, and error bars represent the standard deviation associated with each sample.

and used to obtain mean muscle [Mb] values (table 3), which were used in muscle O_2 store determinations. Average muscle [Mb] increased significantly with age ($F_{4,42} = 92.78, P < 0.001$). Large pups and juveniles had only 63% and 76% of adult [Mb], respectively (table 3). Our value for adult skeletal muscle [Mb] (3.32 ± 0.10 g 100 g^{-1} wet muscle) was slightly higher than the value published by Lenfant et al. (1970; 2.60 g 100 g^{-1} wet muscle) but comparable to the [Mb] reported by Castellini and Somero (1981; 3.13 g 100 g^{-1} wet muscle).

Muscle Mass

Total muscle mass increased linearly with total body mass following the equation

$$M_m = 341.72 \times M_b - 401.34$$

($n = 19$, $r^2 = 0.98$, $P < 0.001$), where M_m is muscle mass and M_b is total body mass. Percent muscle mass increased markedly across age classes ($F_{4,14} = 23.45, P < 0.001$), with juveniles showing slightly lower (30.19% ± 1.43%) but statistically comparable ($P = 0.65$) percent muscle mass to adults (table 3). Percent muscle mass increased 5.8% from small pups to large pups and another 3% from large pups to juveniles. Our measured percent muscle mass for adult sea otters (32.78% ± 2.08%) agrees closely with the value of 33% muscle mass typically assumed for adult marine mammals (Ponganis 2011).

Lung Mass and Lung Volume

Total lung mass increased as a function of total body mass (table 4; fig. 2*A*) following the equation

$$M_l = 30.82 \times M_b + 81.12$$

($n = 20$, $r^2 = 0.88$, $P < 0.001$), where M_l is lung mass in grams and M_b is total body mass in kilograms. In contrast, lung mass as a percentage of overall body mass decreased with increasing body mass (fig. 2*B*) following the equation

$$M_l = -0.00063 \times M_b + 0.05$$

($n = 20$, $r^2 = 0.35$, $P = 0.0038$), where M_l is lung mass as a proportion of body mass and M_b is total body mass in kilograms. Because lung mass did not make up the same proportion of body mass in all age classes (table 4), we did not assume lung volume could be estimated as a constant proportion of body mass based on published adult values. Instead, we used the

Table 3: Muscle parameters for sea otters across five age classes

Age class	*Longissumus dorsi* [Mb] (g 100 g^{-1} wet muscle)	*Gracilis* [Mb] (g 100 g^{-1} wet muscle)	Average [Mb] (g 100 g^{-1} wet muscle)	Muscle mass (% total body mass)
Neonate	.34 ± .05^{A} (9)	.34 ± .06^{A} (9)	.34 ± .06^{A} (9)	21.46 ± .57^{A} (5)
Small pup	1.03 ± .27^{A} (3)	1.06 ± .24^{A} (3)	1.04 ± .25^{A} (3)	21.35 ± .94^{AB} (4)
Large pup	2.10 ± .10^{B} (8)	2.10 ± .16^{B} (8)	2.10 ± .13^{B} (8)	27.19 ± 1.64^{BC} (3)
Juvenile	2.47 ± .30^{B} (5)	2.56 ± .22^{BC} (5)	2.52 ± .24^{B} (5)	30.19 ± .25^{C} (3)
Adult	3.43 ± .10^{C} (22)	3.21 ± .12^{C} (22)	3.32 ± .10^{C} (22)	32.78 ± 2.08^{C} (4)

Note. Data presented are means ± SE. Superscripts display connecting letters report from Tukey's post hoc pairwise comparisons. Sample size (n) is given in parentheses after each value. [Mb] = myoglobin concentration.

Table 4: Lung mass and lung volume parameters of sea otters across five age classes

Age class	Lung mass (g)	Lung mass (g kg^{-1})	DLV (mL kg^{-1})	TLC (mL kg^{-1})
Neonate (5)	115.54 ± 21.88^{A}	46.39 ± 3.36^{AB}	284.33 ± 20.60^{AB}	474.87 ± 34.41^{AB}
Small pup (2)	194.60 ± 44.60^{A}	52.99 ± 7.95^{A}	324.84 ± 48.72^{B}	542.52 ± 81.38^{A}
Large pup (4)	344.50 ± 87.33^{A}	45.28 ± 4.96^{AB}	277.57 ± 30.40^{AB}	463.57 ± 50.76^{AB}
Juvenile (3)	468.90 ± 45.44^{AB}	45.29 ± 1.74^{AB}	277.60 ± 10.64^{AB}	463.62 ± 17.77^{AB}
Adult (6)	763.25 ± 95.01^{B}	33.77 ± 2.50^{B}	206.99 ± 15.31^{B}	345.69 ± 25.58^{B}

Note. Data presented are means ± SE. Superscripts display connecting letters report from Tukey's post hoc pairwise comparisons. Sample size (n) for each age class is given in parentheses. DLV = diving lung volume; TLC = total lung capacity.

mean mass-specific adult lung mass (33.77 ± 2.50 g kg^{-1}) measured in this study, in combination with published values of adult sea otter TLC (345 mL kg^{-1}; Lenfant et al. 1970) and DLV (207 mL kg^{-1}; Ponganis 2011), to calculate lung volume as a function of lung mass. The resulting equations were

$$\text{TLC} = (10.22 \times M_l) \times M_b^{-1},$$

$$\text{DLV} = (6.13 \times M_l) \times M_b^{-1},$$

where M_l is lung mass in grams and M_b is body mass in kilograms. These equations were used in combination with measured lung masses of immature and adult sea otters to determine TLC and DLV across ontogeny (table 4). Mass-specific TLC decreased with age, ranging from 542.52 ± 81.38 mL kg^{-1} in small pups to 345.69 ± 25.58 mL kg^{-1} in adults ($F_{4,15} = 3.54$, $P = 0.032$). Mass-specific DLV also decreased with age, ranging from 324.84 ± 48.72 mL kg^{-1} in small pups down to 206.99 ± 15.31 mL kg^{-1} in adults ($F_{4,15} = 3.54$, $P = 0.032$).

