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2014

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UNIVERSITY OF CALIFORNIA

SANTA CRUZ

**ONTOGENY OF ENERGETIC DEMAND AND DIVING ABILITY IN THE
SOUTHERN SEA OTTER (*ENHYDRA LUTRIS NEREIS*)
AND IMPLICATIONS ON DIVING AND FORAGING BEHAVIOR**

A dissertation in partial satisfaction
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

ECOLOGY AND EVOLUTIONARY BIOLOGY

by

Nicole Marie Thometz

June 2014

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Abstract

Ontogeny of Energetic Demand and Diving Ability in the Southern Sea Otter
(*Enhydra lutris nereis*) and Implications on Diving and Foraging Behavior

by

Nicole M. Thometz

The integration of physiological and behavioral studies can yield valuable information important to the conservation and management of imperiled species. In the following chapters, I examine a suite of physiological characteristics and behavioral attributes of southern sea otters (*Enhydra lutris nereis*) across a variety of life stages and discuss resulting population level consequences in this threatened species. In my first data chapter (Chapter 2), I use open-flow respirometry to determine age- and activity- specific metabolic rates of immature southern sea otters throughout ontogeny. These data are then combined with activity budgets of wild sea otters to determine the energetic cost of pup rearing for adult females. In Chapter 3, I determine age-specific oxygen storage capacity and diving abilities of sea otters from birth through adulthood. Finally, in Chapter 4, I examine the foraging behavior of sea otters off the coast of central California. I found that sea otter pups have elevated mass-specific metabolic rates in comparison to adult conspecifics, which are highest for molting pups and begin to approach adult levels around the average age of weaning (6 mo.). In addition, immature sea otters have limited blood and muscle oxygen stores throughout

dependency, which result in a limited capacity for diving and high dependence on adult females throughout lactation. The high energetic demands of pups result in elevated field metabolic rates (FMR) for lactating females. Female FMR is increased 17% by three weeks postpartum and continues to increase throughout lactation. By the average age of weaning female FMR is increased 96% above pre-pregnancy levels. These heightened energetic demands are reflected in the foraging behavior of wild sea otters. Adult females appear behaviorally constrained by dependent young during an already energetically costly life stage. Both physiological and behavioral data suggest that it takes sea otters approximately two years to develop comparable diving abilities to adults; however, individuals at this stage are likely inefficient foragers when compared to adults. Together these data indicate that late-lactation and the first years post-weaning are the most physiologically challenging life stages for sea otters and that these groups are likely the most sensitive to disturbance and resource limitation. The high energetic demands of dependent pups influence body condition, parental provisioning strategies, and life history decisions in adult females. In addition, high energy demands, physiological limitations, and behavioral naïveté make maintaining positive energy balance difficult for juvenile and sub-adult sea otters. Ultimately, these chapters provide novel information regarding age-specific energy demands, physiological abilities, and foraging behavior of southern sea otters across a variety of life stages, and elucidate mechanisms underlying current population level trends.

Acknowledgements

I had the privilege of working with many amazing people throughout my graduate career and this dissertation¹ would not have been possible without the help of the following people. First, I was very fortunate to have two amazing advisors, Dr. Terrie Williams and Dr. Jim Estes, who were wonderful mentors throughout this entire process. Thank you both for taking a chance on a young scientist right out of undergrad and for continually supporting and believing in me along the way. Terrie, thank you for giving me the opportunity to do fieldwork with you in the Antarctic. It was one of the best experiences of my graduate career. Jim, I appreciate all of the wonderful conversations we have had over the years. I always left your office thinking more critically and at the same time more broadly about my research. Lastly, thank you both for supporting my passion for teaching during my graduate career. You have both taught me so much and have been amazing role models as scientists and as educators.

Dr. Pete Raimondi and Dr. Paul Ponganis made up the remainder of my dissertation reading committee. Pete, thank you for always having your door open. I am so grateful for all of your help over the years. Paul I could not have asked for a

¹ The text of this dissertation includes a reprint of the following previously published material: Thometz, N.M., Tinker, M.T., Staedler, M.M., Mayer, K.A., and Williams, T.M. (2014). Energetic Demands of Immature Sea Otter from Birth to Weaning: Implications for maternal costs, reproductive behavior, and population level trends. *Journal of Experimental Biology* (In Press). The co-authors listed in this publication were directly involved with the development of my first data chapter (Chapter 2). For chapter 2, NMT and TMW conceived and designed the captive experiments; MMS and MTT conceived and designed the field experiments; NMT and KAM performed captive experiments; MMS conducted and oversaw field observations. NMT, MTT, and MMS analyzed the data. NMT wrote the manuscript with editorial advice provided by all authors.

better outside committee member. My thesis is much stronger because of your incredible insight. Dr. Tim Tinker was essentially an unofficial advisor and unofficial committee member, and was there every step of the way. Tim, thank you for everything over the years. Your guidance has been invaluable.

Ultimately, this work would not have been possible without strong collaborations between UC Santa Cruz, U.S. Geological Survey, Monterey Bay Aquarium, and the California Department of Fish and Wildlife. There are so many people to thank from these organizations. First, I need to especially thank Dr. Tim Tinker, Michelle Staedler, Dr. Mike Murray, and Karl Mayer without whom this work would not have been possible. Thank you to Dr. Jim Estes, Andrew Johnson, Marissa Young, Dr. Chris Harrold, Sandrine Hazan, Jessica Fujii, Joe Tomoleoni, Ben Weitzman, Colleen Young, Francesca Batac, Melissa Miller, Erin Dodd, Gena Bentall, Brandi Katz, Erin Lenihan, Max Tarjan, Holly MacCormick, Dr. Laird Henkel, Jack Ames, Dr. Dave Jessup, James Bodkin, George Esslinger, Mike Kenner, Mike Harris, Brian Hatfield, and all of the southern sea otter research alliance. I feel very lucky to have worked with such an amazing group of people. You all inspire me on a daily basis.

When it came to initial training, troubleshooting, and building equipment there are a handful of people I owe a huge thanks to. Dr. Laura Yeates took me under her wing and got me started in the Williams lab, I will be forever grateful. Thank you to Dr. Robin Dunkin, Dr. Luis Huckstadt, Dr. Shawn Noren, Randolph Skrovan, and Nate Moore for all of your help with different aspects of my research. Also, thank you to the administrative staff in our department, Susan Thuringer, Debbie Inferra, Kathy Durkin, and Maria Choy, who made sure everything ran smoothly on a daily basis. I had help

from many volunteers during my time as a graduate student and I am deeply grateful to each of them. I would like to extend a special thanks to Sierra Edhelson who was always enthusiastic and continually went above and beyond the call of duty.

Captive animal work was conducted under US Fish and Wildlife permits MA045447 and MA045447-2 to T.M. Williams. Wild animal work was conducted under US Fish and Wildlife permit MA672624 to M.T. Tinker. Sample collection was conducted under US Fish and Wildlife permits MA045447 and MA045447-2 to T.M. Williams and MA186914-0 to J. Packard. All animal care use protocols were evaluated and approved by Institutional Animal Care and Use Committees at both the University of California Santa Cruz and the Monterey Bay Aquarium. Funding for this project was provided by the U.S. Geological Survey Western Ecological Research Center, Office of Naval Research [N00014-08-1-1273 to T.M. Williams], Peter Read and the Otter Cove Foundation, Dr. Earl A. & Ethyl M. Myers Oceanographic and Marine Trust [to N.M. Thometz], Sooy Fellowship [to N.M. Thometz], National Geographic Young Explorers Grant [9047-11 to N.M. Thometz], the National Science Foundation [DDIG #1210591 to J.A. Estes & N.M. Thometz], and the UC Santa Cruz Chancellor's Dissertation Year Fellowship [to N.M. Thometz].

Thank you to everyone in the Tinker-Estes Lab and Williams Lab for being my friends, teachers, and collaborators over the years. To Cohort '08, I couldn't have asked for a better group of people to share this experience with. Susy Honig and Alexis Jackson, were my companions throughout this entire journey, thank you both for being the most wonderful friends. You two kept me sane during the rough patches. Susy, there is no question that grad school was exponentially better because of you.

My undergraduate mentor from the University of Portland, Dr. Becky Houck, remains to this day one of the most wonderful people I have ever known. Her passion for teaching was unmatched and enthusiasm for wildlife was infectious. I got to where I am today because of her. She left a lasting impression on anyone who ever knew her and I am so thankful for every minute we had together. I will always have a special place in my heart for octopuses.

Thank you to my family for being endlessly supportive of my dreams and ambitions. I am the person I am today because of you. Ashley, I could not ask for a better sister, you have always been my biggest cheerleader, and I appreciate that more than you know (flag!). Mom and Dad, thank you for your unwavering love and support in every aspect of my life. I am so lucky to have the amazing parents I do.

Joe, your love, support, and encouragement have meant the world to me. You are my best friend, teacher, dive buddy, and partner in adventure, travel, and life. Thank you for being right beside me through all the ups and downs.

CHAPTER 1

Introduction

Metabolic energy is critically important for fueling the essential life processes of all organisms. Although a large portion of the variation in energetic demand between species can be accounted for by differences in body mass, considerable variation still exists across taxa (Kleiber, 1947). For example, endotherms generally have higher metabolic requirements than ectotherms due to the maintenance and regulation of body temperature by internal means (Schmidt-Nielsen, 1997). In addition, differences in habitat, diet type, and foraging method can account for much of the variation observed between closely related species (Nagy, 2005). Even within a single species, energetic demands change over the course of a lifetime as individuals transition through different life stages. For mammalian species, immature individuals exhibit heightened mass-specific metabolic demands throughout ontogeny due to the energetic costs associated with growth and development (Brody, 1945). Similarly, adult males experience increased energetic demands when holding territories or competing for mates (Haley, 1994; Mitchell et al., 1976), and females must manage high energetic costs associated with reproduction and lactation (Millar, 1977; Oftedal et al., 1987).

In addition to variability in energetic demands, all individuals within a species do not possess the same physiological capacities for finding and obtaining available resources. For instance, body size can markedly influence the type of resources individuals are capable of exploiting as well as the areas in which they are able to forage (Kooyman, 1989; Scharf et al., 2000; Thompson et al., 1998). Immature individuals are often physiologically limited, behaviorally naïve, and inefficient foragers when compared to adults (Brandt, 1984; Horning and Trillmich, 1999; Jeglinski et al., 2012; Zimmer et al., 2011). Furthermore, foraging opportunities may be restricted for individuals caring for dependent young (Barclay, 1989; Breed et al., 2006; Page et al., 2005). Thus, there are differences in behavioral flexibility, which influence the capacity of individuals to respond to changes within their environment. Ultimately, such differences in energetic demand, foraging capacity, and behavioral flexibility may be reflected in reproductive rates and mortality trends at the population level (Brown et al., 2004; Horning and Trillmich, 1999; Trillmich and Dellinger, 1991).

When trying to understand population level dynamics it is important to have detailed age- and stage-specific physiological and behavioral data. Understanding life histories of threatened species can allow decision makers to focus limited conservation dollars on protecting the most vulnerable life stages in order to have the greatest impact on species recovery (Congdon et al., 1994; Crouse et al., 1987; de Kroon et al., 2000). Therefore, studying the underlying physiological and behavioral constraints of individuals over a lifetime is key to understanding not only how a species functions within its environment, but also, how sensitive or resilient specific life stages may be to past, present, or future disturbances. Unfortunately, for many threatened and

endangered species, obtaining physiological or behavioral data can be extremely difficult, but none-the-less essential for effective protection and management (Cooke et al., 2013; Wikelski and Cooke, 2006). In my dissertation I build from these concepts and examine the physiology and behavior of a threatened marine mammal species across a variety of life stages.

Sea otters were hunted to near extinction during the fur trade of the 18th and 19th centuries; however a few remnant populations survived commercial exploitation (Kenyon, 1969; Riedman and Estes, 1990). Population recovery of the southern sea otter has proceeded relatively slowly (< 5% per year) in comparison to northern sea otter populations in Washington, British Columbia, and Alaska which have all recovered at rates of 17-20% per year (Estes, 1990). Elevated rates of mortality have been implicated in limiting the recovery of this threatened species (Estes et al., 2003; Tinker et al., 2006) and throughout the central portion of the current range resource limitation is a major issue (Tinker et al. 2008). The highest rates of mortality occur in postpartum pups and juveniles during their first year post-weaning (Estes et al., 2003; Tinker et al., 2006). In addition, mortality of prime-age females has increased over the past 20 years and has had a strong influence on the population trajectory (Tinker et al. 2006). Therefore, I examined the energetic requirements, diving abilities, and foraging behavior of southern sea otters from birth through adulthood and assessed the potential consequences of resource limitation for specific life stages. Ultimately, I conducted this work in an attempt to discern underlying mechanisms and gain a better understanding of trends occurring at the population level.

In **Chapter 2**, I use a combination of laboratory and field based techniques to determine age-specific energetic demands of dependent sea otter pups throughout ontogeny and quantify the energetic cost of pup rearing for adult females. I show that immature sea otters have elevated mass-specific metabolic rates in comparison to adult conspecifics and that these additional energetic demands appear to influence body condition, parental provisioning strategies, and life history decisions of adult females as well as play a major role in influencing current trends in mortality.

In **Chapter 3**, I quantify the oxygen storage capacity and diving ability of sea otters from birth through adulthood. I begin by examining the development of blood, muscle, lung, and total body oxygen stores in this small marine mammal. I then use age-specific total body oxygen stores and metabolic rates (Chapter 2) to calculate aerobic dive limits for different sea otter age classes. Sea otters have large lung capacities and I discuss how this influences their development of diving ability and results in interesting differences between sea otters and other marine mammal species. Finally, I estimate the age at which immature sea otters reach comparable diving abilities to adult conspecifics and discuss how this may impact juveniles during their first year post-weaning.

In **Chapter 4**, I examine the foraging behavior of southern sea otters off the coast of central California, keeping in mind the differences in energetic demand (Chapter 2) and physiological ability (Chapter 3) among demographic groups. I use archival time-depth recorders (TDRs) to elucidate detailed foraging dive behavior of individual sea otters and examine variation in behavior due to sex, reproductive stage, and age. Ultimately, I identify the demographic groups which exhibit the highest

foraging effort, most limited behavioral flexibility, and are likely the most vulnerable to further reductions in prey distribution and abundance.

Together, chapters 2, 3, and 4 identify many mechanisms driving sea otter behavior, life history strategies, and trends in mortality. In the final chapter (**Chapter 5**) I synthesize the data presented in all three data chapters and discuss how this dissertation provides further support that physiological and behavioral research can be essential in understanding population level trends and highly useful in effective conservation and management of threatened and endangered species.

CHAPTER 2

Energetic Demands of Immature Sea Otters from Birth to Weaning: Implications for maternal costs, reproductive behavior, and population level trends

Abstract

Sea otters (*Enhydra lutris*) have the highest mass-specific metabolic rate of any marine mammal, which is superimposed on the inherently high costs of reproduction and lactation in adult females. These combined energetic demands have been implicated in the poor body condition and increased mortality of female sea otters nearing the end of lactation along the central California coast. However, the cost of lactation is unknown and currently cannot be directly measured for this marine species in the wild. Here, we quantified the energetic demands of immature sea otters across five developmental stages as a means of assessing the underlying energetic challenges associated with pup rearing that may contribute to poor maternal condition. Activity-specific metabolic rates, daily activity budgets, and field metabolic rates (FMR) were determined for each developmental stage. Mean FMR of pre-molt pups was 2.29 ± 0.81 MJ day⁻¹ and increased to 6.16 ± 2.46 MJ day⁻¹ and 7.41 ± 3.17 MJ day⁻¹ in post-molt pups and dependent immature animals, respectively. Consequently, daily energy demands of adult females increase 17% by three weeks postpartum and continue

increasing to approximately 96% above pre-pregnancy levels by the average age of weaning. Our results suggest that the energetics of pup rearing superimposed on small body size, marine living, and limited on-board energetic reserves conspire to make female sea otters exceptionally vulnerable to energetic shortfalls. By controlling individual fitness, maternal behavior, and pup provisioning strategies, this underlying metabolic challenge appears to be a major factor influencing current population trends in southern sea otters (*Enhydra lutris nereis*).

Introduction

Across mammalian species, the most energetically taxing life history period for females is lactation, which can require as much as three to four times the energy intake needed during non-lactating periods (Millar, 1977; Williams et al., 2007). When nursing, the metabolic demands of dependent young are inextricably linked to their mother, who must provide enough energy to support the needs of her young while managing the metabolic cost of milk production and her own energetic requirements. A variety of factors including duration of lactation, metabolic demands of offspring, energetic reserves of the female, and the availability of prey resources will determine the success of the female in accomplishing this task (Boness and Bowen, 1996; Bowen et al., 2001; Burns et al., 2004; Georges and Guinet, 2000; Verrier et al., 2011a). Ultimately, these factors will impact daily behavioral responses, the level of energy invested in reproduction, and the overall fitness of adult females and offspring (Andersen et al., 2000; Georges and Guinet, 2000; Millar, 1975; Oftedal et al., 1987; Pontier et al., 1993).

As the smallest marine mammal species, sea otters (*Enhydra lutris* - Linnaeus 1758) face extraordinary energetic challenges associated with aquatic living (Riedman and Estes, 1990). High surface area to volume ratios result in elevated heat loss to the environment (Dejours, 1987) which sea otters counter-balance with dense fur insulation and increased metabolism (Costa and Kooyman, 1984; Kenyon, 1969; Morrison et al., 1974; Williams, 1989; Yeates et al., 2007). Consequently, sea otters have one of the highest known mass-specific metabolic rates of any marine mammal and represent an extreme in mammalian metabolism (Costa and Williams, 1999; Morrison et al., 1974; Williams, 1989; Yeates et al., 2007). As a result of these elevated metabolic demands, adult sea otters consume 20-25% of their body mass in food per day (Costa and Kooyman, 1982; Kenyon, 1969; Morrison et al., 1974) and spend an average of 20-50% of the day foraging depending on habitat, sex, reproductive status, and per-capita prey availability (Estes et al., 1986; Ralls and Siniff, 1990; Staedler, 2011; Tinker et al., 2008; Yeates et al., 2007). For adult females, energetic costs associated with reproduction must be added onto these inherently high metabolic demands.