Total Oxygen Storage Capacity and Calculated Aerobic Dive Limits

On a mass-specific basis, blood and muscle O_2 storage capacity increased significantly with age (blood O_2: $F_{4,148} = 76.13$,

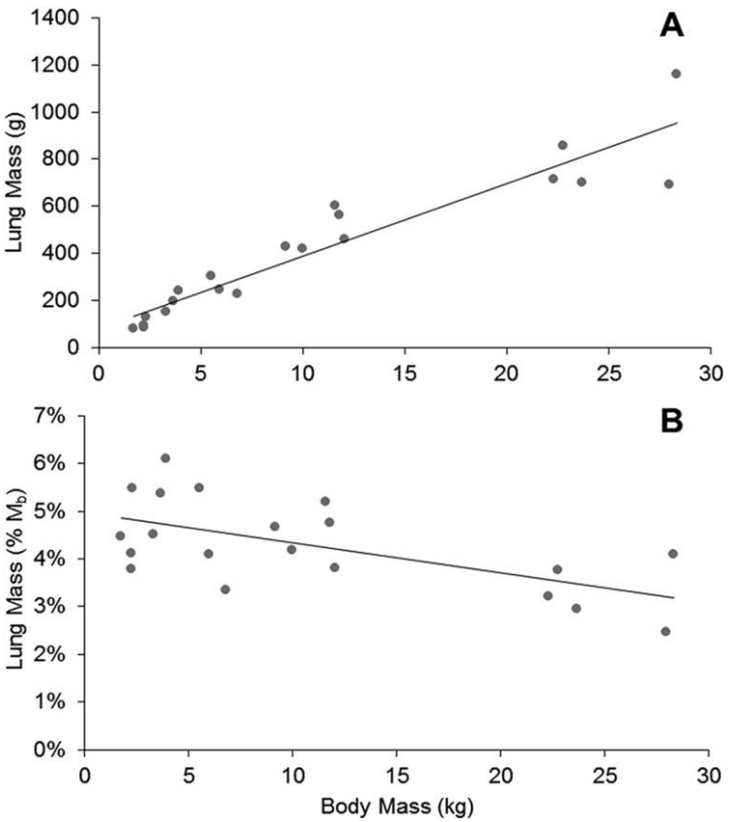

Figure 2. Total lung mass (A) and lung mass as a percentage of body mass (B) in relation to total body mass. Lines display results of least squares linear regression. Total lung mass increased as a function of total body mass following the equation $M_l = 30.82 \times M_b + 81.12$ ($n = 20$, $r^2 = 0.88$, $P < 0.001$), where M_l is lung mass in grams and M_b is total body mass in kilograms. Lung mass as a percentage of body mass decreased as a function of total body mass following the equation $M_l = -0.00063 \times M_b + 0.05$ ($n = 20$, $r^2 = 0.35$, $P = 0.0038$).

$P < 0.001$; muscle O_2: $F_{4,42} = 116.63$, $P < 0.001$; table 5). Neonates and small pups did not differ in blood ($P = 0.93$) or muscle ($P = 0.44$) O_2 storage capacity. Large pups had lower blood O_2 storage capacity than juveniles ($P < 0.001$) but comparable muscle O_2 storage capacity ($P = 0.19$). Adults had greater blood and muscle O_2 storage capacities than all other age classes ($P < 0.001$), including juveniles ($P = 0.0003$). In contrast, mass-specific lung O_2 stores decreased across age classes ($F_{4,15} = 3.54$, $P = 0.03$; table 5). Small pups displayed the highest mass-specific lung O_2 stores (DLV: 48.73 ± 7.31 mL kg^{-1}; TLC: 81.38 ± 12.21 mL kg^{-1}) and were the only age group that differed significantly from adults ($P = 0.047$).

Total body O_2 storage capacities (mL O_2 kg^{-1}) of neonates and small pups were 77%–89% and 86%–100% of adult values, respectively, due to very large mass-specific lung O_2 stores (table 5). Juveniles had mass-specific total body O_2 stores that were equal to adult values if we assumed DLV; this changed to juveniles having higher mass-specific total body O_2 stores than adults when we assumed TLC. Although young sea otters displayed comparable O_2 storage capacity to adults, heightened mass-specific metabolic rates associated with immaturity counterbalanced O_2 stores and resulted in reduced cADLs for younger age classes (table 5). The cADL of small pups was 60%–70% of adult cADL, while large pups displayed a cADL that was 82%–90% of adult cADL, depending on whether DLV or TLC was assumed. Juvenile sea otters had comparable cADL values to adult sea otters regardless of the lung volume assumed (table 5).

Discussion

Although sea otters are shallow benthic foragers, making 69% of their dives to depths of 20 m or less (Bodkin et al. 2004), they have large mass-specific total body O_2 stores in comparison to other marine divers (fig. 3). Regardless of the lung volume assumed while diving (TLC or DLV), adult sea otters have greater mass-specific total body O_2 stores than many cetacean, otariid, and diving bird species. However, the large lungs of sea otters and the dual functions they serve complicate comparisons of mass-specific body O_2 storage capacity and diving ability across taxa. For example, gray, ringed, harbor, harp, and ribbon seals all exhibit smaller mass-specific body O_2 stores than sea otters (fig. 3), but in general, average and maximum dive times and depths of phocid seals greatly exceed those of sea otters (Ponganis 2011). Similarly, when comparing penguin species, which have analogous buoyancy issues to sea otters, the deepest-diving emperor penguin has lower mass-specific total body O_2 stores than shallower-diving king and Adélie penguins (fig. 3). Despite lower mass-specific total body O_2 stores, emperor penguins have higher combined mass-specific blood and muscle O_2 stores (Kooyman and Ponganis 1998; Sato et al. 2002). Similar to many phocid and otariid species, the deep-diving emperor penguin is likely capable of such deep dives because of high blood and muscle O_2 capacity and minimization of air in the respiratory system while diving (Ponganis et al. 1997, 1999).

When comparing blood and muscle O_2 stores across taxa and excluding the lung O_2 store, sea otters still have a greater mass-specific O_2 storage capacity than many marine divers, including bottlenose dolphins, sub-Antarctic fur seals, northern fur seals, Steller sea lions, walruses, and a variety of diving bird species (fig. 3). Furthermore, despite being one of the smallest marine mammal species and a relatively shallow diver, sea otter hematological parameters are comparable to the highest values reported for deep-diving sea lions. Adult sea otter PV (84.07 ± 7.35 mL kg^{-1}), BV (173.47 ± 9.85 mL kg^{-1}), and Hct (56.49% ± 1.36%) are all remarkably similar to values described in Australian sea lions (PV = 83.7 ± 12.5 mL kg^{-1}; BV = 178.3 ± 30.9 mL kg^{-1}; Hct = 51.7% ± 0.5%; Fowler et al. 2007), New Zealand sea lions (PV = 75.1 ± 4.1 mL kg^{-1}; BV = 152.7 ± 6.7 mL kg^{-1}; Hct = 51.0% ± 2.0%; Costa et al. 1998), and Galapagos sea lions (PV = 78.4 ± 12.3 mL kg^{-1}; BV = 186.0 ± 30.4 mL kg^{-1}; Hct = 57.7% ± 2.6%; Villegas-Amtmann and Costa 2010). In addition, sea otter blood O_2 storage capacity falls within the lower range reported for phocid seals (fig. 3).

It has been suggested that the impressively high blood O_2 storage capacity of New Zealand sea lions relative to other otariids is an adaptation that enables this species to make relatively deep and long-duration benthic foraging dives (Gales and Mattlin 1997; Costa et al. 1998). A similar mechanism may underlie the high blood O_2 storage capacity reported here for sea otters. Sea otters appear to have maximized mammalian hematological values that would lead to increased diving capacity. Conversely, sea otter muscle O_2 storage capacity is comparable to the lowest values reported for otariids and well below the average observed in phocid seals and cetaceans (fig. 3). Rather than being solely an adaptation for diving in sea otters, increased BV and blood O_2 storage capacity may be necessary to compensate for large lungs and, thus, a large gas exchange surface area.

In addition to an exceptionally large lung capacity, the lean body composition of sea otters may also contribute to their seemingly high mass-specific O_2 storage capacity when compared to other marine mammals. Although sea otters exhibit large mass-specific body O_2 stores, high positive buoyancy makes diving energetically expensive for this species when compared to other marine mammals (Williams 1989). Consequently, their rate of O_2 use while diving is high (Yeates et al. 2007). Unlike other marine divers, which use neutral and negative buoyancy to reduce overall dive costs (Williams 1989; Crocker et al. 1997; Williams et al. 2000; Miller et al. 2004; Yeates et al. 2007; Aoki et al. 2011; Nousek-McGregor et al. 2014), sea otters rarely achieve neutral or negative buoyancy while diving (Cashman 2002). As a result, sea otters consume body O_2 stores at a substantially faster rate than other marine mammals during routine diving and foraging activities (Yeates et al. 2007).

Development of Oxygen Stores

Hematological development in sea otters closely resembles patterns observed in other marine (Horning and Trillmich

Table 5: Blood, muscle, lung, and total oxygen storage capacity, diving metabolic rate, and calculating aerobic dive limits (cADL) of sea otters across five age classes

Age class	Total blood O_2 store (mL kg^{-1})	Total muscle O_2 store (mL kg^{-1})	Lung O_2 store DLV/TLC (mL kg^{-1})	Total body O_2 storage capacity DLV/TLC (mL kg^{-1})	Diving metabolic rate (mL O_2 min^{-1} kg^{-1})	cADL DLV/TLC (min)
Neonate	$20.95 \pm .39^{A}$	$.95 \pm .16^{A}$	$42.65 \pm 3.09^{AB}/71.23 \pm 5.16^{AB}$	64.55/93.13	35.38	1.82/2.63
Small pup	$20.17 \pm .59^{A}$	2.93 ± 1.24^{A}	$48.73 \pm 7.31^{A}/81.38 \pm 12.21^{A}$	71.83/104.48	30.82	2.33/3.39
Large pup	$27.76 \pm .68^{B}$	7.93 ± 1.47^{B}	$41.63 \pm 4.56^{AB}/69.54 \pm 7.62^{AB}$	77.32/105.23	24.34	3.18/4.32
Juvenile	$31.67 \pm .70^{C}$	10.12 ± 2.19^{B}	$41.64 \pm 1.60^{AB}/69.54 \pm 2.67^{AB}$	83.43/111.33	23.05	3.62/4.83
Adult	$37.66 \pm .44^{D}$	14.67 ± 2.03^{C}	$31.05 \pm 2.30^{B}/51.85 \pm 3.84^{B}$	83.43/104.18	21.6	3.86/4.82