Typically, female sea otters give birth to a single pup once a year with lactation lasting an average of six months (Jameson and Johnson, 1993). For other marine mammal species, lactation may be as short as four days as observed in hooded seals, or last several years as reported for some toothed whales [i.e. bottlenose dolphin (*Tursiops sp*), sperm whale (*Physeter macrocephalus*), and beluga whale (*Delphinapterus leucas*)] (Boness et al., 2002). Provisioning strategies in marine mammals can range from strict capital breeding to strict income breeding (Jonsson, 1997). Capital breeders,

such as phocid seals and mysticete whales, acquire sufficient energetic stores prior to giving birth and typically fast throughout lactation (Boness et al., 2002; Lockyer, 1984; Lockyer, 2007; Stearns, 1992; Trillmich, 1996). In contrast, income strategists, such as otariid seals and many odontocete whales, have minimal energetic reserves at parturition and must forage throughout lactation (Boness and Bowen, 1996; Huang et al., 2009; Perrin and Reilly, 1984; Stearns, 1992).

Along this continuum, sea otters represent extreme income strategists among marine mammals. High metabolic demands likely prevent female sea otters from building up large fat reserves prior to giving birth. This species also lacks a blubber layer that is typically utilized by many marine mammals for both insulation and energy storage (Kenyon, 1969; Williams and Worthy, 2002). Lacking this reserve, female sea otters must spend a large proportion of time foraging throughout lactation (Gelatt et al., 2002; Staedler, 2011) to continually support the increasing metabolic demands of a growing pup. The cost is likely considerable and despite foraging throughout lactation, female sea otters are reported to lose body mass over the course of pup dependency (Monson et al., 2000). However, to date neither the energetic demands of immature sea otters nor the cost of lactation for adult females have been quantified.

The extreme metabolic demands of female sea otters provide a unique opportunity to examine mammalian limits to maternal provisioning in a wild carnivore. Because lactation occurs at sea it is currently not feasible to measure sea otter maternal investment directly via milk transfer. Instead, we used a combination of laboratory methods and field-based observations to quantify the energetic requirements of sea otters throughout ontogeny. These data were used in combination with previously

published values for adult female metabolic rates and activity budgets to assess the underlying energetic challenges associated with pup rearing that may contribute to low maternal physiological condition near the end of lactation. Specifically, we quantified activity-specific metabolic rates, daily activity-budgets, and field metabolic rates (FMR) of southern sea otters (*Enhydra lutris nereis*) in five developmental stages (Table 2.1) from birth through weaning. From these data, we estimated both daily and cumulative energetic demands superimposed on adult females rearing dependent young, and assessed the physiological and ecological implications of age-specific energy demands on both immature and adult female sea otters.

Methods

Experimental Design

Resting and active metabolic rates were determined for southern sea otters at the Monterey Bay Aquarium (Monterey, CA) through the Sea Otter Research and Conservation (SORAC) program from February 2009 to March 2011. Five developmental stages, defined in relation to molting period and foraging proficiency (Payne and Jameson, 1984), were examined (Table 2.1). Observations of routine daily behaviors of wild pup and juvenile sea otters were collected off the coast of central California using radio telemetry and high powered spotting scopes (Questar Inc., New Hope, PA, USA) between January 2000 and October 2008 for independent juvenile animals, and March 2007 and December 2008 for dependent animals. Combining activity-specific metabolic rates measured in captivity with empirically-derived

activity budgets of wild sea otters, we calculated FMR for each of the five developmental stages.

Laboratory studies

Animals

Seven wild born sea otter pups (1 male; 6 females) were measured during metabolic trials. These individuals stranded in central California as pups and were brought to the Monterey Bay Aquarium to be reared in captivity within the SORAC program and released back into the wild upon maturity. Therefore, individuals were measured during the time they were housed at the Monterey Bay Aquarium and no individuals were specifically captured for use in this study. Longitudinal measurements were made for individuals ranging in age from seven days to 248 days. Subjects were weighed before each trial (Hygeia EBSL-20, Guang-Dong, China, or SR Scales SRV945, Tonawanda, NY). Individuals were housed in salt water pools (800 gallons) at the Monterey Bay Aquarium, which were maintained with seasonal, ambient salt water ($T_{\text{water}} = 10^{\circ}\text{C} - 14^{\circ}\text{C}$). Nursing pups were fed a formula consisting of Esibilac powder (PetAg Inc., Hampshire, IL), water, and surf clam (*Spisula solidissima*). Pups received solid food at 4 weeks and formula was completely deleted from the diet by 8 weeks. Solid diets consisted of whole or partial prey items, including white shrimp (*Litopenaeus setiferus*), surf clam, squid (*Loligo opalescens*), manila clams (*Venerupis philippinarum*), mussels (*Mytilus edulis*), and crabs (*Cancer productus* and *Cancer antennarius*).

Respirometry and Metabolic Demand

Age-specific energetic demands associated with various behaviors were determined by measuring oxygen consumption of pups throughout development following the methods of Williams et al. (2004) as adapted by Yeates et al. (2007). Pups were fasted an average of seven hours prior to metabolic trials to ensure individuals were in a post-absorptive state (Costa and Kooyman, 1984). Individuals were placed beneath one of two clear acrylic domes to accommodate metabolic measurements in-air and in-water. A haul-out dome (0.8 m L x 0.6 m W x 0.3 m H) was secured onto a solid wooden base for in-air trials, while a separate dome was attached to a PVC piping frame which allowed it to float on the water surface (1.1 m L x 0.8 m W x 0.3 m H) for in-water trials. Metabolic trials were conducted indoors under seasonal environmental conditions ($T_{\text{air}} = 14^{\circ}\text{C} - 22^{\circ}\text{C}$; $T_{\text{water}} = 10^{\circ}\text{C} - 14^{\circ}\text{C}$) that were representative of conditions wild sea otters experience along the central coast of California. Observers monitored trials by a closed circuit video camera and recorded the behavior of animals. Oxygen consumption was determined for three different activity levels: (1) resting, (2) moderately active, and (3) highly active. An animal was considered highly active when rapidly moving around the dome during in-air trials or when diving, swimming submerged, or rapidly swimming at the surface during in-water trials. Trial length ranged from 30 minutes to 120 minutes during which the animal was allowed to rest or move freely. Oxygen consumption was measured continuously. Specific behaviors and associated oxygen consumption rates were considered at a steady state if maintained for a minimum of five minutes.

The rate of oxygen consumption was determined using an open flow respirometry system designed for aquatic mammals (Williams et al., 2004). Air was pulled through the metabolic dome at 30-80 L min⁻¹ by a mass flow controller (Flow kit 500H, Sable Systems, Henderson, NV, USA). The exact flow of air through the dome was dependent on animal mass and was recorded continuously during each trial. Sub-samples of dome exhaust were dried (Drierite, W. A. Hammond Drierite, Xenia, OH, USA), scrubbed of CO₂ (Baralyme, Chemetron Medical Division, Allied Healthcare Products, St Louis, MO, USA), and dried again, before entering an oxygen analyzer (model FC1-B, Sable Systems, Henderson, NV, USA). The oxygen content of the dome was maintained above 20.10% by modifying the flow rate to avoid hypoxic conditions. Oxygen content of the dome exhaust was logged every 1.0 second on a laptop computer. Flow rates were corrected to standard temperature and pressure (STPD) and V O₂ determined using standard methods (Withers, 1977). V O₂ for specific behaviors was calculated by dividing the amount of oxygen used while performing a given behavior by its respective duration (DATACAN V Software, Sable Systems International, Henderson, NV, USA). Before each trial the oxygen analysis system was calibrated with dry ambient air (20.94% O₂). The system was calibrated once a week with nitrogen gas according to Fedak et al. (1981).

Field studies

Animals

Twenty-six free-ranging sea otters, including dependent young (1-180 days old; n=12) and juvenile individuals (6 months-2.5 years old; n=14), were observed to determine daily activity budgets. Adult females and juvenile sea otters were captured,

tagged, and implanted with intra-abdominal VHF radio transmitters (7.6 cm x 10.2 cm x 2.5 cm, 120 g ; Advanced Telemetry Systems Inc., Isanti, MN, USA) along the Monterey Peninsula and San Simeon, CA. Dependent young were not implanted with radio transmitters and instead were located using the VHF signal of their mother. Individuals were captured and surgically implanted following standardized procedures (Ames et al., 1986; Monson et al., 2001; Williams and Siniff, 1983). Two colored plastic flipper tags (Temple Tags, Temple, TX, USA) were attached in the webbing of each hind flipper for visual identification in the field (Ames et al., 1986). Dependent and juvenile sea otters were observed along the central coast of California and behavioral data were collected by direct observation utilizing a 50 x 80 field spotting scope (Questar Inc., New Hope, PA, USA) from shore. By relocating tagged females at frequent intervals, pup date of birth could be determined \pm 1 day and behavioral data from immature animals could be obtained throughout dependency.

Activity Budgets

Daily activity budgets of dependent sea otters were collected from birth until weaning in congruence with an associated study (Staedler, 2011). Instantaneous behaviors (Altmann, 1974) of pups were recorded during daylight hours, twice weekly for six hour intervals when pups were 0-10 weeks of age and once weekly thereafter until weaning. Six hour observation intervals took place equally throughout different times of day to cover all daylight hours. Behavior was recorded at 10 minute intervals with instantaneous behaviors classified into one of 10 categories: resting on mother or in water, nursing on mother or in water (in which any part of the pup was in water), foraging, being groomed by mother, passive grooming self (slowly rubbing fur while

on back), active grooming self (vigorously rubbing fur and somersaulting), swimming, and other (e.g. interacting with conspecifics). Behavior at night could not be directly observed for dependent pups and instead the proportion of behaviors during night time hours was assumed to be similar to activity observed during the day. This was a valid assumption because sea otters are cathemeral organisms and rest, forage, groom and perform various other behaviors at regular intervals throughout a 24 hour period (Ralls and Siniff, 1990).

For juvenile animals, radio-tagged otters were located opportunistically over the study period for collection of 24-hour activity sessions, with one to five sessions recorded for each individual. Behavior was measured using a combination of direct observation and radio telemetry. The temporal pattern of the VHF signal was used to determine behavior following standardized methods (Loughlin, 1980a). For example, while an otter was resting at the surface the VHF signal was a constant pulse, while foraging the signal disappeared when the otter was submerged, and during behaviors such as active grooming and swimming the signal became more variable. The instantaneous behavior of a focal animal was recorded at 10 minute intervals over the 24-hour recording session.

Daily activity budgets for dependent pups and juvenile sea otters were then calculated as the proportion of all records associated with each behavior. Any period when behavior was recorded as “unidentified” due to poor transmitter signal quality were removed prior to analysis. Thus, the reported activity budgets represent proportions of known activities. To reduce the potential for bias, we limit our analyses to activity sessions where <10% of the intervals were unidentified.

Analysis

Field Metabolic Rates

FMR values for all five developmental stages were calculated by combining empirically-derived, age- and behavior-specific metabolic costs with activity budgets of wild sea otters. Each behavior observed in the wild was associated with an activity level (resting, moderately active and highly active) for which $\dot{V}O_2$ had been measured in either air or water. For example, foraging or swimming attempts by pre-molt pups were considered moderately active behaviors, whereas in older age groups, foraging and swimming behaviors were classified as highly active. Passive grooming was considered a moderately active behavior for all age groups and nursing was associated with resting metabolic rates (RMR). For the majority of behaviors, in-water $\dot{V}O_2$ values were used. However, for any behavior during which a pup would be completely onboard an adult female (e.g. resting on mother), an in-air metabolic value was used. Active grooming could not be empirically measured during respirometry trials; therefore, 2.2 times RMR in-water was assigned for active grooming behaviors for all age classes based on previously published values for sea otters (Williams, 1989; Yeates et al., 2007). $\dot{V}O_2$ ($\text{ml O}_2 \text{ kg}^{-1} \text{ min}^{-1}$) values for each age class and behavior were converted to energetic demand (MJ day^{-1}) using a factor of $20.08 \text{ kJ l O}_2^{-1}$ (Schmidt-Nielsen, 1997). The amount of energy required for each behavior was multiplied by the percentage of time an individual in a specific age group spent performing that behavior each day. FMR values were determined by summing the energetic costs of each behavior over a 24 hour period. The means, variance and standard deviations of these estimates were determined using standard procedures for calculating point estimates of

independent variables (Quinn and Keough, 2003). Daily energetic costs calculated from detailed activity budgets were collapsed into general behavioral categories (resting, nursing, foraging, grooming, swimming, and other) for final FMR calculations. Average daily energetic demands of adult female sea otters with and without young were calculated as described above, utilizing previously published activity budgets (Staedler, 2011) and behavior-specific metabolic rates (Williams, 1989) of adult female southern sea otters and assuming an average female body mass of 19.89 kg (Williams, 1989).

Statistical Analyses

We evaluated the functional relationship between age and mass-specific metabolic rates by fitting linear and non-linear functions using maximum likelihood methods. We created separate plots of mass-specific O_2 consumption ($V \cdot O_2$) versus pup age (in days) for each activity level (resting, moderately active and highly active behavior). We then evaluated 3 alternative fitted trend lines for each data set: 1) a simple linear regression model, $V \cdot O_2 = a + b \times \text{age}$; 2) an exponential model, $V \cdot O_2 = a \times e^{b \times \text{age}}$; and 3) a piecewise linear regression (Toms and Lesperance, 2003), $V \cdot O_2 = a + b \times \text{age} + c \times f(\text{age} - K)$, where $f(x) = x$ for $x \geq 0$ and $f(x) = 0$ for $x < 0$, and K is a functional breakpoint or “knot value” (note that the piecewise regression model can be re-expressed as two simple polynomial functions, one for ages $\leq K$ and another for ages $> K$). We set K equal to 180 days, as this is the average age of weaning when dependent pups transition to the independent juvenile stage (Riedman et al 1994). We present results for the model having the highest coefficient of determination (R^2) after adjusting for the number of fitted parameters, as measured by the degrees-of-freedom-adjusted

R^2 , or \hat{R}^2 (Ronchetti, 1985). Due to sample size limitations, for this analysis we combined data from both sexes and from in-air and in-water measurements.

Linear mixed-effects models were used to compare mass-specific metabolic rates among developmental stages and activity level, while accounting for non-independence of longitudinal data, random-effects due to differences between individual animals, and unequal sample sizes. In-air and in-water metabolic data were analyzed separately owing to differences in the number of developmental stages sampled. For this analysis, developmental stage and activity level were treated as fixed effects and individual differences were treated as random effects. Bonferroni post hoc tests were used to identify pairwise differences within fixed effects. The effect of sex could not be tested because of limited sample sizes, and thus data for both sexes were pooled. Linear mixed-effects models were performed using SYSTAT 13 (Systat Software Inc. Richmond, CA, USA). Results are presented as means \pm 1 s.d. and were considered significant at $P \leq 0.05$.

Results

Metabolic Rates

Mass-specific metabolic rates of sea otters decreased as a function of age, irrespective of activity state (Fig. 2.1; Tables 2.2-2.3). For resting behavior, the relationship between rate of oxygen consumption ($\dot{V}O_2$) and age of pups was best described by a simple linear regression ($\hat{R}^2=0.552$; Fig. 2.1A):

$$\dot{V}O_2 = 25.85 - 0.062 \times \text{age}. \quad (1)$$

For moderately active behavior, the relationship between $\dot{V}O_2$ and age was best described by a piecewise linear regression:

$$\dot{V}O_2 = 35.08 - 0.089 \times \text{age} , \quad (2)$$

$$\dot{V}O_2 = 19.06 - 0.006 \times (\text{age} - 180) , \quad (3)$$

for animals ≤ 180 and >180 days of age, respectively ($\hat{R}^2=0.633$; Fig. 2.1B). For highly active behavior, the relationship between $\dot{V}O_2$ and age was best described by a piecewise linear regression:

$$\dot{V}O_2 = 40.51 - 0.098 \times \text{age} \quad (4)$$

$$\dot{V}O_2 = 22.87 - 0.004 \times (\text{age} - 180) \quad (5)$$

for animals ≤ 180 and >180 days of age, respectively ($\hat{R}^2=0.493$; Fig. 2.1C). The functional relationships for moderately active behavior and highly active behavior indicate that mass-specific metabolic needs of immature sea otters reach an asymptote around the time of weaning, with average values ($\dot{V}O_2 \sim 19$ and 22 for moderate and highly active behavior, respectively) that are consistent with those measured for adult sea otters (Williams, 1989; Yeates et al., 2007). It is likely that RMR also reaches an asymptotic value shortly after weaning, and the lack of statistical support for a breakpoint in this case probably reflects sample size limitations (only 3 data points were recorded for resting animals >200 days of age).

In-air metabolic rates were measured for the three youngest developmental stages and across activity levels (Table 2.2). Developmental stage ($F_{2,35} = 19.90$, $P < 0.001$) and activity level ($F_{2,35} = 24.04$, $P < 0.001$) both had a significant effect on mass-specific metabolic rates. The RMR of pre-molt pups was 28% higher than that of

post-molt pups ($P < 0.001$). Although pre-molt pups displayed higher resting and moderately active mass-specific metabolic rates than molting pups, the values were not significantly different ($P = 0.33$). In-air highly active metabolic rates of molting pups were 26% higher than those of post-molt pups ($P < 0.001$). Differences between in-air moderately active and highly active metabolic rates were not statistically significant ($P = 1.00$). Only one pre-molt pup was observed being highly active during in-air trials, and therefore no mean value was reported (Table 2.2).

In-water metabolic rates were measured for all five developmental stages and across activity levels (Table 2.3). As with in-air measurements, developmental stage ($F_{4,137} = 51.23$, $P < 0.001$) and activity level ($F_{2,137} = 59.18$, $P < 0.001$) had significant effects on in-water mass-specific metabolic rates. Within each age class, resting, moderately active, and highly active metabolic rates differed significantly from one another ($P < 0.001$). The metabolic rates of pre-molt pups and molting pups were significantly different from those of all other developmental stages ($P < 0.005$), but were not significantly different from one another ($P = 1.00$). The RMR of pre-molt pups was 47% higher than that of dependent immature individuals ($P < 0.005$), while the moderately active metabolic rate of molting pups was 42% higher than that of dependent immature sea otters ($P < 0.005$). The metabolic rate of highly active molting pups was 14% higher than that of post-molt pups and 42% higher than that of juvenile individuals. Mass-specific metabolic rates of dependent immature animals differed significantly from all developmental stages ($P \leq 0.005$) except juveniles ($P = 0.24$). Only one pre-molt pup was observed being highly active during in-water trials and therefore no mean value was reported (Table 2.3).

Activity Budgets

Detailed activity budgets were determined for all five developmental stages (Table 2.4) and used in FMR calculations (Table 2.5). The amount of time each day spent engaged in specific activities changed markedly with age. In general, individuals greatly reduced time resting and grooming with age and spent more time foraging (Fig. 2.2). Pre-molt pups spent on average $58 \pm 28\%$ of their day resting, while post-molt pups, dependent immature animals, and juvenile animals spent on average 28-29% of their day resting. Grooming time declined throughout immature age classes, reaching its lowest level for juvenile sea otters. Conversely, the percent time spent performing energetically expensive behaviors (e.g. foraging, swimming) increased with age. Dependent immature animals foraged 2.8 hours more per day than molting pups, while juveniles foraged 3 hours more than post-molt pups and 2.5 hours more than dependent immature animals (Fig. 2.2). Swimming only accounted for 9-10% of the daily budgets of molting pups, post-molt pups, and dependent immature animals, but comprised 16% of the activity budget of juvenile animals. Nursing was the most consistent behavior throughout dependency and accounted for 13-16% of the activity budget of all dependent sea otters.