Note. Metabolic values adapted from Thometz et al. (2014) for immature age classes and Yeates et al. (2007) for adults. Data presented are means ± SE. Diving lung volume (DLV) and total lung capacity (TLC) indicate the assumed lung volume. Superscripts display connecting letters report from Tukey's post hoc pairwise comparisons.

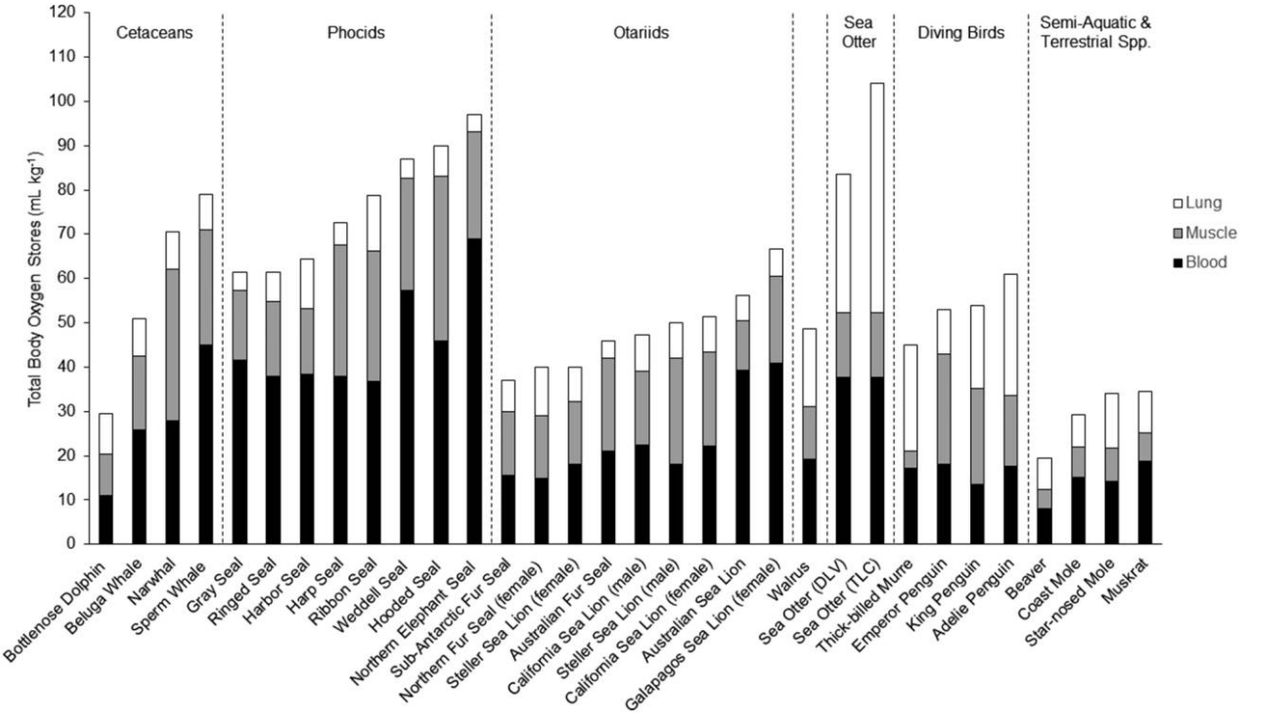

Figure 3. Mass-specific total body O_2 stores of adult sea otters (assuming both diving lung volume [DLV] and total lung capacity [TLC]) in comparison to a variety of marine, semiaquatic, and terrestrial divers. The height of each bar represents the total O_2 storage capacity for a given species, while each stacked bar indicates the proportional contribution of blood (black), muscle (gray), and lung (white) O_2 stores to total body O_2 storage capacity. Species compared include the bottlenose dolphin (Noren et al. 2001, 2002), beluga whale (Shaffer et al. 1997), narwhal (Williams et al. 2011), sperm whale (Kooyman and Ponganis 1998), gray seal (Noren et al. 2005), ringed seal (Lydersen et al. 1992), harbor seal (Burns et al. 2005), harp seal (Burns et al. 2007), ribbon seal (Lenfant et al. 1970), Weddell seal (Burns and Castellini 1996; Kooyman and Ponganis 1998), hooded seal (Burns et al. 2007), northern elephant seal (Kooyman and Ponganis 1998), sub-Antarctic fur seal (Verrier et al. 2011), northern fur seal (Shero et al. 2012), Steller sea lion (Richmond et al. 2006), Australian fur seal (Spence-Bailey et al. 2007), California sea lion (Weise and Costa 2007), Australian sea lion (Fowler et al. 2007), Galapagos sea lion (Villegas-Amtmann and Costa 2010), walrus (Lenfant et al. 1970), thick-billed murre (Croll et al. 1992), emperor penguin (Kooyman and Ponganis 1998), king and Adélie penguins (Sato et al. 2002), beaver (McKean and Carlton 1977), coast and star-nosed moles (McIntyre et al. 2002), and muskrat (MacArthur et al. 2001).

1997; Costa et al. 1998; Ponganis et al. 1999; Noren et al. 2002; Burns et al. 2005, 2007; Richmond et al. 2005; Clark et al. 2007; Fowler et al. 2007; Weise and Costa 2007; Verrier et al. 2011), semiaquatic (MacArthur et al. 2001), and terrestrial (Seal et al. 1967; McIntyre et al. 2002; Mohri et al. 2007) species. Of note, the BV of adult sea otters reported in this study is nearly two times higher than the previously published value (Lenfant et al. 1970). Ponganis et al. (1993) discussed how reduced cardiac output and splenic dilation due to inhaled anesthesia used by Lenfant at al. (1970) likely resulted in slow mixing of tagged erythrocytes and reduced Hct levels and thus provided an underestimate of actual BV. Our methods, which used a combination of fentanyl and midazolam rather than inhalational anesthesia, would have resulted in normal cardiac output (Yaster et al. 1990; Parworth et al. 1998) and constriction of the spleen due to mild hypercarbia (Rasmussen et al. 1978), which would have increased mixing of Evans blue dye in the circulatory system and maintained high Hct levels, resulting in more accurate PV and BV estimates. Indeed, our values are more comparable to recent estimates of PV and BV for northern sea otters (J. Burns, personal communication). For a relatively shallow diving marine mammal, sea otters have high mass-specific BVs, but the general pattern of PV and BV development in immature sea otters is similar to trends reported for other marine mammal species (Ponganis et al. 1993; Costa et al. 1998; Burns et al. 2005; Richmond et al. 2006; Fowler et al. 2007; Weise and Costa 2007).

Although adult marine divers are known to have muscle Mb concentrations that are 10–30 times higher than those exhibited by terrestrial mammals (Castellini and Somero 1981; Kooyman and Ponganis 1998; Ponganis 2011), there is a prolonged period of Mb development that has been documented for a wide variety of marine divers (Ponganis et al. 1999; Noren et al. 2001; Richmond et al. 2006; Kanatous et al. 2008). The development of muscle Mb is thought to be slow in comparison to Hb development and driven by an increase in diving and foraging activity (Ponganis et al. 1999; Noren et al. 2001; Geiseler et al. 2013). Noren et al. (2001) examined differences in Mb development between species that have a terrestrial postpartum phase (seals and penguins) and species that are born directly

into the marine environment (dolphins). They concluded that despite differences in the location of postpartum development (land or sea), the completion of Mb development occurred during the onset of independent foraging. In addition, Geiseler et al. (2013) identified increased muscular activity as the major driver of Mb development in the *longissimus dorsi* of immature hooded seals.

As sea otters are born directly into the marine environment and lack a terrestrial postpartum phase (Kenyon 1969; Payne and Jameson 1984), our results support the idea that the completion of muscle Mb development occurs during the initial phases of independent foraging, as muscle activity is increasing, regardless of whether an individual is born directly into the marine environment. Sea otters are born with minimal muscle Mb (10% of adult [Mb]), a result similar to what has been reported for neonate bottlenose dolphins (10% of adult [Mb]; Noren et al. 2001) and 1-mo-old Australian fur seals (9% of adult [Mb]; Spence-Bailey et al. 2007). Muscle Mb appears to develop most rapidly between the small pup and large pup age classes, doubling in a span of 3 mo. This period also corresponds to the initiation of diving and foraging (Payne and Jameson 1984) and, thus, increasing muscular activity in sea otters. As benthic foragers, sea otter pups' frequent diving attempts alongside mothers likely facilitate the development of muscle Mb throughout their dependency. Around weaning, sea otters exhibit mean [Mb] that are 63% and 76% of the adult value for large pups and juveniles, respectively. Therefore, it appears that sea otters require 1.5–2.5 yr to develop comparable muscle mass and [Mb] to adults. The result is limited muscle O_2 storage capacity for immature sea otters during their first year postweaning.

In general, the lung mass determined for adult sea otters (3.39% $\pm$ 0.25% body mass) in this study agrees with the value determined for northern sea otters (3.86% body mass) by Tarasoff and Kooyman (1973). Given that we found a decrease in relative lung mass with age and body size in sea otters (fig. 2) and that the previous study (Tarasoff and Kooyman 1973) included both immature and adult individuals, our mean is likely closer to the true value for adult sea otters. The decrease in the ratio of lung mass to body mass with age in sea otters may be partially related to ventilation and flotation requirements of their young. In contrast to sea otters, the ratio of lung mass to body mass has been found to be greater in adult harbor porpoises than in neonates (McLellan et al 2002).

Our estimations of immature sea otter lung volumes are based on the assumption of a constant lung volume-to-lung mass ratio throughout development. This assumption resulted in a decrease in the calculated mass-specific lung volume with age in sea otters, similar to declines observed in several terrestrial species (Avery and Cook 1961; Bartlett and Areson 1977; Nardell and Brody 1982; Gomes et al. 2001; Kornecki et al. 2005). For example, in a study that examined O_2 storage capacity in star-nosed moles, juveniles exhibited significantly greater mass-specific total lung capacities than adults (McIntyre et al. 2002). However, this decrease in mass-specific lung volume with age contrasts with the increase in mass-specific lung volume with age reported in both harp and hooded seals (Burns et al. 2007). These differences raise the question as to whether the relationship of lung mass to lung volume is constant throughout development.