Energetic Demands and Field Metabolic Rates

In-air (Table 2.2) and in-water (Table 2.3) activity-specific metabolic rates were combined with detailed activity budgets (Table 2.4) to determine daily energetic demands for each developmental stage. Total daily energetic demands increased 3-fold from birth to weaning (Table 2.5). Although resting required little energy compared with other behaviors, it accounted for 55% and 32% of the FMR of pre-molt and

molting pups, respectively (Table 2.5). Swimming had a large impact on the energetic expenditure of juveniles, accounting for 18% of FMR. The largest contributing factor to FMR in older age classes was foraging behavior, accounting for 36% and 47% of FMR for dependent immature and juvenile animals, respectively. The FMR of dependent immature and juvenile animals were the highest of all age classes measured in this study (Table 2.5). On a mass-specific basis, molting pups displayed the greatest energetic demand ($0.87 \pm 0.24 \text{ MJ day}^{-1} \text{ kg}^{-1}$) of all developmental stages and juveniles exhibited comparable daily mass-specific demands ($0.59 \pm 0.11 \text{ MJ day}^{-1} \text{ kg}^{-1}$) to adults (Fig. 2.3).

Discussion

Energetic Demands of Immature Sea Otters

The unique, longitudinal data set described in this study details the metabolic demands of sea otter pups and provides the first energetic profile of developmental and, thus, baseline maternal investment costs for this species. As might be expected, pre-molt pups displayed the lowest total FMR of all age classes because of a combination of inactivity and small body size (Table 2.3). Although molting pups displayed the second lowest FMR (Table 2.3), they maintained the highest mass-specific daily energetic demands of all age classes (Fig. 2.3), presumably as a result of shedding of their natal pelage and growing adult coats. Once past the molt stage, sea otter pups spent much of their time learning to dive and forage. The increase in energetically expensive behaviors combined with decreased time spent resting with age (Fig. 2.2), concomitant with increasing body mass, resulted in higher overall energetic

requirements for post-molt pups and dependent immature sea otters than for younger age classes (Table 2.5).

Juvenile sea otters had the highest overall energy requirements of all developmental stages (Table 2.5) and exhibited mass-specific energetic demands similar to those of adults (Fig. 2.3). These newly weaned sea otters no longer have the benefit of obtaining food from their mothers and are likely inefficient foragers in comparison to adult conspecifics. As a result, juvenile sea otters competing with adults for limited prey resources may face considerable energetic hurdles when trying to meet metabolic demands during the first years post-weaning (Burns, 1999). We hypothesize that of all immature age classes, high metabolic demands have the greatest impact on juvenile sea otters during their first year post-weaning.

It is important to note that FMR values presented here were calculated from metabolic measurements taken while animals were post-absorptive. Sea otters in the wild are infrequently post-absorptive and as a consequence continually incur energetic costs associated with the digestion and absorption of prey (Costa and Kooyman, 1984; Kenyon, 1969; Payne and Jameson, 1984; Yeates et al., 2007). The relationship between activity, food digestion and thermoregulatory demands will also affect daily costs of free-ranging sea otters. In part, temporary increases in metabolic rate associated with digestion may offset thermal demands and minimize the need for activity-based heat production, potentially decreasing thermal energetic costs (Costa and Kooyman, 1984; Yeates, 2006). Thus, when taking into consideration the frequency in which sea otters feed and the energetic cost of digestion, values presented

here are likely conservative estimates of daily energetic requirements for wild sea otters.

Energetic Consequences for Adult Females

Average daily activity budgets of females with and without pups (Table 2.6) from Staedler (2011) were used in combination with behavior-specific metabolic rates of adult female sea otters from Williams (1989) to determine daily behavior-specific energetic demands of adult female sea otters with and without pups (Table 2.7). Daily metabolic requirements of dependent sea otters from the present study (Table 2.5) were then used as a measure of additional energetic demands on females and used to determine FMRs of adult female sea otters with pups (Fig. 2.4). We found that by three weeks postpartum, a pup increases the daily energetic demands of its mother by 17% (Fig. 2.4). Female sea otters in both California (Table 6; Staedler 2011) and Alaska (Gelatt et al., 2002) have been shown to markedly reduce foraging activity during the first weeks postpartum. As a result, females must initially rely on energy stored on-board to support the additional metabolic demands of nursing and caring for a new pup. Daily energetic demands of a pup quickly increase throughout dependency and by four months postpartum daily costs are 78% higher for a female with a pup compared with one without. By the end of lactation, which occurs approximately six months postpartum, a dependent pup increases the daily energetic demands of its mother by 96% (Fig. 2.4). At this stage of development, the immature sea otter begins to forage and contribute in part to its own energy demands (Payne and Jameson, 1984). However, these animals also continue to nurse frequently (Fig. 2.2), solicit for food, and share

and steal prey from their mothers (Payne and Jameson, 1984; Staedler, 2011), making them a substantial energetic burden for adult females until weaning occurs.

The total energetic impact of lactation and dependency on mothers can be quantified by summing the sequential costs for each pup developmental stage. Within the first 40 days postpartum a female sea otter must provide 100 MJ to a new pup, and this energetic investment continues to increase substantially as development and growth of the pup continues. By 140 days, when a pup reaches the dependent immature age class, its mother has invested over 625 MJ; if a female successfully raises her pup to weaning (~180 days), she will have invested nearly 930 MJ. This energetic investment is in addition to the adult female's own elevated daily metabolic demands (Fig. 2.4). If at parturition an adult female is energy neutral (that is, bringing in sufficient prey to satisfy her own daily energetic needs and relying on on-board energy stores for her pup), she would lose 29 kg in body mass by the time her pup was weaned at 180 days. This value corresponds to an impossible >100% reduction in body mass and certain mortality. Obviously, female sea otters must markedly increase foraging effort during pup rearing. Bowen et al. (2001) found that despite significant increases in both foraging effort and food intake throughout lactation harbor seals incur a 32% mass loss by late-lactation; 97% of which was due to the depletion of fat reserves. Because sea otters lack large fat reserves to draw upon during lactation (Kenyon, 1969; Williams and Worthy, 2002), increased food intake during lactation is essential for successful pup rearing.

When compared with pinnipeds, sea otters invest considerably more energy into their young over the course of lactation than would be predicted by their body size (Fig.

2.5). Female sea otters invest 128% more energy per kilogram throughout pup dependency than would a similarly sized phocid seal. Likewise, the level of investment is 28% higher for a sea otter than a similarly sized otariid (Fig. 2.5). Given their size, limited energetic reserves, and high basal metabolic demands, our data support the classification of sea otters as extreme income breeders (Stearns, 1992) among marine mammals.

Ecological Implications for Mother and Offspring

The reproductive schedule of female sea otters is fairly rigid, with females giving birth to a pup once a year regardless of current body and environmental conditions (Riedman et al., 1994). As a result, female sea otters are thought to utilize a “bet-hedging” strategy, either keeping or abandoning a pup postpartum depending upon physiological and environmental factors (Monson et al., 2000; Stearns, 1992). Our results suggest that this key life history decision has an energetic foundation: a female sea otter must balance the probability of successfully weaning a pup given her current body energy stores and her likelihood of obtaining sufficient energy to sustain both herself and pup growth, against the potential loss of future reproductive opportunities. For females in poor condition at parturition (or that are experiencing low foraging success), the optimal decision may be to “cut losses”, abandoning the current pup in favor of improving future reproductive opportunities.

Females that go through with pup rearing face a second key life history decision in terms of the timing of weaning. Early weaning (<180 days) reduces a female’s energetic investment in the current pup, thereby reducing her energetic deficit at the end of lactation and leaving her in better physical condition before giving birth to her

next pup. However, early weaning exacerbates the energetic challenges faced by juveniles as a result of high mass-specific energetic demands (Fig. 2.1) combined with physiological and behavioral immaturity, which can reduce foraging efficiency (Burns, 1999; Noren et al., 2001; Noren et al., 2002). Conversely, delayed weaning increases the likelihood of pup survival because pups with longer dependency periods gain an energetic advantage through increased foraging experience, increased body mass, and lower mass-specific metabolic demands at time of weaning (Beauplet et al., 2005; Horning and Trillmich, 1997; Trillmich and Dellinger, 1991). However, delayed weaning requires a greater energetic investment by the female (Fig. 2.4). If the energetic deficit accrued by a female during lactation is too large and exceeds her capacity to replenish energetic reserves before a subsequent pup is born, the female will have to abandon the next pup early in development (Monson et al., 2000).

The trade-offs described above imply two key decision points for an adult female: (1) whether or not to abandon a pup shortly after parturition and (2) at what age to wean a pup. Interestingly, the timing of these two energetically-based decision points for adult females coincides with the two age classes that commonly experience the highest rates of mortality in the wild: postpartum pups and juveniles in their first year post-weaning (Estes et al., 2003; Tinker et al., 2006). A study on northern sea otters (*Enhydra lutris kenyoni*) in Prince William Sound, Alaska concluded that female body condition was the major driver leading to postpartum mother-pup separations (Garshelis and Garshelis, 1987). In a different study conducted on northern sea otters, Monson et al. (2000) suggested that females experiencing unfavorable environmental or physical conditions may abandon a pup early in development before a substantial

energetic investment was made. Given the rapid increase in energetic investment for a female to raise a pup to weaning (Fig. 2.4) we conclude that current and future female energetic expenditures are the major drivers behind the majority of pup losses occurring shortly after birth in both northern and southern sea otters (Garshelis and Garshelis, 1987; Jameson and Johnson, 1993; Riedman et al., 1994; Tinker et al., 2006).

Current Implications for Southern Sea Otters

Population recovery of the southern sea otter has been slow and marked by occasional periods of decline since near extirpation during the fur trade of the 18th and 19th centuries (Kenyon, 1969; Tinker et al., 2006). Numerous studies have attributed the sluggish recovery of southern sea otters to elevated mortality, with the highest mortality rates occurring in postpartum pups and juveniles in their first year post-weaning, but with a disproportionately high mortality rate also observed in prime-age females (Estes et al., 2003; Tinker et al., 2006). Because adult female mortality in particular has a profound influence on the population trajectory of the southern sea otter (Gerber et al., 2004; Tinker et al., 2006), it is of concern for overall population stability and growth.

Linked to these trends is the density-dependent reduction in prey resource availability for southern sea otters in areas of high population density (Tinker et al., 2008), which contributes to poor female body condition at the end of lactation. Often adult female deaths occur immediately before or after pup weaning (Tinker et al., 2006; USGS, unpublished), when nutritional deficiencies and poor body condition leave females particularly vulnerable to mating trauma, infection, and disease. Termed, “end-lactation syndrome”, this condition has been observed more frequently over the past 20

years, especially in areas of high sea otter abundance (USGS, unpublished). Our study identifies the underlying energetic mechanism driving the reduction in female body condition as lactation progresses. We hypothesize that the extremely high energetic cost of pup rearing for this species, in combination with localized prey limitations occurring along the California coast (Tinker et al., 2008), results in a physiological tipping point, manifested as an increase in end-lactation female mortality.

Conclusions

Meeting the remarkably high energetic demands of immature sea otters throughout dependency is physiologically challenging for lactating females and often results in a substantial reduction in maternal body mass by pup weaning (Monson et al., 2000). The consequences of increased energetic demands during pup rearing appear to be exacerbated in areas of limited prey availability, where such demands may result in extremely poor body condition or mortality for females at the end of lactation. Although this study focused on southern sea otters in California, these data are broadly applicable to northern sea otters in Alaska, British Columbia, and Washington; particularly in areas of high sea otter abundance where resources are likely limited. We propose that the extreme energetic demands described here have cascading implications for parental provisioning strategies, life history decisions, population dynamics, and current trends in mortality in wild sea otters.

Table 2.1: Developmental stages and classifications for immature sea otters in the present study.

Age Class	Age (days)	Description
Pre-Molt Pup	1-45	Natal pelage, extremely buoyant, unable to dive
Molting Pup	46-90	Shedding natal pelage, short diving attempts
Post-Molt Pup	91-135	Adult pelage, short diving and foraging attempts, rarely feeds on own captured prey
Dependent Immature	136-180	Adult pelage, makes regular foraging dives, contributes moderately to own energetic demands
Juvenile	181-250	Adult pelage, weaned from adult female

Age classes adapted from those described by Payne and Jameson (1984)

Table 2.2: Body mass and mass-specific metabolic rates (means \pm s.d.) of 6 post-absorptive sea otters measured dry on a haul-out across three developmental stages.

Age Class	Mass (kg)	Resting (ml O ₂ min ⁻¹ kg ⁻¹)	Moderately Active (ml O ₂ min ⁻¹ kg ⁻¹)	Highly Active (ml O ₂ min ⁻¹ kg ⁻¹)
Pre-Molt Pup	3.09 \pm 0.81	23.18 \pm 3.85	31.93 \pm 3.15	---
Molting Pup	5.67 \pm 1.05	22.16 \pm 3.90	28.29 \pm 4.58	30.61 \pm 2.26
Post-Molt Pup	9.08 \pm 1.01	17.49 \pm 0.59	21.00 \pm 1.30	23.67 \pm 1.04

Table 2.3: Body mass and mass-specific metabolic rates (means \pm s.d.) of seven post-absorptive sea otters measured in water across five developmental stages.

Age Class	Mass (kg)	Resting (ml O ₂ min ⁻¹ kg ⁻¹)	Moderately Active (ml O ₂ min ⁻¹ kg ⁻¹)	Highly Active (ml O ₂ min ⁻¹ kg ⁻¹)
Pre-Molt Pup	3.09 \pm 0.81	26.03 \pm 3.34	31.36 \pm 1.55	---
Molting Pup	5.67 \pm 1.05	24.89 \pm 7.98	32.55 \pm 5.31	35.38 \pm 3.03
Post-Molt Pup	9.08 \pm 1.01	16.88 \pm 1.75	23.43 \pm 2.78	30.82 \pm 3.69
Dependent Imm.	12.06 \pm 1.02	16.08 \pm 2.26	21.19 \pm 4.24	24.34 \pm 5.76
Juvenile	14.42 \pm 1.39	13.63 \pm 1.08	18.98 \pm 2.28	23.05 \pm 3.74

Table 2.4: Detailed activity budgets of dependent (n = 12) and juvenile (n = 14) sea otters.

Activity	Pre-Molt Pup	Molting Pup	Post-Molt Pup	Dependent Imm.	Juvenile
Resting on mother	29.2 ± 21.2%	5.0 ± 8.0%	1.4 ± 4.2%	1.6 ± 7.7%	0.0 ± 0.0%
Resting in water	28.9 ± 19.2	34.7 ± 22.7	26.9 ± 20.8	26.7 ± 23.2	28.9 ± 16.6
Nursing on mother	11.6 ± 9.3	6.5 ± 6.5	3.2 ± 9.4	0.9 ± 1.6	0.0 ± 0.0
Nursing in water	1.4 ± 3.7	5.6 ± 6.5	13.1 ± 12.2	13.9 ± 9.6	0.0 ± 0.0
Foraging	2.2 ± 5.3	19.3 ± 16.3	28.7 ± 22.3	31.1 ± 26.1	41.4 ± 17.3
Groomed by mother	16.8 ± 12.8	8.4 ± 7.4	5.4 ± 9.6	3.5 ± 4.0	0.0 ± 0.0
Passive grooming	3.1 ± 4.6	3.1 ± 4.0	2.9 ± 2.7	2.7 ± 3.6	1.6 ± 2.6
Active grooming	2.4 ± 4.0	7.6 ± 7.2	6.8 ± 5.9	6.9 ± 5.6	7.0 ± 5.2
Swimming	4.3 ± 6.3	9.7 ± 11.2	9.4 ± 12.6	9.3 ± 14.0	16.0 ± 7.5
Other	0.0 ± 0.0	0.0 ± 0.0	2.2 ± 3.0	3.4 ± 3.7	5.0 ± 5.0

Values are presented as the percent of a 24 hour day spent engaged in a specific activity (means ± s.d.)

Table 2.5: Behavior-specific daily energetic demands and field metabolic rate (FMR) of southern sea otters across developmental stages (means \pm s.d.).

Age Class	Resting (MJ day ⁻¹)	Nursing (MJ day ⁻¹)	Foraging (MJ day ⁻¹)	Grooming (MJ day ⁻¹)	Swimming (MJ day ⁻¹)	Other (MJ day ⁻¹)	Total/FMR (MJ day ⁻¹)
Pre-Molt Pup	1.28 \pm 0.65	0.27 \pm 0.22	0.06 \pm 0.15	0.56 \pm 0.37	0.12 \pm 0.18	0.00 \pm 0.00	2.29 \pm 0.81
Molting Pup	1.60 \pm 1.11	0.47 \pm 0.38	1.12 \pm 0.95	1.24 \pm 0.78	0.52 \pm 0.61	0.00 \pm 0.00	4.94 \pm 1.84
Post-Molt Pup	1.26 \pm 0.95	0.73 \pm 0.70	2.32 \pm 1.84	1.14 \pm 0.80	0.58 \pm 0.78	0.14 \pm 0.19	6.16 \pm 2.46
Dependent Imm.	1.59 \pm 1.40	0.83 \pm 0.56	2.64 \pm 2.36	1.31 \pm 0.80	0.79 \pm 1.21	0.25 \pm 0.28	7.41 \pm 3.17
Juvenile	1.65 \pm 0.96	0.00 \pm 0.00	3.98 \pm 1.80	1.00 \pm 0.68	1.54 \pm 0.67	0.40 \pm 0.36	8.56 \pm 2.31
Adult Male*	4.20	---	6.10	2.40	1.60	1.40	15.70

*Values for adult male sea otters adapted from Yeates et al. (2007)

Table 2.6: Simple activity budgets of female sea otters with and without pups adapted from Staedler (2011).

Activity	Female with no pup	Female with 0-3 wk old pup	Female with >3-10 wk old pup	Female with >10 wk old pup
Resting	51.7 ± 4.9%	72.1 ± 9.6%	56.3 ± 5.80%	44.8 ± 2.2%
Active Other	8.7 ± 3.8	6.4 ± 2.4	5.7 ± 2.1	6.7 ± 1.3
Foraging	39.7 ± 6.6	21.5 ± 9.5	38.1 ± 5.1	48.6 ± 1.8

Activity budgets were determined from time-depth recorders implanted in wild sea otters. Any behavior not identified as “resting” or “foraging” was classified as “active other”. Values are presented as the percent of a 24 hour day spent engaged in a specific activity (means ± s.d.).

Table 2.7: Mean behavior-specific daily energetic demands of female southern sea otters with and without pups. Not including additional costs associated with lactation.

Female Offspring Status	Resting (MJ day ⁻¹)	Active Other (MJ day ⁻¹)	Foraging (MJ day ⁻¹)	Total (MJ day ⁻¹)
Female with no pup	4.01	0.97	5.38	10.37
Female with 0-3 wk old pup	5.60	0.91	2.92	9.42
Female with >3-10 wk old pup	4.37	0.80	5.17	10.34
Female with >10 wk old pup	3.48	0.95	6.58	11.01

Values were calculated utilizing adult female activity budgets (see Table 2.6) from Stuedler (2011) in combination with behavior-specific metabolic rates of female sea otters from Williams (1989). Average adult female body mass of 19.89 kg (Williams 1989) was assumed for adult female energetic demand calculations. Behavior-specific metabolic rates from Williams (1989) used in the above calculations were: “resting” = 13.5 ml O₂ min⁻¹ kg⁻¹, “active other” = 24.74 ml O₂ min⁻¹ kg⁻¹ (average of grooming, swimming, and diving behaviors), and “foraging” = 23.58 ml O₂ min⁻¹ kg⁻¹ (average of swimming and diving behaviors).

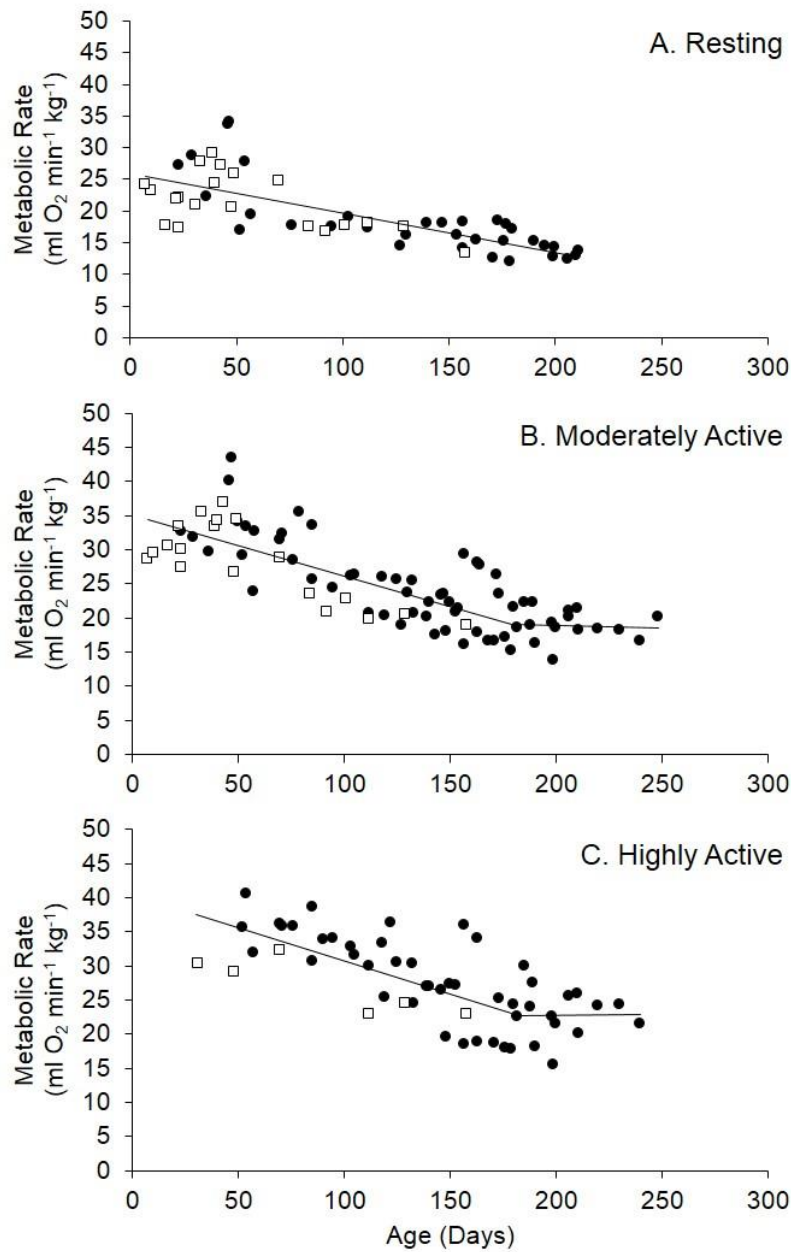


Figure 2.1: Metabolic rate in relation to age for southern sea otters. Values for resting (A), moderately active (B), and highly active (C) sea otters are compared. Each point represents results from a single respirometry trial, data points for all study animals ($n = 7$) are included, with in-air (open squares) and in-water (filled circles) measurements identified. Lines represent the results of piecewise linear regression analyses with breakpoints at the average age of weaning (~ 180 days) representing the point at which metabolic rates begin to plateau at approximately adult levels. Regressions and statistics are presented in the Results.

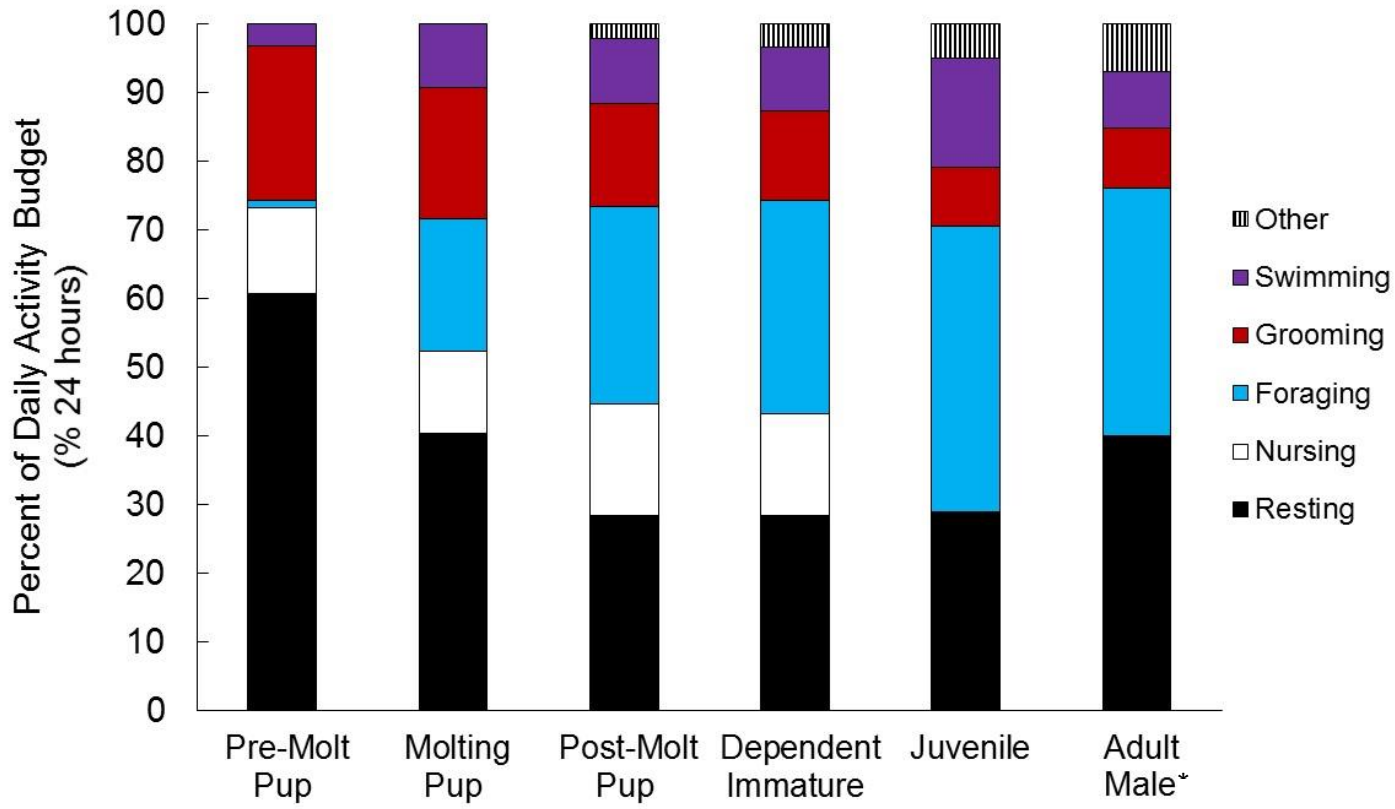


Figure 2.2: Daily activity budgets for wild sea otters across developmental stages from pup to adult. Data for dependent (n = 12) and juvenile (n = 14) sea otters in this study are compared to data for *adult male sea otters from Yeates et al. (2007). Daily activity budgets were calculated as the proportion of all records associated with six behavioral categories: resting, nursing, foraging, grooming, swimming, and other. The reported activity budgets represent proportions of 24 hour periods of known activities as described in the methods.

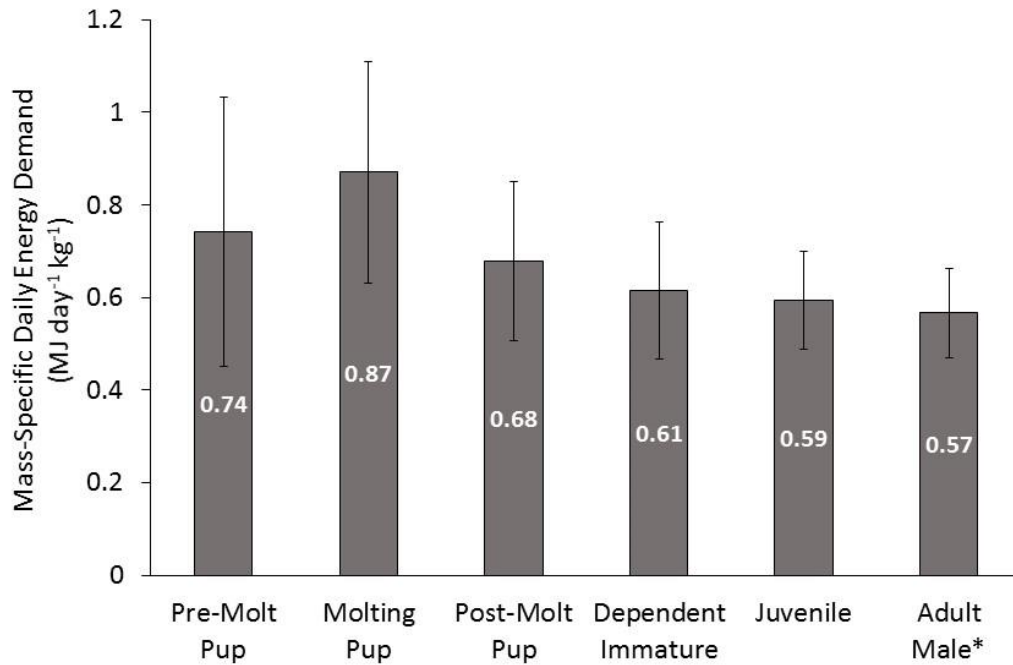


Figure 2.3: Mass-specific daily energetic demands of wild sea otters across developmental stages in comparison to adult males. *Adult male mass-specific energetic demand adapted from Yeates et al. (2007). Height of bars and lines represent means \pm 1 s.d. Age class means displayed within bars.

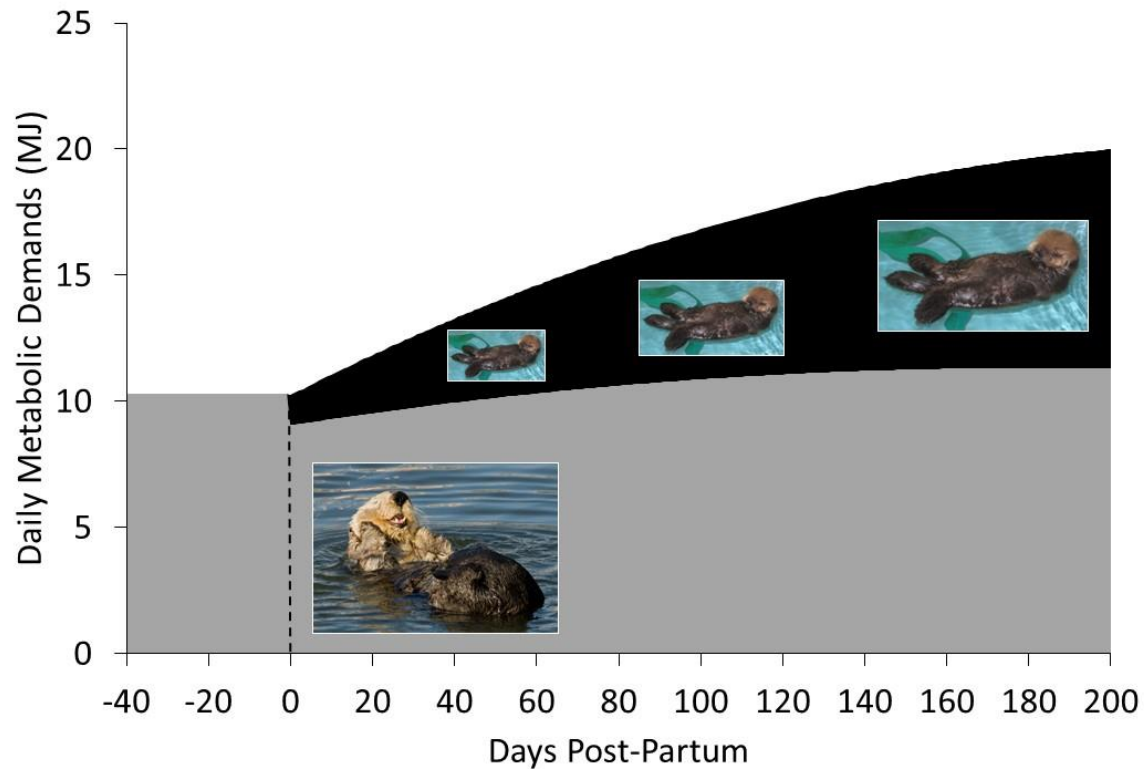


Figure 2.4: Daily metabolic demands of an adult female sea otter in relation to days postpartum. Time in days, spanning 40 days before birth to 200 days postpartum, is displayed on the x-axis. Average daily energetic demands of an adult female (grey, Table 2.7) are distinguished from the additional energetic demands of a dependent pup (black, Table 2.5). The daily metabolic demand (equivalent to FMR) of a postpartum female is represented by the combination of female (grey) and pup (black) energetic demands on a given day. Dashed line denotes pup birth. Note that average age of weaning is 180 days. Photo credit: J. Tomoleoni (adult) and N. Thometz (pup).

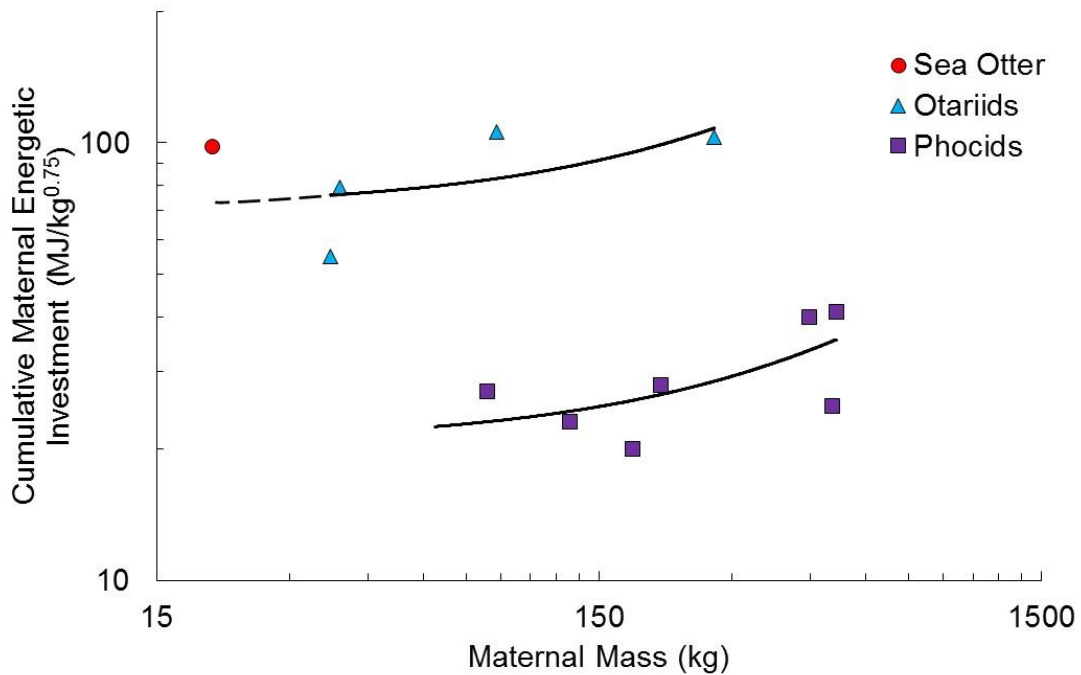


Figure 2.5: Total maternal energetic investment over the course of pup dependency in relation to average adult female body mass for marine mammals. Sea otters from the present study (red circle), are compared to otariids (blue triangles) and phocid seals (purple squares). Pinniped data are adapted from Boness and Bowen (1996). Points represent cumulative energetic investment in a pup by an adult female by the average age of weaning. Least squares linear regression equations are $y = 0.136x + 70.889$ ($r^2 = 0.41$) and $y = 0.0289x + 20.615$ ($r^2 = 0.43$) for otariids and phocids, respectively. The dashed line is an extension of the otariid regression and is provided for comparison with the sea otter.

CHAPTER 3

Ontogeny of Oxygen Storage Capacity and Diving Ability in the Southern Sea Otter (*Enhydra lutris nereis*): The costs and benefits of large lungs

Abstract

Given their small size, large lung capacities, and dense pelage, sea otters (*Enhydra lutris*) experience diving constraints more similar to many diving bird species than to other marine mammals. We examined the ontogeny of blood, muscle, and lung oxygen stores and calculated aerobic dive limits (cADL) in southern sea otters (*Enhydra lutris nereis*) to examine the consequences of large lungs on the development of diving ability. Total oxygen storage capacity matures rapidly in sea otters, with immature animals exhibiting total body oxygen stores comparable to adults well before the age of weaning. This result is driven by exceptional lung capacity at birth, combined with a decrease in mass-specific lung volume with age. Blood and muscle oxygen stores remain well below adult values prior to weaning, as large pups exhibit 69% and 54% of adult values, respectively. High positive buoyancy, associated with large lung volumes and air trapped within pelage, disproportionately impacts different age classes and greatly influences the ontogeny of diving ability in sea otters. Slow muscle development likely limits the ability of immature sea otters to dive against high buoyant

forces, making them unable to efficiently dive with total lung capacity (TLC) and instead must utilize a smaller diving lung volume (DLV). The cADL of juveniles is 3.03 minutes, assuming DLV, while adult cADL is 4.14 minutes, assuming TLC. Thus, despite seemingly fast development of total body oxygen storage capacity, our results suggest that immature sea otters do not reach adult diving capacities until after their first year post-weaning.