There have been few examinations of this relationship in mammals (Avery and Cook 1961; Fisher and Mortola 1980; Kornecki et al. 2005), and although the ratio is generally greater in adults than in neonates, the ratio appears to plateau early in development in the few longitudinal studies available (Nardell and Brody 1982; Gomes et al. 2001). Marked differences in lung structure and maturity at birth between terrestrial and marine mammals (Engel 1953), a lack of data regarding marine mammal respiratory development, and the exceptional size of sea otter lungs in all age classes make applying patterns from the available published literature to sea otters difficult. We recognize that assuming a constant relationship between lung volume and lung mass may result in an overestimation of neonatal lung volume; however, we feel our assumption is valid, especially given that most of our age classes were beyond the neonatal stage.

We hypothesize that the large contribution of lung mass to body mass in immature sea otters is a consequence of the dual roles sea otter lungs must play. Neonates do not make diving attempts, are completely dependent on adult females, and rely on the positive buoyancy provided by both their lungs and natal pelage to float at the surface (Payne and Jameson 1984). Therefore, large lung capacities at birth benefit both neonates and females caring for neonates by increasing positive buoyancy, allowing neonates to float passively at the surface while a female is foraging at depth. In older age classes, large lungs are both beneficial and costly. Due to increased positive buoyancy, large lungs are a benefit at the surface as they allow individuals to float high out of the water, thus limiting heat loss to the marine environment while performing essential behaviors such as resting, feeding, and nursing. Large lungs are also beneficial at depth because they function as an important O_2 store (Kenyon 1969; Denison and Kooyman 1973; Tarasoff and Kooyman 1973). In contrast, large lungs become an energetic burden at depth for foraging sea otters because high positive buoyancy associated with large lung capacity increases the effort required while diving (Cashman 2002; Yeates et al. 2007). Therefore, although they closely resemble other marine mammal species in blood and muscle development, it appears that sea otters differ in the development of, dependence on, and utilization of the lung.

Total body O_2 storage capacity and the proportional contribution of each O_2 store to total body O_2 storage capacity changes markedly across developmental stages in sea otters (fig. 4). When assuming DLV, the relative contribution of lung O_2 storage capacity to total O_2 storage capacity decreases from 66% in neonates to 37% in adults (fig. 4). The proportional contribution of blood O_2 storage capacity increases from 32.5% in neonates to 45% in adults, which differs from patterns reported for a number of otariid (Richmond et al. 2006; Spence-Bailey et al. 2007; Weise and Costa 2007), phocid (Burns et al. 2005; Noren et al. 2005; Clark et al. 2007), and cetacean (Noren et al.

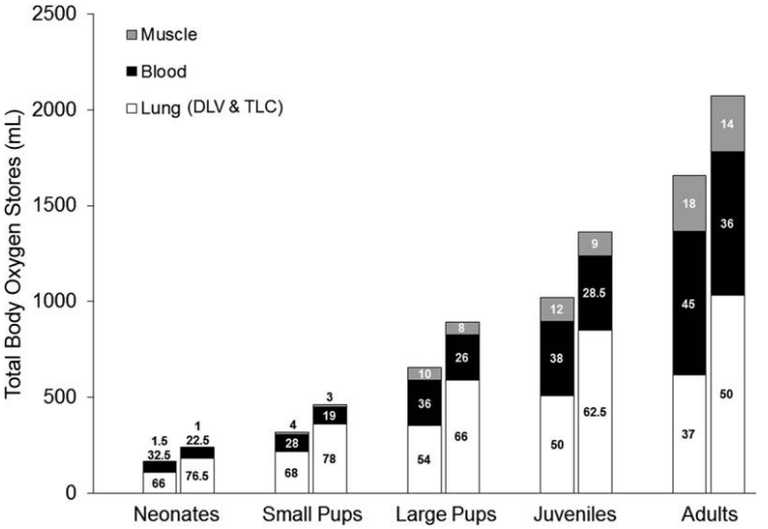

Figure 4. Total O_2 storage capacity of sea otters across age classes assuming both diving lung volume (DLV; left bar) and total lung capacity (TLC; right bar). The height of each bar represents total body O_2 storage capacity, while each stacked bar indicates the proportional contribution of lung (white), blood (black), and muscle (gray) O_2 stores to the total O_2 storage capacity of each age class. Numbers display the percentage of total body oxygen stores made up of lung, blood, and muscle oxygen stores for each age class assuming either DLV or TLC.

2002) species. In comparison, the proportional contribution of the muscle O_2 store increases 12-fold from neonates to adults, with increased diving behavior, foraging attempts, and muscle activity (Payne and Jameson 1984; Thometz et al. 2014) likely driving this physiological change throughout development (Noren et al. 2001; Geiseler et al. 2013).