Introduction

The ontogeny of diving ability has been examined in a variety of marine divers, with the general result that individuals demonstrate poor diving ability at birth, followed by physiological and behavioral developments that closely track the dependency period of a given species (Burns et al., 2005; Clark et al., 2007; Costa et al., 1998; Fowler et al., 2007; Kooyman et al., 1983; Noren et al., 2002; Noren et al., 2005; Ponganis et al., 1999; Richmond et al., 2006; Shero et al., 2012; Verrier et al., 2011b; Weise and Costa, 2007). The majority of these studies have examined the development of blood and muscle oxygen stores in phocid and otariid species, with limited empirical data existing for other marine mammal groups. Sea otters (*Enhydra lutris*) differ taxonomically, physiologically, and behaviorally from other marine mammals (Reynolds and Rommel, 1999) and likely display unique characteristics in the ontogeny of diving ability.

Sea otters have the highest mass-specific metabolic rate of any marine mammal, are voracious benthic foragers, and may spend as much as half the day foraging to meet daily energetic demands (Estes et al., 1982; Morrison et al., 1974; Ralls and Siniff,

1990; Staedler, 2011; Tinker et al., 2008; Yeates et al., 2007). They lack a blubber layer, which is used by nearly all other marine mammals for thermoregulation and energy storage (Costa and Williams, 1999; Kenyon, 1969). Instead sea otters rely solely on fur for insulation and have the densest pelage of any mammal, trapping a substantial layer of air next to the skin (Fish et al., 2002; Williams et al., 1992). Furthermore, sea otters have exceptional mass-specific lung capacities when compared to other marine and terrestrial mammals (Lenfant et al., 1970; Snyder, 1983; Tarasoff and Kooyman, 1973). The combination of air-trapped within dense pelage and large lung capacities result in considerable positive buoyant forces experienced by sea otters (Cashman, 2002; Fish et al., 2002).

Although a benefit when resting, grooming, manipulating prey, and caring for young at the water surface (Costa and Kooyman, 1982; Kenyon, 1969), high positive buoyancy can be a burden at depth, making foraging energetically expensive for sea otters in comparison to other marine mammals, which may be able to rely on neutral or negative buoyancy when diving (Williams, 1989; Williams, 2000; Yeates et al., 2007). In this regard, sea otters are more similar to diving bird species, which must dive with air in both the respiratory system and trapped in plumage (Ponganis and Kooyman, 2000; Stephenson, 1995; Wilson et al., 1992). For diving birds, there are inherent trade-offs between oxygen availability in the respiratory system, positive buoyant forces, and the energetic cost of diving (Sato et al., 2002; Wilson et al., 1992). 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 utilizing 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; Kooyman et al., 1983). Therefore, ADL is a good metric of the time an animal is capable of foraging on a single dive. Although, directly determined by measuring post-diving blood lactic levels (Kooyman et al., 1980; Kooyman et al., 1983), ADL can be calculated (cADL) by dividing total body oxygen stores (blood, muscle, lung) by oxygen demand (Kooyman, 1989). For immature marine divers, a combination of heightened oxygen demand and limited oxygen stores result in shorter cADLs in comparison to adult conspecifics (Burns, 1999; Kooyman et al., 1983; 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 stores in the southern sea otter (*Enhydra lutris nereis*) across 5 age classes: neonates, small pups, large pups, juveniles, and adults (Table 3.1). 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 oxygen stores. Total body oxygen stores (this study) were

used in combination with age-specific metabolic rates (Thometz et al., 2014) of southern sea otters to determine a cADL for each age class. Lastly, we calculated positive buoyancy due to lung volume and pelage for all age classes. It appears 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.

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). 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; Garshelis, 1984; Kenyon, 1969). For statistical comparisons, study animals were grouped into 5 different age classes consistent with age estimates and total body length measurements (Table 3.1). Birth dates (± 1 week) were known for a sub-set of study animals and were used to validate age classifications.

Hematology

Hematological records of captive, wild, and wild-rehabilitated southern sea otters, spanning a 13 year period from 1997 to 2010, were obtained from MBA. Blood values used in this study included red blood cell (RBC) count, hemoglobin (Hb), hematocrit (HCT), and mean corpuscular hemoglobin content (MCHC). Blood samples taken by MBA veterinary staff were sent to IDEXX Reference Laboratories (IDEXX

Laboratories INC.) or ANTECH Diagnostics (Irvine, CA) for analysis, and/or analyzed in-house at MBA using a VetScan HM5 (Abaxis, Union City, CA) to validate laboratory results. Sixty-seven sea otters (32 male, 35 female) ranging in age from one day to eleven years old, were included in analyses. We excluded blood samples from sick or injured animals as identified by MBA veterinary staff, leaving 395 blood samples in the present 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

Plasma volume (PV) and blood volume (BV) were determined using the Evans blue dye technique (El-Sayed et al., 1995; Foldager and Blomqvist, 1991). Ten immature sea otters (5 male, 5 female), ranging in age from 11 days to one year 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 prior to injection of Evans blue dye (Sigma Diagnostics, St. Louis, MO, USA). Next a pre-determined amount (0.5 g kg^{-1}) of Evans blue dye was injected into the blood stream and the syringe was flushed with blood and saline repeatedly to ensure complete delivery of the solution. Three serial blood samples (2-3 ml) were taken at 7 to 10 minute 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 harvested 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 minutes to determine HCT in duplicate. All vacutainers were spun for 15 minutes at 3,500 rpm to separate red blood cells from plasma. The supernatant was pipetted into 5 ml cryovials and stored at -80°C until analysis. The photometric absorbance of plasma samples was measured at 624 and 740 nm 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 (Costa et al., 1998; El-Sayed et al., 1995; Foldager and Blomqvist, 1991). Blood volume was calculated using measured plasma volume and HCT following the equation:

$$BV = PV \times (1-HCT)^{-1} \quad (1)$$

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. Myoglobin 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*) and harbor porpoise (*Phocoena phocoena*) muscle of known Mb content were used as assay controls bounding upper and lower Mb concentrations.

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 Co., San Leandro, CA) or hanging scale (model I-20W OHAUS, Florham Park, NJ). Morphological measurements were taken prior to each dissection. Carcasses were carefully pelted, internal organs examined, specific organs (heart, lungs, liver, & kidneys) weighed, and all organs were 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 or not 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 (Ponganis, 2011) and total lung capacity (TLC) = 345 ml/kg (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 oxygen storage capacity was determined by summing blood, muscle and lung oxygen stores (Kooyman, 1989; Lenfant et al., 1970). Blood oxygen stores were determined following the methods of Kooyman (1989) as explained by Ponganis et al. (1993) and were calculated by summing arterial and venous oxygen stores:

$$\text{Arterial O}_2 = 1/3 \times (\text{BV}) \times (0.95 - 0.20 \text{ saturation}) \times (\text{O}_2 \text{ capacity}) \quad (2)$$

$$\text{Venous O}_2 = 2/3 \times (\text{Arterial O}_2 \text{ Content} - 5\% \text{ by volume}) \quad (3)$$

where BV is blood volume (ml kg⁻¹) and 1/3 and 2/3 are estimated proportions of arterial and venous blood, respectively (Lenfant et al., 1970). O₂ capacity is Hb x 1.34 mL O₂ Hb⁻¹, where 1.34 mL O₂ Hb⁻¹ is the oxygen binding capacity of Hb (Kooyman, 1989). Arterial saturation was assumed to be 0.20 to 0.95 for equation 2, while venous saturation was assumed to be 0.15 to 0.90 (Kooyman, 1989) for equation 3. Muscle oxygen storage capacity was calculated using the equation:

$$\text{Muscle O}_2 = [\text{Mb}] \times (1.34 \text{ mL O}_2 \text{ g Mb}^{-1}) \times (\text{body mass} \times p) \quad (4)$$

where 1.34 mL O₂ g Mb⁻¹ is the oxygen binding capacity of Mb (Kooyman, 1989) and p is the proportion of muscle mass. Lung oxygen storage capacity was calculated using the equation:

$$\text{Lung O}_2 = V_1 \times 0.15 F_{\text{O}_2} \quad (5)$$

where V₁ is lung volume and 0.15 F_{O₂} is the assumed oxygen extraction efficiency of lungs (Kooyman, 1989); and was calculated two ways, assuming DLV and TLC, which gave a range of potential lung volumes sea otters may utilize while diving.

Calculated Aerobic Dive Limit (cADL)

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

$$\text{cADL} = \text{Total O}_2 \text{ stores (muscle O}_2 + \text{blood O}_2 + \text{lung O}_2) \times (\text{diving MR})^{-1} \text{ (6)}$$

where age-class specific diving metabolic rates ($\text{ml O}_2 \text{ min}^{-1} \text{ kg}^{-1}$) reported in Thometz et al. (2014) and Yeates et al. (2007) were used to calculate aerobic dive limits for immature and adult sea otters, respectively. Diving metabolic rates from Thometz et al. (2014) may be conservative estimates of diving metabolic rates in the wild as animals were post-absorptive when measured, which may result in slightly elevated cADLs. Adult diving metabolic rate from Yeates et al. (2007) was measured with two adult male sea otters that were post-prandial. Metabolic rate (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 of male and female southern sea otters from the literature (Williams, 1989; Yeates et al., 2007).

Statistical Analyses

Sample size limited our ability to examine the difference 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, plasma volume, blood volume, *longissimus dorsi* [Mb], *gracilis* [Mb], average [Mb], muscle mass percentage, lung mass, and the total O₂ 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 a paired T-test. Linear regression analyses were used to describe changes in plasma volume, blood volume, 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 INC., 2012). Means are reported \pm standard error (\pm SE). 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 ($F_{4,152} = 54.76$, $P < 0.0001$), and HCT ($F_{4,148} = 49.04$, $P < 0.0001$) all increased significantly throughout ontogeny (Table 3.2). 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 likely mature at or around the average age of weaning (6 months). Neonates and small pups were not significantly different from one another in any blood parameter ($P > 0.05$ for all comparisons) and displayed approximately 66%, 64%, and 64% of adult values for RBC, Hb, and HCT, respectively. Large pups differed greatly from all other age classes (Table 3.2) with regards to RBC, Hb, and HCT, suggesting that sea otters undergoing rapid hematological development around 3-5 months 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 plasma volume and total blood volume increased significantly with body mass. Total plasma volume increased linearly following the equation:

$$PV \text{ (ml)} = 74.67 \times M_b + 64.25 \quad (7)$$

($n = 13$, $r^2 = 0.96$, $P < 0.0001$) where PV is plasma volume and M_b is total body mass.

Total blood volume increased linearly following the equation:

$$BV \text{ (ml)} = 170.03 \times M_b - 105.98 \quad (8)$$

($n = 13$, $r^2 = 0.98$, $P < 0.0001$) where BV is blood volume and M_b is total body mass.

Mass-specific plasma volume (Table 3.2) 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 plasma volume decreased as a function of body mass following the equation:

$$PV \text{ (ml kg}^{-1}\text{)} = -1.19 \times M_b + 98.85 \quad (9)$$

($n = 13$, $r^2 = 0.41$, $P = 0.04$). Mass-specific blood volume 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:

$$BV \text{ (ml kg}^{-1}\text{)} = 0.98 \times M_b + 145.44 \quad (10)$$

($n = 13$, $r^2 = 0.24$, $P = 0.08$). We report that adult sea otter mass-specific blood volume is $173.48 \pm 9.85 \text{ ml kg}^{-1}$, which is nearly 2 times higher than the previously published value (91 ml kg^{-1} ; Lenfant et al. 1970).

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. 3.1). Therefore, [Mb] values for both muscles within individuals were averaged and used to obtain mean muscle [Mb] values (Table 3.3), 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 59% and 72% of adult [Mb], respectively (Table 3.3). Our value for adult skeletal muscle [Mb] ($3.32 \pm 0.10 \text{ g } 100 \text{ g muscle}^{-1}$) was slightly higher than the value published by Lenfant et al. (1970) (2.60 g%), but comparable the [Mb] reported by Castellini and Somero (1981) ($3.13 \text{ g } 100 \text{ g muscle}^{-1}$).

Muscle Mass

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

$$M_m = 341.72 \times M_b - 401.34 \quad (11)$$

($n = 19$, $r^2 = 0.98$, $P < 0.001$) where M_m 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 ($30.19 \pm 1.43\%$), but statistically comparable ($P = 0.65$)

percent muscle mass to adults (Table 3.3). Percent muscle mass increased 4% from small pups to large pups and another 5% from large pups to juveniles. Our measured percent muscle mass for adult sea otters ($32.78 \pm 2.08\%$) 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 3.4; Fig. 3.2A) following the equation:

$$M_l = 30.82 \times M_b + 81.12 \quad (12)$$

($n = 20$, $r^2 = 0.88$, $P < 0.001$) 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. 3.2B) following the equation:

$$M_l = -0.00063 \times M_b + 0.05 \quad (13)$$

($n = 20$, $r^2 = 0.35$, $P = 0.0038$) where M_l is lung mass (g) and M_b is total body mass (kg). Because lung mass did not make up the same proportion of body mass in all age classes (Table 3.4) we did not assume lung volume could be estimated as a constant proportion of body mass based on published adult values. Instead, we used mean mass-specific adult lung mass ($33.77 \pm 2.50 \text{ g kg}^{-1}$, $F_{4,15} = 3.54$, $P = 0.032$) 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:

$$\text{TLC} = (10.22 \times M_l) \times M_b^{-1} \quad (14)$$

$$DLV = (6.13 \times M_l) \times M_b^{-1} \quad (15)$$

Where M_l is lung mass (g) and M_b is body mass (kg). These equations were used in combination with measured lung masses of immature and adult sea otters to determine TLC and DLV across ontogeny (Table 3.4). Mass-specific TLC decreased with age, ranging from 542.52 ± 81.38 ml kg^{-1} in small pups to 345.69 ± 30.10 ml kg^{-1} in adults ($F_{4,15} = 2.831$, $P = 0.065$). Mass-specific DLV also decreased with age, ranging from 324.84 ± 48.72 ml kg^{-1} in small pups down to 206.98 ± 18.02 ml kg^{-1} in adults ($F_{4,15} = 2.831$, $P = 0.065$).

Total Oxygen Storage Capacity and cADL

On a mass-specific basis, blood and muscle oxygen storage capacity increased significantly with age (blood O_2 : $F_{4,148} = 90.72$, $P < 0.001$; muscle O_2 : $F_{4,42} = 116.63$, $P < 0.001$) (Table 3.5). Neonates and small pups did not differ in blood ($P = 0.65$) or muscle ($P = 0.44$) oxygen storage capacity. Large pups had lower blood oxygen storage capacity than juveniles ($P < 0.001$), but comparable muscle oxygen storage capacity ($P = 0.19$). Adults had greater blood and muscle oxygen storage capacities than all other age classes, including juveniles ($P < 0.001$). In contrast, mass-specific lung oxygen stores decreased across age classes ($F_{4,15} = 3.54$, $P = 0.03$) (Table 3.5). Small pups displayed the highest mass-specific lung O_2 stores (DLV: 48.72 ± 7.31 ml kg^{-1} ; TLC: 81.38 ± 17.26 ml kg^{-1}) and were the only age group that differed significantly from adults ($P = 0.047$).

Total body oxygen storage capacity (ml O_2 kg^{-1}) of neonates and small pups were 81-94% and 92-105% of adult values, respectively, due to very large mass-specific lung O_2 stores (Table 3.5). Juveniles, whether assuming DLV or TLC, had

mass-specific total body O₂ stores that were above adult values. Although young sea otters displayed comparable oxygen storage capacity to adults, heightened mass-specific metabolic rates associated with immaturity reduced the cADL of younger age classes (Table 3.5). The cADL of small pups was 64-74% of adult cADL, while large pups displayed a cADL that was 84-91% of adult cADL, depending if DLV or TLC was assumed, respectively. Juvenile sea otters had comparable cADL values to adult sea otters regardless of the lung volume assumed (Table 3.5).

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 remarkable total body O₂ stores in comparison to other marine divers (Fig. 3.3). Regardless of the lung volume assumed (TLC or DLV), adult sea otters have greater mass-specific total body O₂ 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 total body O₂ storage capacity and diving ability across taxa. For example, grey (Noren et al., 2005), ringed (Lydersen et al., 1992), and harbor (Burns et al., 2005) seals all exhibit smaller total body O₂ stores than sea otters, but average and maximum dive times and depths of phocid seals in general greatly exceed those of sea otters (Ponganis, 2011). Similarly, when comparing penguin species, which have analogous buoyancy issues, the deepest diving emperor penguin has lower total body O₂ stores than the more shallow diving king and Adelie penguins (Fig. 3.3). However, despite lower total body O₂ stores, emperor penguins have higher combined blood and muscle O₂ 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 O₂ capacity and minimization of air in the respiratory system while diving (Ponganis et al., 1997; Ponganis et al., 1999).

When comparing combined blood and muscle O₂ stores across taxa and excluding the lung O₂ store, sea otters have a greater O₂ storage capacity than many marine divers including bottlenose dolphins (Noren et al., 2002), sub-Antarctic fur seals (Verrier et al., 2011b), Steller sea lion females (Richmond et al., 2006), northern fur seals (Lenfant et al., 1970), walruses (Lenfant et al., 1970), thick-billed murrelets (Croll et al., 1992) and both king and Adelie penguins (Sato et al., 2002). Sea otters have relatively large blood and muscle O₂ stores among marine divers, but despite this, high positive buoyancy makes diving more energetically expensive for sea otters in comparison to other marine mammals (Williams, 2000; Yeates et al., 2007), as sea otters are very rarely neutrally or negatively buoyant while diving (Cashman, 2002), which could act to reduce overall dive costs.

Development of Oxygen Stores

Hematological development in sea otters closely resembles patterns observed in other marine (Burns et al., 2005; Burns et al., 2007; Clark et al., 2007; Costa et al., 1998; Horning and Trillmich, 1999; Noren et al., 2002; Noren et al., 2005; Ponganis et al., 1999; Richmond et al., 2005; Verrier et al., 2011b; Weise and Costa, 2007), semi-aquatic (MacArthur et al., 2001; McIntyre et al., 2002), and terrestrial species (Mohri et al., 2007; Seal et al., 1967). Of note, the blood volume 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 et al. (1970) likely resulted in slow mixing of tagged erythrocytes, reduced HCT levels, and thus provided an underestimate of actual blood volume. Our methods, which used a combination of fentanyl and midazolam rather than inhalational anesthesia, would likely have resulted in normal cardiac output (Parworth et al., 1998; Yaster et al., 1990) 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. For a relatively shallow diving marine mammal, sea otters have high mass-specific blood volumes, but the general pattern of plasma and blood volume development in immature sea otters was similar to trends reported for other marine mammal species (Burns et al., 2005; Costa et al., 1998; Fowler et al., 2007; Ponganis et al., 1993; Richmond et al., 2006; Weise and Costa, 2007).

Although adult marine mammals are known to have muscle myoglobin concentrations that are 10-30% higher than those exhibited by terrestrial mammals (Castellini and Somero, 1981; Kooyman and Ponganis, 1998), there is a prolonged period of [Mb] development that has been documented for a wide variety of marine divers (Kanatous et al., 2008; Noren et al., 2001; Ponganis et al., 1999; Richmond et al., 2006). 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 (Noren et al., 2001; Ponganis et al., 1999). Noren et al. (2001) examined differences in Mb development between species that have a terrestrial post-parturition phase (seals &

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.

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] occurs during the initial phases of independent foraging regardless of whether or not an individual is born directly into the marine environment. Sea otters are born with minimal muscle myoglobin (10% of adult [Mb]), a result similar to what has been reported for neonate bottlenose dolphins (10% adult [Mb]; Noren et al. 2001) and one month-old Australian fur seals (9% 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 months, a period that also corresponds to the initiation of diving and foraging (Payne and Jameson, 1984). As benthic foragers, frequent diving attempts alongside their mothers likely facilitate the rapid development of muscle [Mb] in pups throughout dependency. At weaning, sea otters have between 63% and 76% of adult [Mb], which corresponds to the mean values determined for large pups and juveniles, respectively. Therefore, it likely takes sea otters 1.5-2.5 years to develop comparable muscle mass and [Mb] to adults, which results in limited muscle O₂ storage capacity for immature sea otters during their entire first year post-weaning.

The lung mass determined for adult sea otters ($3.39 \pm 0.25\%$ body mass) in this study agrees closely with the value determined for northern sea otters (3.86% body

mass) by Tarasoff and Kooyman (1973); however, when taking into consideration that Tarasoff and Kooyman (1973) combined both immature and adult sea otters in their study, our mean is likely closer to the true value for adult sea otter lung mass. The pattern we observed was different from studies that have examined age related changes in the lungs of other marine mammals, which show increases in mass-specific lung volume (Burns et al. 2007) and increases in the proportion of body mass made up by the lungs (McLellan et al., 2002) as a function of age. In contrast, sea otters displayed an age-related decrease in the ratio of lung mass to body mass, a pattern more commonly observed in small terrestrial mammals (Bartlett and Areson, 1977), but appears exaggerated in sea otters. The large contribution of lung mass to body mass in immature sea otters is likely a consequence of the conflicting roles sea otter lungs must play. In terrestrial mammals, the ratio of lung surface area to oxygen consumption also decreases over the course of development (Bartlett and Areson, 1977). Although we did not directly measure lung surface area in this study, we suspect sea otters would display a similar pattern, which would be useful for immature sea otters managing extremely high oxygen demands (Thometz et al., 2014). Although they closely resemble other marine mammal species in the development of blood and muscles, it appears that sea otters differ in the development of, dependence on, and utilization of the lung.

Total body O₂ storage capacity and the proportional contribution of each oxygen store to total body O₂ storage capacity changes greatly across developmental stages in sea otters (Fig. 3.4). When assuming DLV, the proportional contribution of muscle increases ten-fold from neonates to adults, while the contribution of lungs decrease

from 77% in neonates down to 45% in adults (Fig. 3.4). Increases in diving and foraging behavior with age (Thometz et al., 2014) likely facilitate the development of muscle mass and [Mb]. Neonates do not make diving attempts and rely on buoyancy provided by both the lung and natal pelage to float at the surface (Payne and Jameson, 1984). Large lung capacities at birth benefit both neonates and females caring for neonates by increasing positive buoyancy, allowing a neonate to float passively at the surface while its mother is foraging at depth. In older age classes the positive buoyant force provided by the lungs is both beneficial and costly. In older sea otters, large lungs are beneficial at the surface as they provide positive buoyancy to perform essential behaviors, they are beneficial at depth because they function as an important O₂ store (Denison and Kooyman, 1973; Kenyon, 1969; Tarasoff and Kooyman, 1973), but they are energetically costly for foraging sea otters at depth as high positive buoyancy increases the effort required while diving (Cashman, 2002; Yeates et al., 2007).

Lung Volume and Buoyancy

We modeled the positive buoyant forces due to lung capacity and pelage across age classes (Table 3.6) utilizing equation 2 from Skrovan et al. (1999):

$$B_D = V_D g + B_B$$

In which B_D is the buoyant force (N) at depth, V_D is air volume (L) at depth, 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, 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 the loss of air from the fur while diving was not accounted for.

Mass-specific positive buoyant forces of sea otters are not equal across age classes (Table 3.6). In fact, mass-specific positive buoyant forces experienced by a neonate are twice those experienced by an adult sea otter; and this is likely an underestimate as it did not account for the 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 buoyant forces make diving more challenging. A large pup diving to 10 m with TLC will experience positive buoyant forces of $3.98 N kg^{-1}$ in comparison to $2.84 N kg^{-1}$ in an adult sea otter (Table 3.6). If that same animal dove with DLV instead of TLC it would reduce its positive buoyancy by 21.3%. 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 3.3; Fig. 3.4) that may not be physically capable of diving against high positive buoyancy with the same ability as adults. In fact, 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. 3.5; USGS unpublished data).

Implications on Calculated Aerobic Dive Limit

Our results suggest that young sea otters in their first year post-weaning have comparable diving abilities to adults (Table 3.5); however, high positive buoyancy from large lung volumes and the added buoyancy of air in the pelage may complicate these results and act to reduce the diving and foraging abilities of younger individuals despite calculated values. For example, the mass-specific positive buoyancy experienced by a juvenile sea otter diving with TLC is 1.74 N kg^{-1} greater than that experienced by an adult diving with TLC. If that juvenile dives with DLV instead of TLC it will experience comparable mass-specific positive buoyant forces to an adult diving with TLC (Table 3.6). Although juveniles have higher total body O_2 stores than adults, muscle [Mb] and muscle mass do not reach adult levels until after the first year post-weaning (Table 3.5), which will limit their ability to dive against high positive buoyancy. Therefore, we suggest that immature sea otters likely do not dive with TLC, and instead rely on a smaller DLV, which will ultimately reduce positive buoyant forces to comparable levels to adults, with the consequence of reducing total body O_2 stores while diving. Indeed, this assumption appears to be supported by dives times of dependent sea otters in the wild (Fig. 3.5). In contrast, adult sea otters are likely capable of diving with TLC due to increased musculature (Table 3.3), decreased ratio of lung volume to body mass (Fig. 3.2; Table 3.4), and decreased surface area to body volume ratio, which reduces positive buoyancy associated with air in pelage.

Immature sea otters have high mass-specific metabolic rates relative to adult conspecifics (Thometz et al., 2014) which result in increased oxygen utilization while diving and reduced cADLs. The cADL of neonates is approximately half the adult cADL (Table 3.5). When comparing cADLs of large pups and juveniles to adults, they exhibit 84-91% and 95-102% of adult values, respectively. However, as suggest above, immature sea otters likely dive with DLC rather than TLC as a means to reduce mass-specific positive buoyancy to forces more comparable to adults (Table 3.6; Fig. 3.5). Thus, cADL calculations for immature age classes in sea otters should be made assuming DLV, while adult cADL should be calculated assuming TLC. When calculating and comparing cADL in this manner, large pups and juveniles have cADLs that are 2.68 and 3.03 minutes, respectively, and adult cADL is 4.14 minutes. Thus, juvenile cADL is only 73% of the adult value and immature sea otters likely do not have comparable diving abilities to adults until after their first year post-weaning. Given that the majority of dives made by adult male sea otters are shorter than 2 minutes and dives by adult females are an average of 1 minute (Bodkin et al., 2004), our cADL values appear to be reasonable.

Sato et al. (2002) showed that Adelie 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 reducing 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 3.6), both adult and immature sea otters

likely 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), they may use appropriate lung volumes for specific dive depths to reduce positive buoyancy on shallower or shorter dives when maximum lung volume is not necessary.

Conclusions

Despite comparable total body O₂ stores of juveniles and adults (Table 3.5), we propose that high positive buoyancy associated with large lung capacities and dense pelage prevent sea otters from reaching adult diving capacities until after their first year post-weaning. Juveniles continue to display limited muscle mass (Table 3.3), as well as, mass-specific blood and muscle O₂ stores (79.1% and 69% of adult values, respectively) throughout their first year post-weaning (Table 3.5), which may limit their ability to dive against high positive buoyancy. Furthermore, mass-specific positive buoyant forces experienced while diving are disproportionately higher for immature sea otters than for adults (Table 3.6). Immature sea otters diving with smaller lung volumes are able to substantially reduce positive buoyancy while diving (Table 3.6), which reduces the energetic cost of diving. Therefore, in sea otters it appears that the most appropriate way to calculate age-specific cADL is to assume DLV for immature animals and TLC for adults. Due to the dual function and unique development of their lungs, sea otters provide a unique case of the ontogeny of diving ability in a marine mammal species.

Table 3.1: Age class descriptions including age and body length ranges used to classify animals in this study. Average body mass and body length of study animals (not including animals from MBA hematological records) are displayed (means \pm SE) and sample size (n) is given in parentheses.

Age Class	Age Range	Body Length Range (cm)	Body Mass (kg)	Body Length (cm)
Neonate	>1 month	45 – 69	2.55 \pm 0.18 (16)	59.03 \pm 1.79 (16)
Small Pup	1 – 2 months	70 – 84	4.43 \pm 0.30 (9)	75.48 \pm 2.12 (9)
Large Pup	3 – 5 months	85 – 99	8.50 \pm 0.62 (11)	93.51 \pm 1.39 (11)
Juvenile	6 months – 1.5 years	100 – 110	12.24 \pm 0.99 (10)	105.34 \pm 0.84 (10)
Adult	> 1.5 years	>110	19.89 \pm 1.01 (29)	118.86 \pm 3.83 (29)

Table 3.2: Summary of hematological variables, plasma volume, and blood volume for each age class (means \pm SE). Superscripts display connecting letters report from Tukey's post-hoc pairwise comparisons. Sample size (n) is given in parentheses.

Age Class	RBC ($10^6/\mu\text{L}$)	Hb (g dL ⁻¹)	Hct (%)	MCHC (g dL ⁻¹)	PV (mL kg ⁻¹)	BV (mL kg ⁻¹)
Neonate	3.49 \pm 0.08 ^A (30)	12.01 \pm 0.22 ^A (30)	35.93 \pm 0.89 ^A (30)	33.60 \pm 0.41 ^A (30)	100.87 \pm 5.49 ^A (3)	152.83 \pm 8.31 ^A (3)
Small Pup	3.42 \pm 0.10 ^A (29)	12.25 \pm 0.36 ^A (29)	35.86 \pm 1.05 ^A (29)	34.21 \pm 0.27 ^A (29)	92.58 \pm 10.39 ^{AB}	144.22 \pm 7.22 ^A (2)
Large Pup	4.58 \pm 0.09 ^B (45)	16.03 \pm 0.39 ^B (45)	48.01 \pm 1.21 ^B (45)	33.49 \pm 0.24 ^A (45)	77.81 \pm 1.91 ^{AB} (2)	151.55 \pm 6.30 ^A (2)
Juvenile	4.99 \pm 0.11 ^C (40)	18.04 \pm 0.40 ^C (40)	53.54 \pm 1.26 ^C (40)	33.74 \pm 0.21 ^A (40)	72.60 \pm 0.57 ^B (3)	153.69 \pm 1.88 ^A (3)
Adult	5.15 \pm 0.06 ^C (9)	19.01 \pm 0.23 ^C (9)	56.49 \pm 1.36 ^C (9)	33.81 \pm 0.48 ^A (9)	84.07 \pm 7.35 ^{AB} (3)	173.47 \pm 9.85 ^A (3)

Table 3.3: Summary of muscle parameter values for each age class (means \pm SE). Superscripts display connecting letters report from Tukey’s post-hoc pairwise comparisons. Sample size (n) is given in parentheses after each value.

Age Class	<i>Longissimus dorsi</i> [Mb] (g 100 g muscle ⁻¹)	<i>Gracilis</i> [Mb] (g 100 g muscle ⁻¹)	Average [Mb] (g 100 g muscle ⁻¹)	Muscle Mass (% total body mass)
Neonate	0.34 \pm 0.05 ^A (9)	0.34 \pm 0.06 ^A (9)	0.34 \pm 0.06 ^A (9)	21.46 \pm 0.57 ^A (5)
Small Pup	1.03 \pm 0.27 ^A (3)	1.06 \pm 0.24 ^A (3)	1.04 \pm 0.25 ^A (3)	21.35 \pm 0.94 ^{AB} (4)
Large Pup	2.10 \pm 0.10 ^B (8)	2.10 \pm 0.16 ^B (8)	2.10 \pm 0.13 ^B (8)	27.19 \pm 1.64 ^{BC} (3)
Juvenile	2.47 \pm 0.30 ^B (5)	2.56 \pm 0.22 ^{BC} (5)	2.52 \pm 0.24 ^B (5)	30.19 \pm 0.25 ^C (3)
Adult	3.43 \pm 0.10 ^C (22)	3.21 \pm 0.12 ^C (22)	3.32 \pm 0.10 ^C (22)	32.78 \pm 2.08 ^C (4)

Table 3.4: Summary of lung mass and lung volume parameters for each age class (means \pm SE). Superscripts display connecting letters report from Tukey's post-hoc pairwise comparisons. Sample size (n) for each age class is given in parentheses.

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

Table 3.5: Age-specific oxygen storage capacity of blood, muscle, and lungs (mean \pm SE), total body oxygen storage capacity, diving metabolic rate, and cADL of southern sea otters. DLV and TLC denote the assumed lung volume. Superscripts display connecting letters report from Tukey’s post-hoc pairwise comparisons.

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	11.92 \pm 0.22 ^A	0.95 \pm 0.16 ^A	42.65 \pm 3.09 ^{AB} / 71.23 \pm 5.16 ^{AB}	55.52 / 83.95	35.38	1.57 / 2.37
Small Pup	11.15 \pm 0.33 ^A	2.93 \pm 1.24 ^A	48.73 \pm 7.31 ^A / 81.38 \pm 17.26 ^A	62.81 / 94.16	30.82	2.04 / 3.06
Large Pup	15.72 \pm 0.38 ^B	7.93 \pm 1.47 ^B	41.63 \pm 4.56 ^{AB} / 69.54 \pm 7.62 ^{AB}	65.28 / 91.80	24.34	2.68 / 3.77
Juvenile	18.06 \pm 0.40 ^C	10.12 \pm 2.19 ^B	41.64 \pm 1.60 ^{AB} / 69.54 \pm 4.62 ^{AB}	69.82 / 97.25	23.05	3.03 / 4.22
Adult	22.83 \pm 0.26 ^D	14.67 \pm 2.03 ^C	31.05 \pm 2.30 ^B / 51.85 \pm 3.84 ^B	68.70 / 89.45	21.6	3.18 / 4.14

* Values adapted from Thometz et al. (2014) for immature age classes and Yeates et al. (2007) for adults

Table 3.6: Estimated mass-specific positive buoyant forces experienced by sea otters due to air in pelage and lungs. Equations 1 & 2 from Skrovan et al. (1999) were used to determine total positive buoyancy due to pelage and lungs across a range of depths (0 m, 5 m, 10 m, 20 m), not net buoyancy, as the density and corresponding negative buoyancy of sea otter tissue was not accounted for. Positive buoyant forces were calculated assuming both TLC and 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).

Depth	Neonate ($N kg^{-1}$)		Small Pup ($N kg^{-1}$)		Large Pup ($N kg^{-1}$)		Juvenile ($N kg^{-1}$)		Adult ($N kg^{-1}$)	
	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

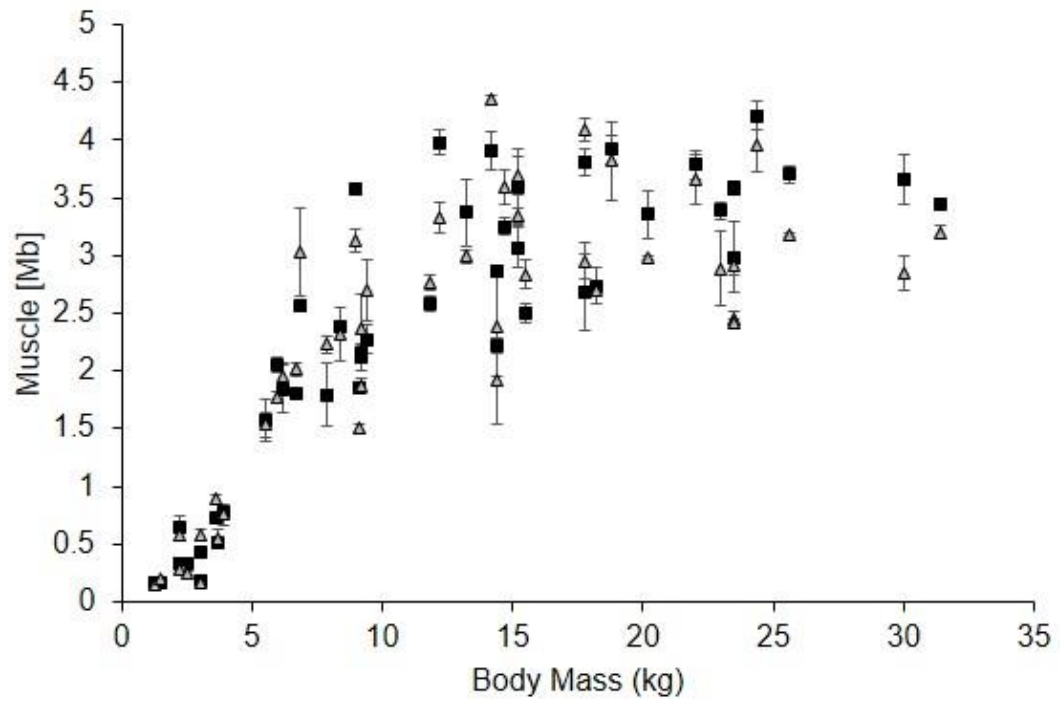


Figure 3.1: Muscle [Mb] of two major locomotor muscles, the *longissimus dorsi* (black squares) and *gracilis* (grey triangles), in relation to body mass. Samples were run in triplicate and error bars represent the standard deviation associated with each sample.