Lung Volume and Buoyancy

We modeled the positive buoyant forces due to lung capacity and pelage across age classes (table 6) using equation (2) from Skrovan et al. (1999), $B_D = V_D g + B_B$, where B_D is the buoyant force (N) at depth, V_D is air volume (L) at depth, $\boldsymbol{g}$ is acceleration due to gravity (9.8 m s^{-2}), and B_B is the buoyant force of the body without air (N). B_B was not determined or estimated for sea otters in this study, and therefore, only total positive buoyancy due to air in the lungs and pelage was determined (not net buoyancy). The total amount of air in the lung (L), assuming both TLC and DLV, at each age class was taken from this study. The average amount of air trapped in sea otter pelage (L) for each age class was calculated assuming a relationship between surface area and body mass in sea otters of $SA = 0.111M_b^{0.535}$ (Cashman 2002), in which SA is surface area (m^2) and M_b is body mass (kg), and assuming that the amount of air trapped in pelage is 8.48 L m^{-2} (Fish et al. 2002). Equation (1) from Skrovan et al. (1999), $V_D = V_S/(1 + 0.1h)$, where V_D is volume at depth, V_S is volume at the surface, and h is depth, was used to determine changes in lung and pelage air volume with depth. Changes in buoyancy at depth due to loss of air from fur while diving were not accounted for.

Mass-specific positive buoyant forces of sea otters are not equivalent across age classes (table 6). Rather, mass-specific positive buoyant forces experienced by a neonate are twice those experienced by an adult sea otter. This is likely a conservative estimate as it did not account for the potential higher buoyancy of natal pelage in comparison to adult fur (Kenyon 1969; Payne and Jameson 1984). For a new pup not yet diving or foraging, such positive forces are solely beneficial, but for older dependent animals and juveniles, high positive buoyancy makes diving more challenging. A large pup diving to 10 m with TLC will experience a positive buoyant force of 3.98 N kg^{-1} in comparison to 2.84 N kg^{-1} in an adult sea otter (table 6). If that same animal dived with DLV instead of TLC, it would reduce its positive buoyancy by 23%. Although reducing positive buoyancy will inherently reduce lung O_2 stores while diving, this trade-off may be beneficial for younger animals with developing muscles (table 3; fig. 4) that may not be physically capable of diving against high positive buoyancy with the same efficiency as adults. Indeed, observations of known-age pups diving in the wild show that these young animals generally dive well within age-specific cADLs based on DLV (fig. 5).

Implications on Calculated Aerobic Dive Limit

Our results suggest that young sea otters in their first year postweaning have comparable oxygen stores to adults (table 5). However, high positive buoyancy from large lung volumes and added buoyancy from air in pelage may complicate the calculation of ADLs in this species. For example, the mass-specific positive buoyancy experienced by a juvenile sea otter diving to 5 m with TLC is 1.15 N kg^{-1} greater than that of an adult diving to 5 m with TLC. If instead a juvenile dives with a DLV, it will experience comparable mass-specific positive buoyant forces to an adult diving with TLC (table 6). Although juveniles have comparable total body O_2 stores to adults,

Table 6: Estimated mass-specific positive buoyant forces experienced by sea otters due to air in pelage and lungs

	Neonate (N kg^{-1})		Small pup (N kg^{-1})		Large pup (N kg^{-1})		Juvenile (N kg^{-1})		Adult (N kg^{-1})	
Depth (m)	TLC	DLV	TLC	DLV	TLC	DLV	TLC	DLV	TLC	DLV
0	10.62	8.76	9.14	7.32	7.95	6.13	7.42	5.60	5.68	4.33
5	7.08	5.84	6.09	4.88	5.30	4.09	4.94	3.73	3.79	2.88
10	5.31	4.38	4.57	3.66	3.98	3.07	3.71	2.80	2.84	2.16
20	3.54	2.92	3.05	2.44	2.65	2.04	2.47	1.87	1.89	1.44

Note. Author's calculations used data and equations from Skrovan et al. (1999), Cashman (2002), and Fish et al. (2002). Equations (1) and (2) from Skrovan et al. (1999) were used to determine total positive buoyancy due to pelage and lungs across a range of depths (0, 5, 10, and 20 m), not net buoyancy, as the density and corresponding negative buoyancy of sea otter tissue were not accounted for. Positive buoyant forces were calculated assuming both total lung capacity (TLC) and diving lung volume (DLV). The amount of air in lungs (L) was determined in this study. Average air trapped in pelage (L) was calculated assuming a relationship of $SA = 0.111M_b^{0.535}$ (Cashman 2002), in which SA is surface area (m^2) and M_b is body mass (kg), and assuming that the amount of air trapped in pelage is 8.48 L m^{-2} (Fish et al. 2002).

muscle [Mb] and muscle mass do not reach adult levels until after the first year postweaning (table 5), which will limit their ability to support aerobic dives against high positive buoyancy. We hypothesize that immature sea otters rarely make foraging dives with TLC and instead must rely on smaller diving lung volumes for routine dives. This will ultimately reduce positive buoyant forces (table 6), with the consequence of reducing total body O_2 stores while diving. Indeed, this assumption appears to be supported by dive times of dependent sea otters in the wild (fig. 5). Conversely, adult sea otters are likely capable of making routine dives with TLC due to increased musculature (table 3), a decreased ratio of lung volume to body mass (fig. 2; table 4), and a decreased ratio of surface area to body volume. As a result, mass-specific positive buoyancy is lower in adult sea otters, which reduces the energetic cost of diving.

Immature sea otters have high mass-specific metabolic rates relative to adult conspecifics (Thometz et al. 2014) that result in increased O_2 use while diving and reduced cADLs. Diving metabolic rates from Thometz et al. (2014) that were used for immature cADL calculations may be conservative estimates of the diving metabolic rates of immature wild sea otters, as animals were postabsorptive when measured. Sea otters are known to exhibit a significant increase in metabolism following feeding events due to the energetic costs associated with the digestion and absorption of prey (Costa and Kooyman 1984). Costa and Kooyman (1984) found that sea otter metabolic rates

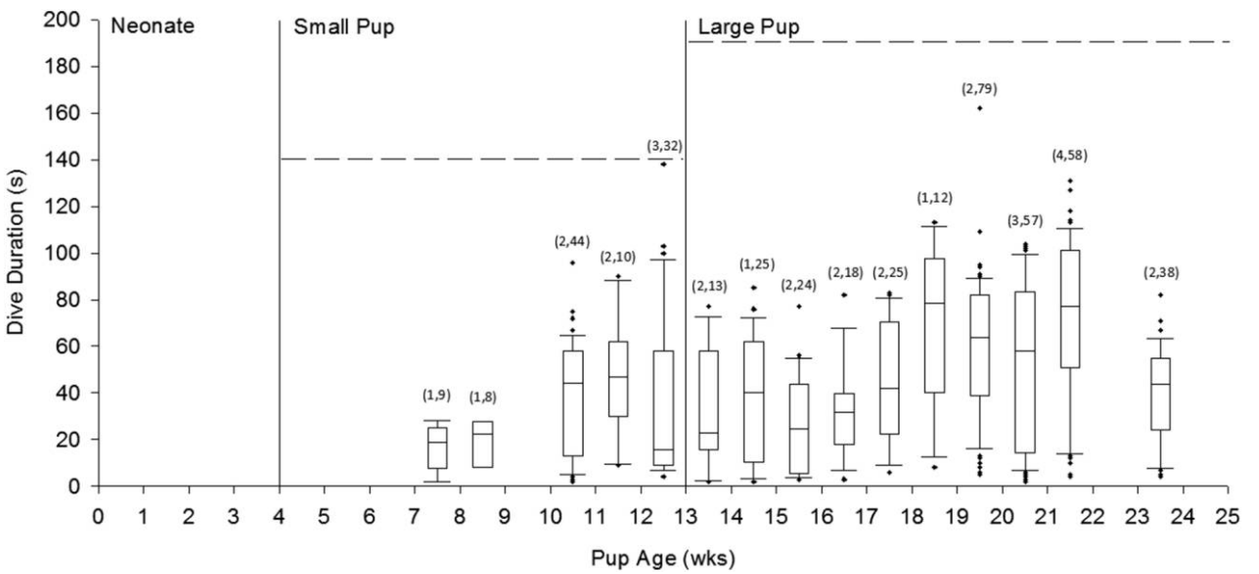

Figure 5. Boxplot of dive durations (s) for immature sea otters, collected from nine known-age dependent sea otters off the coast of Big Sur, California. Data from all individuals were pooled into weekly age groups. The middle horizontal line of each box denotes the median, boxes define quartiles, and vertical lines display the range, with the exception of outliers (diamonds). The number of individuals observed diving at a specific age (n) and the total number of dives observed for a specific age (x) are displayed above each box (n, x). Dependent age classes, as defined in this study, are identified at the top of the figure. Horizontal dashed lines denote the age-specific calculated aerobic dive limits (assuming diving lung volume) of small and large pups. All dive times of immature sea otters were collected opportunistically by researchers examining the behavior of adult female sea otters in Big Sur, California (USGS, unpublished data).

remain elevated 4–5 h following the ingestion of prey, with a peak in metabolism occurring at around 82 min. Because sea otters in the wild are likely seldom postabsorptive (Kenyon 1969; Riedman and Estes 1990; Tinker et al. 2008; Thometz et al. 2014), immature sea otter cADL values reported here may overestimate the cADL of postprandial immature sea otters in the wild. This was not a problem for the diving metabolic rate of adult sea otters from Yeates et al. (2007) as this was determined in animals that were postprandial. Thus, the range of cADL values reported here for adult sea otters is an accurate reflection of wild sea otter diving limits. In view of this, the difference between immature and adult sea otter cADLs reported here is a conservative estimate.

The cADL of neonates is approximately half the adult cADL (table 5). The cADLs of large pups and juveniles to adults are 82%–90% and 94%–100% of adult values, respectively. However, as suggested above, immature sea otters may need to dive with a smaller DLV rather than TLC to reduce mass-specific positive buoyancy while diving (table 6; fig. 5). In view of this, cADL calculations for immature age classes in sea otters should be made assuming DLV, while adult cADL should be calculated assuming TLC. Recalculating in this manner, large pups and juveniles have cADLs that are 3.18 and 3.62 min, respectively, and adult cADL is 4.82 min. Thus, juvenile cADL is only 75% of the adult value, and immature sea otters likely do not have comparable diving abilities to adults until after their first year postweaning. Because the majority of dives made by adult male sea otters are shorter than 2 min and dives by adult females are an average of 1 min (Bodkin et al. 2004), these cADL values appear reasonable.

Sato et al. (2002) showed that Adélie and king penguins modify the amount of air they take down on a given dive depending on dive depth. Bringing less air on shallower dives reduces the energetic cost of diving by mitigating positive buoyant forces. We propose that a similar behavioral mechanism may be used by diving sea otters. Given their relatively shallow mean foraging depths, short mean dive durations (Bodkin et al. 2004), and the positive buoyancy associated with large lung volumes and air trapped within dense pelage (table 6), we hypothesize that both adult and immature sea otters do not dive with the largest lung volume possible on the majority of their dives. Instead, similar to what has been observed for penguins (Sato et al. 2002; Wilson and Zimmer 2004), sea otters may use appropriate lung volumes for specific dive depths to reduce positive buoyancy on shallower or shorter dives when maximum lung volumes are not necessary.

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