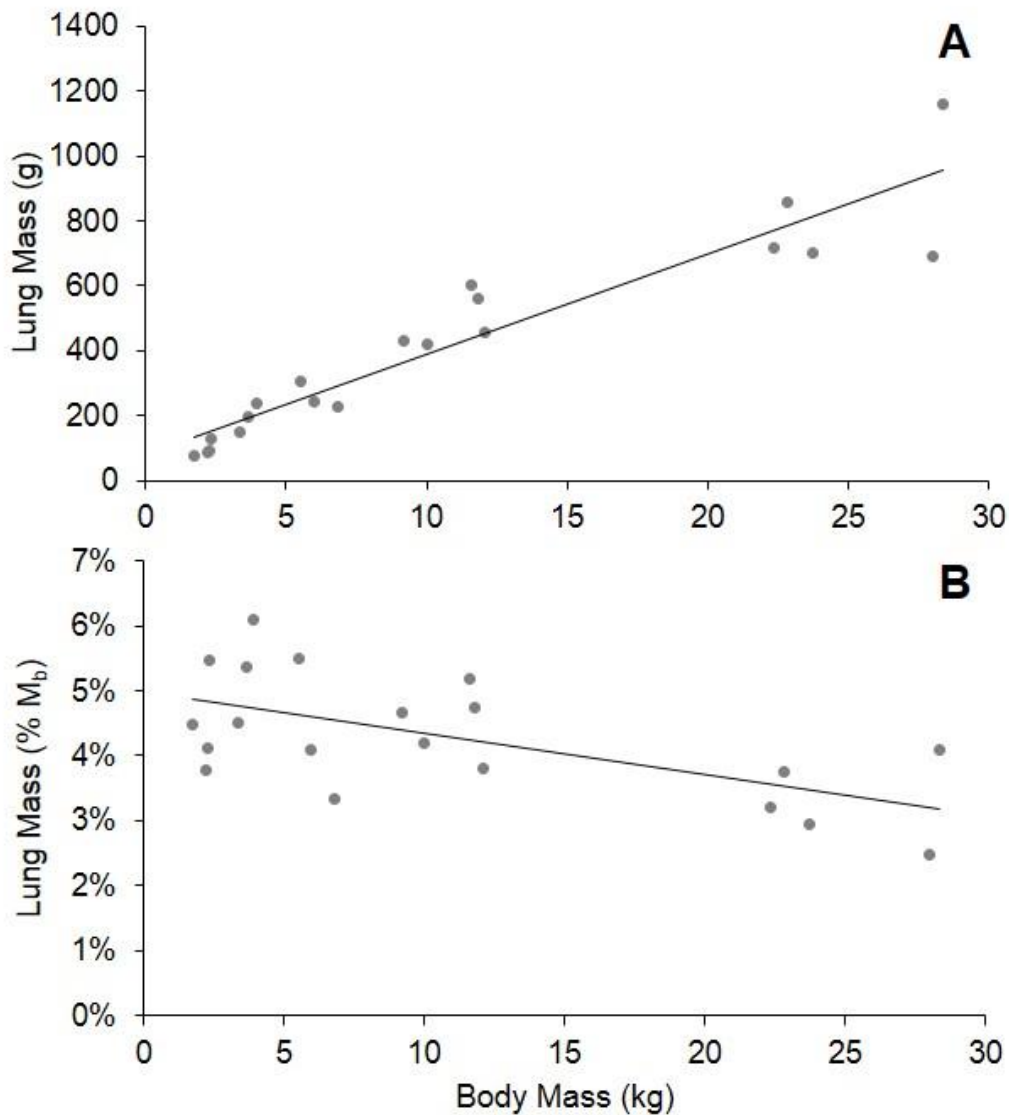


Figure 3.2: The relationships between (A) total lung mass and total body mass and (B) lung mass as a percentage of body mass and total body mass. Lines display results of least squares linear regression. Total lung mass increased as a function of total body mass following the equation: $M_l = 30.82 \times M_b + 81.12$ ($n = 20$, $r^2 = 0.88$, $P < 0.001$). Lung mass as a percentage of body mass decreased as a function of total body mass following the equation: $M_l = -0.00063 \times M_b + 0.05$ ($n = 20$, $r^2 = 0.35$, $P = 0.0038$).

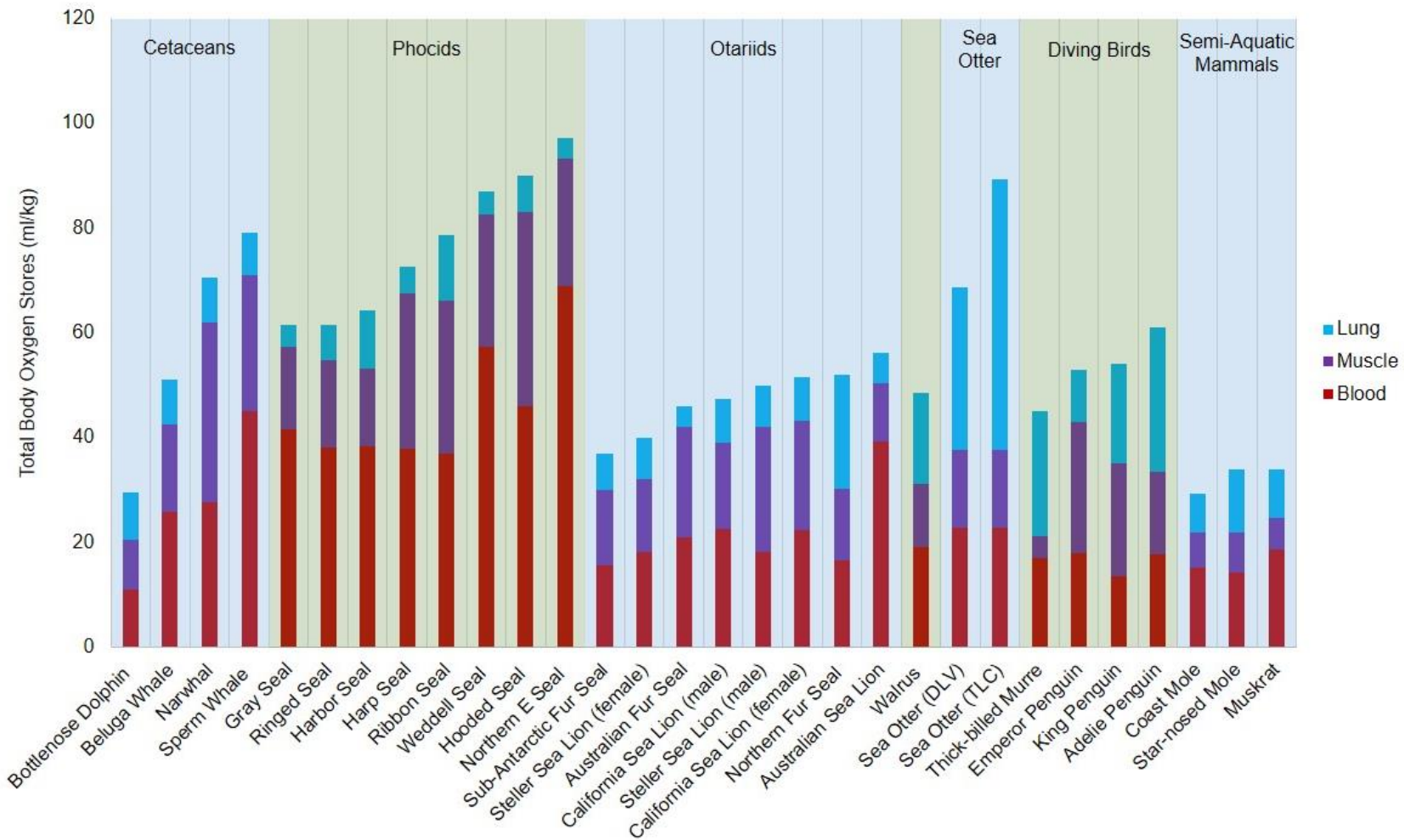


Figure 3.3: Mass-specific total body oxygen stores of adult sea otters (assuming both DLV and TLC) in comparison to a variety of marine divers and semi-aquatic mammals. The height of each bar represents the total oxygen storage capacity for a given species, while

each stacked bar indicates the proportional contribution of lung (blue), blood (red), and muscle (purple) oxygen stores to total body oxygen storage capacity. Species compared include the bottlenose dolphin (Noren et al., 2001; Noren et al., 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., 2011b), Steller sea lion (Richmond et al., 2006), Australian fur seal (Spence-Bailey et al., 2007), California sea lion (Weise and Costa, 2007), northern fur seal (Lenfant et al., 1970), Australian sea lion (Fowler et al., 2007), walrus (Lenfant et al., 1970), thick-billed murre (Croll et al., 1992), emperor penguin (Kooyman and Ponganis, 1998), king & Adelie penguins (Sato et al., 2002), coast mole (McIntyre et al., 2002), star-nosed mole (McIntyre et al., 2002), and muskrat (MacArthur et al., 2001).

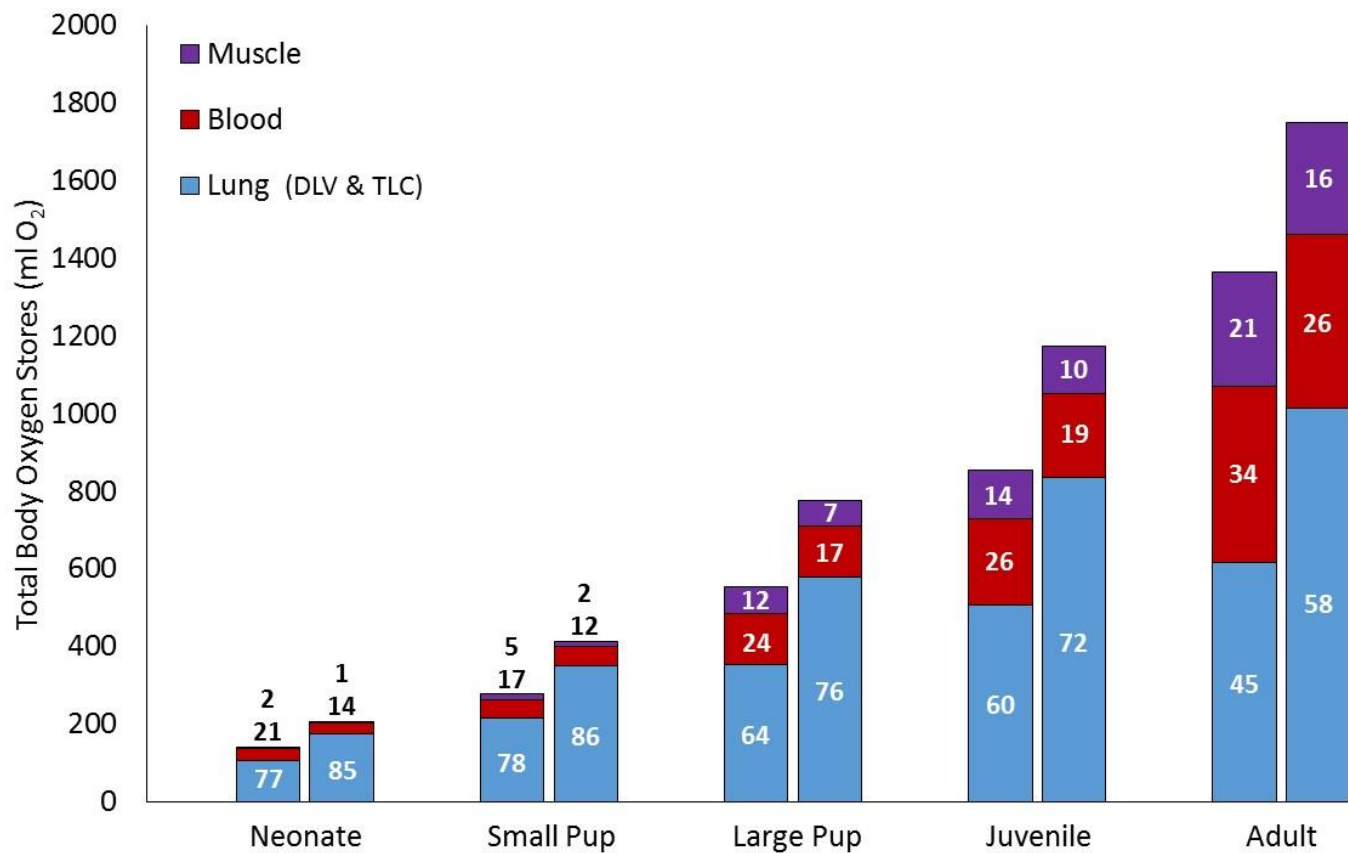


Figure 3.4: Total oxygen storage capacity of sea otters across age classes assuming both DLV (left bar) and TLC (right bar). The height of each bar represents total body oxygen storage capacity, while each stacked bar indicates the proportional contribution of lung (blue), blood (red) and muscle (purple) oxygen stores to the total oxygen 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.

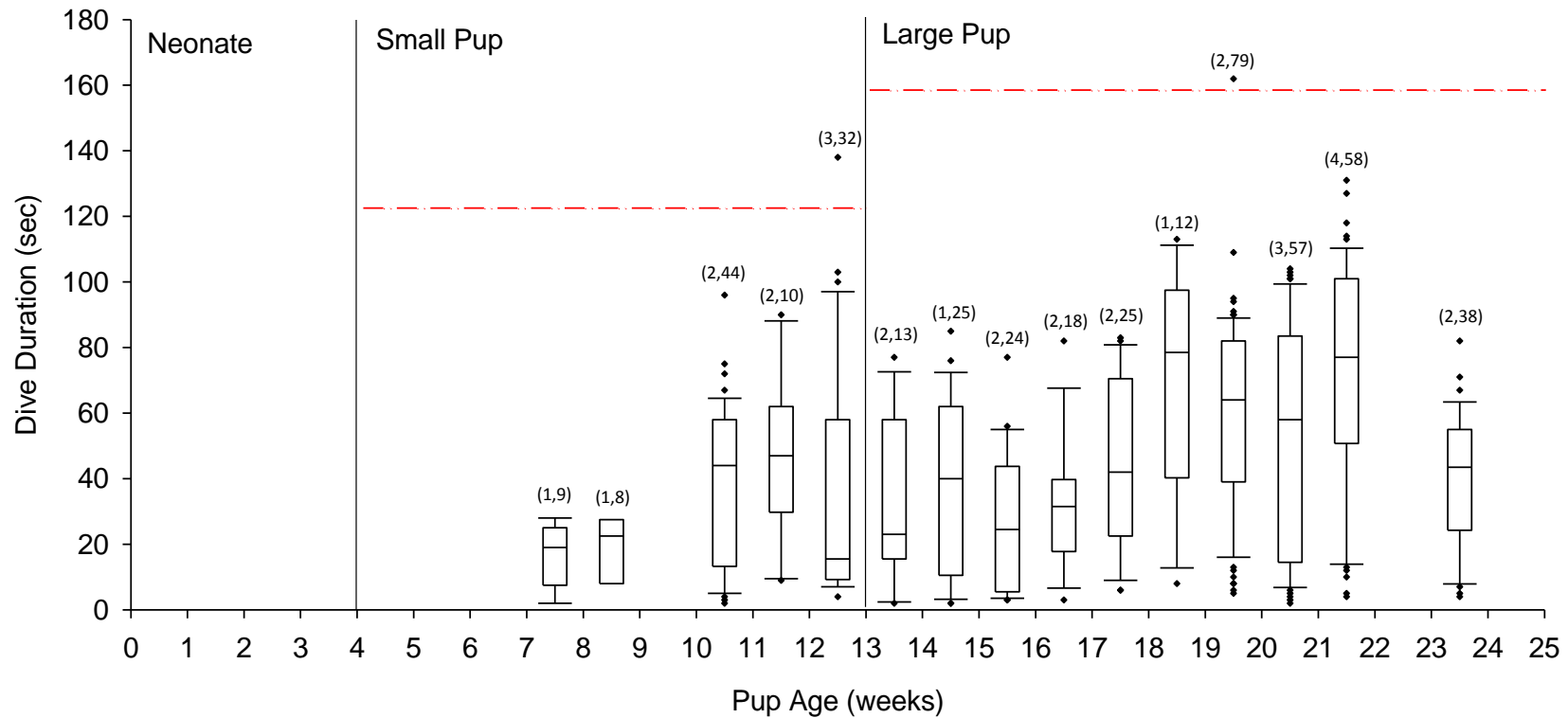


Figure 3.5: Box plot of dive durations (s) for immature sea otters, collected from 9 known-age dependent sea otters off the coast of Big Sur, CA. Data from all individuals were pooled into weekly age groups. The middle horizontal line of each box denotes the median, boxes define quartiles, and vertical 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 (#) are displayed above each box (n,#). Dependent age classes, as defined in the current study, are identified at the top of the figure. Horizontal red dash-dot lines denote the age-specific cADLs (assuming DLV) 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, CA (USGS, unpublished data).

CHAPTER 4

Diving and Foraging Behavior of Sub-Adult and Adult Sea Otters in Central California

Abstract

Archival time-depth recorders (TDRs) were used to examine detailed foraging behavior of southern sea otters off the coast of central California. Of particular interest was the influence of sex, reproductive stage, and age on variation in dive attributes and foraging behavior. Between 2007 and 2010, 96 sea otters were instrumented with TDRs and between 2008 and 2011, 38 of those TDRs were recovered for analysis. During the first three weeks postpartum, females markedly reduced percent time foraging, mean foraging bout duration, and the range of depths they utilized while foraging. In contrast, females with large pups (>10 weeks old) exhibited the longest mean foraging bout duration (138.13 ± 11.27 min), highest mean number of dives per bout (59.29 ± 6.86), and shortest post-dive interval (78.03 ± 6.23 s) of all adult groups. In addition, females with pups increased ascent rates, descent rates, and bottom time to dive time ratios in comparison to females without pups. Sub-adults (1-3.5 years) and females with large pups were the only groups to spend a greater proportion of each day foraging compared with inactive. The mean dive depth of sub-adults (7.87 ± 1.74 m) was comparable to

adult females without pups (7.65 ± 0.89 m), the mean foraging bout duration of sub-adults (138.60 ± 14.98 min) was nearly identical to adult females with large pups, and the maximum dive duration of sub-adults (295.20 ± 25.25 s) was the longest of all groups, even exceeding that of males (280.50 ± 29.66 s). Males exhibited the deepest maximum dive depth (68.38 ± 4.35 m) and broadest range of foraging depths when compared with all other groups. Ultimately, females with large pups and sub-adults exhibited the greatest foraging effort across a variety of parameters including, percent time foraging, foraging bout duration, number of dives per bout, and post-dive intervals. This suggests that these life stages may have the least behavioral flexibility to respond to further reductions in the distribution and abundance of prey resources.

Introduction

Sea otters (*Enhydra lutris*) are voracious benthic foragers with exceptionally high metabolic demands and limited energetic reserves (Bodkin et al., 2004; Costa and Kooyman, 1982; Costa and Kooyman, 1984; Kenyon, 1969; Morrison et al., 1974; Thometz et al., 2014; Williams, 1989; Yeates et al., 2007). For all marine foragers, diving ability and behavior are critical components of successful foraging (Kooyman, 1989); however, sea otters may be especially challenged, both physiologically and behaviorally, given the volume of prey resources they must consume on a daily basis (Costa and Kooyman, 1984; Riedman and Estes, 1990; Yeates et al., 2007). Recent studies have shown that daily energetic demands and foraging abilities of sea otters differ markedly by life stage (Thometz et al., 2014; Chapter 3). Furthermore, factors such as population density, sex, reproductive stage, and prey specialization are known

to influence sea otter foraging behavior (Bodkin et al., 2004; Esslinger et al., 2014; Estes et al., 1982; Gelatt et al., 2002; Tinker et al., 2007; Tinker et al., 2008). How these physiological and behavioral factors act synergistically to influence foraging in wild sea otters is not well understood.

Although the average daily metabolic demands of sea otters are already staggering in comparison to other marine mammals (Costa and Williams, 1999), these demands increase substantially during key life stages (Thometz et al., 2014). In sea otters, lactating females exhibit the highest daily energy demands of all life stages (Thometz et al. 2014). Because adult females give birth to a single pup once a year and dependency lasts for an average of six months, females may spend nearly half of their adult lives in this exceedingly demanding physiological state (Jameson and Johnson, 1993; Riedman et al., 1994). Increased energy demands during lactation (Thometz et al., 2014) coupled with the additional behavioral demands associated with rearing young (Jameson and Johnson, 1993; Staedler, 2011) act to influence foraging behavior in adult females. In both northern (Gelatt et al., 2002) and southern (Staedler, 2011) populations, females greatly reduce percent time foraging in the first weeks postpartum and then progressively increase percent time foraging throughout pup dependency. We hypothesize that lactating females alter many other aspects of routine diving and foraging behavior in relation to increased energetic demands, age-specific foraging abilities of dependent young, and parental provisioning responsibilities.

Age-related differences in foraging behavior and efficiency are common across a wide variety of species (Brandt, 1984; Breed et al., 2011; Buckley and Buckley, 1974; Field et al., 2005; Horning and Trillmich, 1999; Jeglinski et al., 2012; Vaillant et al.,

2013). The development of diving behavior has been described for dependent sea otter pups (Payne and Jameson, 1984), but less is known about the foraging behavior and efficiency of juveniles (Ralls and Siniff, 1990) and sub-adults. Research has shown that sea otters exhibit limited physiological capacities during dependency and do not develop comparable diving abilities to adults until after their first year post-weaning (Chapter 3). Therefore, prolonged physiological development and behavioral immaturity may result in marked differences in the foraging behavior of juvenile, sub-adult, and adult sea otters.

At a broader scale, population density significantly influences the amount of time sea otters spend foraging per day in both northern and southern populations. For example, sea otters in areas close to or at carrying capacity forage for significantly greater amounts of time than those well under carrying capacity (Estes et al., 1978; Estes et al., 1982; Estes et al., 1986; Gelatt et al., 2002; Riedman and Estes, 1990; Tinker et al., 2008). This is the result of a progressive reduction in prey resources with increasing otter abundance (Estes et al., 1978). Thus, southern sea otters (*Enhydra lutris nereis*) forage for approximately 25% of the day off the coast of San Nicolas Island, a low sea otter density area with abundant resources, compared to 40% in central California where sea otter density is high and prey resources are limited (Tinker et al., 2008). In addition, studies have demonstrated that sea otter foraging behavior is influenced by a number of other variables including sex, reproductive stage, and prey specialization (Bodkin et al., 2004; Estes et al., 1982; Gelatt et al., 2002; Staedler, 2011; Tinker et al., 2007; Tinker et al., 2008).

Bodkin et al. (2004) was the first to utilize time-depth recorders (TDR) to describe detailed characteristics of sea otter diving behavior. The study was conducted in Port Althorp, Alaska and examined differences between adult male and adult female sea otters. Most recently, Esslinger et al. (2014) examined differences in adult sea otter foraging effort across a variety of variables including sex, reproductive stage, temperature, and daylight in Prince William Sound, Alaska. The authors found that the majority of variation in foraging effort between study animals was due to within-otter variation, with reproductive stage and environmental factors contributing significantly to the observed variance (Esslinger et al., 2014). Here we expand on these previous studies to investigate how physiological and behavioral constraints experienced during specific life stages may act to influence dive attributes and foraging behavior of sea otters in central California.

Using TDRs implanted in wild sea otters we specifically examined the relative contribution of sex, reproductive status, and age to observed variation in the foraging behavior of southern sea otters in Monterey and Big Sur, California. Study animals were classified into six demographic groups: females without pups, females with very small pups (>3 weeks), females with small pups (3-10 weeks), females with large pups (>10 weeks), sub-adults (1-3.5 years), and adult males. Using these data, we tested three hypotheses: (1) owing to their larger body size and lack of parental investment, males exhibit the greatest range of foraging depths and durations, (2) reproductive stage influences diving and foraging behavior of adult females, and (3) sub-adults exhibit limited diving and foraging behaviors in comparison to adult conspecifics. Lastly, given that sea otters are believed to be resource limited in the central portion of their

current range (Tinker et al., 2008), we quantified how often individuals reached or exceeded calculated aerobic dive limits (cADL; Chapter 3) while foraging to determine if sea otters in resource limited habitats are pushing their physiological limits in order to meet daily energy demands.

Methods

Study Animals and TDR Implantation

Ninety-eight free-ranging sea otters were captured, tagged, and implanted with intra-abdominal VHF radio transmitters (7.6 cm x 10.2 cm x 2.5 cm, 160 g; Advanced Telemetry Systems Inc., Isanti, MN, USA) and time-depth recorders (TDRs, 67 mm x 17 mm x 17 mm, 27 g, Wildlife Computers, Redmond, WA) along the Monterey Peninsula (n = 53) and Big Sur (n = 45), CA from September 2007 to December 2010. Individuals were captured and surgically implanted following standardized procedures (Ames et al., 1986; Monson et al., 2001; Williams and Siniff, 1983). Two colored plastic flipper tags (Temple Tags, Temple, TX, USA) were attached in the webbing of each hind flipper for visual identification in the field (Ames et al., 1986). Sea otters were observed from shore and behavioral data were collected by direct observation utilizing a 50 x 80 spotting scope (Questar, Inc., New Hope, PA, USA).

Thirty eight of the 98 implanted TDRs were retrieved (25 adult females; 8 adult males; 5 sub-adult females) between August 2008 and November 2011 (Table 4.1). TDRs were retrieved by recapturing a study animal and removing its instruments using standardized procedures (Ames et al., 1986; Monson et al., 2001; Williams and Siniff, 1983), or recovered during routine necropsy after a study animal had died and its

carcass was found and retrieved from the study area. Archived data from TDRs ranged from 66 days to over 1094 days, dependent on an individual otter's survival and the battery life of the TDR, for a total of 377,496 hours of recorded data. Data from both Big Sur (8 adult females; 2 adult males; 1 sub-adult female) and Monterey (17 adult females; 6 adult males; 4 sub-adult females) sea otters were used in analyses. Individuals greater than 3.5 years old were classified as adults, while individuals between the ages of 1-3.5 years were classified as sub-adults (Tinker et al., 2006).

Because TDRs record diving behavior for an average of 1–2 years, many females in this study gave birth to (and raised until weaning) one or more pups during the period of TDR data logging. This allowed for an examination of the effects of reproductive stage on diving behavior. Specifically, females were categorized into one of four groups based on the presence and age of their pup: female with no pup (F_{NP}), female with very small pup (F_{VSP} , pup ≤ 3 weeks old), female with small pup (F_{SP} , pup $> 3 \leq 10$ weeks old), and female with large pup (F_{LP} , pup > 10 weeks old). Data sets for each female were divided into the appropriate category based on their reproductive stage ($F_{NP} - F_{LP}$) on each day of TDR data logging and we treated each of these data sets as an independent record for statistical analyses. Together with similar records from male (M) and sub-adult (SA) animals, 6 demographic groups were used for statistical comparisons. Of the sub-adult study animals, two gave birth during the course of TDR data logging but neither were successful at rearing their pup past the first few weeks postpartum. Therefore, only foraging data from periods when sub-adult individuals were without pups were used in statistical comparisons to maintain consistency among sub-adults.

TDR Analyses

Raw TDR data were downloaded from the TDR instruments and pre-processed using the software “Instrument Helper” (version 3.0; Wildlife Computers, Redmond WA) to correct depth readings for drift from the zero mark, and compile the depth readings (at 2 s intervals) into distinct dives and contiguous surface intervals. For each identified dive, a series of six descriptive parameters was calculated (all depths in meters and all time in seconds): maximum dive depth (DEP), duration of the sub-surface interval (DT), duration of time spent at the bottom of the dive (BT, = 90% of maximum dive depth and thus not necessarily on the ocean floor), duration of the post-dive surface interval (PDI, the number of seconds elapsed until the next dive), descent rate (DRT, vertical swim speed from surface to bottom) and ascent rate (ART, vertical swim speed from bottom to surface). These parameters were used to classify dives as feeding or non-feeding dives (i.e. dives conducted during grooming, traveling, or social behavior), following previously published methods (Bodkin et al. 2004; Bodkin et al. 2007). Briefly, a logistic classification function was used to categorize each recorded dive based on a combination of the above described parameters:

$$\log\left(\frac{P}{1-P}\right) = \beta_1 + \beta_2(DT) + \beta_3\left(\frac{BT}{DT}\right) + \beta_4(ART) + \beta_5(DT)(ART) + \beta_6\left(\frac{BT}{DT}\right)(DRT) \quad (1)$$

where β_i is a vector of parameters fit by maximum likelihood, and all dives with $P > 0.5$ were classified as feeding dives. Equation 1 was initially fit to a sub-sample of 5,000 confirmed dives (dives made by study animals which an observer was able to visually confirm as either 1 = feeding or 2 = non-feeding), and then validated by application to

a second sub-sample of 1,000 confirmed dives, to ensure a classification accuracy of >99%. Best fit values for β_i were:

$$[-5.115, 0.258, 0.487, -0.210, 0.063, 3.543]$$

We applied equation 1 to each TDR record to classify all dives, and then sub-divided the entire TDR record into contiguous “bouts” of similar activity states (inactive/resting = R, feeding = F, and active-other = AO).

The net result of the above pre-processing steps was a sample of 86,441 feeding bouts comprising 3,857,794 foraging dives. Because individual dives within a feeding bout tended to be auto-correlated with respect to some dive parameters, we used averages across feeding bouts as the basic statistical unit in all further analyses to ensure independence. For each distinct feeding bout we calculated a series of 12 statistics (dive attributes) with MATLAB 8.0 programming software (The Mathworks Inc., Natick, MA, USA). The dive attributes of interest included mean values for: number of dives per bout, foraging bout duration (min), dive depth (m), dive duration (s), post-dive interval (PDI; s), variance in post-dive interval (Variance PDI; s), bottom time (BT; s), the ratio of bottom time to dive time (BT/DT), maximum dive depth (m), maximum dive duration (s), descent rate (m/s), and ascent rate (m/s). For every 24-hour period of each TDR record, we calculated a time-activity budget by summing the cumulative time spent in each of the three activity states (F, AO, & R). Time-activity budgets were then summarized for all study animals, with each sea otter classified by demographic group and location.

Statistical Analyses

Foraging dive attributes were first analyzed using a three-factor multivariate permutational analysis of variance (PERMANOVA), in which demographic group was a fixed effect (six levels: F_{NP}, F_{VSP}, F_{SP}, F_{LP}, SA, M), location was a random effect (two levels: Big Sur and Monterey), and the interaction between class and location was evaluated as a third factor. We then used a two-factor PERMANOVA to test for differences between dive attributes by class and location without the interaction term. All dive attributes were normalized before analyses, Bray-Curtis similarity and 999 permutations were used. Pairwise comparisons for the reduced model (two-factor PERMANOVA) were made *a posteriori*. Similarity percentages (SIMPER) analyses were used to identify the relative contribution of each variable to explaining the overall variance determined by the model. The SIMPER routine was also used to determine the relative contribution of each dive attribute to differences observed in all pairwise comparisons between demographic groups. All PERMANOVA and SIMPER analyses were made using PRIMER 6 software with PERMANOVA+ (Plymouth Marine Laboratory, UK) (Anderson et al., 2008)

Differences in activity budgets by both demographic group and location were tested utilizing MANOVA with Pella's Trace to determine significance. Location was not significant and therefore dropped in subsequent analyses. Single-factor ANOVAs with Tukey's post-hoc comparisons were run to examine differences among demographic groups for each activity state (F, AO, & R). All MANOVA and ANOVA analyses were completed using JMP®10 statistical software program (SAS Institute

INC., 2012). All means are reported \pm standard error (\pm SE) and results were considered significant if $P < 0.05$.

Results

Across all individuals and locations, mean dive depth was 9 m and mean dive duration was 60 s. Of all foraging dives, 99% occurred within 37 m in Big Sur and 39 m in Monterey. The deepest recorded dive in the current study was made by an adult male in Big Sur to 87 m, while the longest recorded dive was 472 s by a sub-adult in Monterey. In contrast to previous reports of diving behavior in northern sea otters (Bodkin et al. 2004), none of the individuals in this study exhibited a bimodal pattern of foraging depth; however, this was likely due to a difference in prey resources between northern and southern populations, rather than a fundamental difference in the behavior or diving capacities of these two sub-species.

When examining the data across all individuals, certain variables were inherently correlated. The number of dives per foraging bout increased linearly as foraging bout duration increased ($y = 0.4768x - 8.2042$; $n = 87$; $r^2 = 0.78$; $P < 0.001$). Similarly, mean dive duration increased linearly with mean dive depth ($y = 3.3148x + 32.946$; $n = 87$; $r^2 = 0.80$; $P < 0.001$), however the ratio of bottom time to dive time remained consistent across the range of mean dive depths ($n = 87$, $r^2 = 0.002$, $P = 0.62$). When examining surface intervals between dives, variance in PDI increased linearly as mean PDI increased ($y = 0.6245x + 29.916$; $n = 87$; $r^2 = 0.75$; $P < 0.001$). Recognizing these inherent correlations, we found that specific foraging dive attributes differed

greatly by demographic group and proceeded to examine these differences utilizing PERMANOVA and SIMPER analyses.

Dive Attributes - Analyses

Based on our three-factor model, dive attributes differed significantly by demographic group ($pseudo-F_{5,75} = 8.21$, $p = 0.001$), but not by location ($pseudo-F_{1,75} = 0.89$, $p = 0.44$) or by their interaction ($pseudo-F_{5,75} = 0.48$, $p = 0.96$). With the reduced model, demographic group remained highly significant ($pseudo-F_{5,80} = 5.01$, $p = 0.001$) and location remained non-significant ($pseudo-F_{1,80} = 1.97$; $P = 0.115$). Because location was not significant in either model, that term was dropped from additional analyses and we evaluated the proportional contribution of all foraging dive attributes to the characterization of each demographic group utilizing SIMPER analysis (Fig. 4.1). Mean dive duration was one of the most important variables for characterizing all demographic groups except F_{VSP} . Mean dive depth and bottom time contributed significantly to the characterization of all female groups except F_{VSP} (Fig. 4.1). Mean PDI, variance PDI, BT/DT, and descent rate, cumulatively contributed (c.c.) 56.7% to the characterization of F_{VSP} (Fig. 4.1B). F_{LP} and sub-adults were the only groups in which mean number of dives per bout was among the highest contributing dive attributes (F_{LP} : 9.92%; SA: 16.41%) to their characterization (Fig. 4.1D & 4.1E). Maximum dive duration alone contributed 34.5% to the characterization of adult males (Fig. 4.1F).

Pairwise tests were run for all demographic groups and nearly all (10 of 15 comparisons) were significantly different ($p < 0.05$) from one another (Table 4.2), with the overall difference between males and sub-adults being marginally significant ($t =$

1.59, $p = 0.062$). Subsequently, the proportional contribution of each variable to the differences observed in pairwise comparisons was examined by SIMPER analysis (Table 4.2). Dive attributes that contributed the most to differentiating F_{NP} and F_{VSP} were variance PDI, ascent rate, descent rate, and mean PDI (c.c. = 44.85%). For adult males, maximum dive depth and maximum dive duration were the two highest contributing variables (c.c. = 29.94-39.79%) in all pairwise comparisons with adult female groups (Table 4.2). Maximum dive depth and maximum dive duration also contributed greatly to the differentiation between sub-adults and F_{VSP} (c.c. = 24.84%), and sub-adults and F_{SP} (c.c. = 28.22%). Mean number of dives per bout contributed greatly to the differentiation of sub-adults and F_{VSP} (11.48%), as well as sub-adults and F_{SP} (8.49%) (Table 4.2).

Dive Attributes – Overall Patterns

To examine differences in dive attributes and foraging behavior in adult sea otters by sex alone, we compared males to females without pups (F_{NP}). Mean foraging bout duration differed markedly between males and F_{NP} (Fig. 4.2A), as foraging bout durations of F_{NP} lasted on average 18 min longer than males (Table 4.3). In addition, F_{NP} made more dives per foraging bout than male conspecifics (Fig. 4.2B; Table 4.3). Males exhibited deeper mean dive depths and longer mean dive durations than F_{NP} (Table 4.3), and more prominent differences were observed in maximum dive depths and dive durations (Fig. 4.2C & 4.2D; Table 4.3) between males and F_{NP} . Lastly, males exhibited longer and more variable PDIs than F_{NP} (Table 4.3).

Reproductive stage strongly influenced the diving and foraging behavior of adult females and as lactation progressed, females exhibited similar changes in foraging

behavior across a number of parameters (Fig. 4.2). For example, females reduced mean foraging bout duration (Fig. 4.2A) and mean number of dives per foraging bout (Fig. 4.2C) when caring for very small pups. Lactating females progressively increased foraging bout duration and number of dives per foraging bout over the course of dependency, to the extent that F_{LP} had a greater mean foraging bout duration and made more dives per foraging bout than F_{NP} (Fig. 4.2A & 4.2B; Table 4.3). Mean dive duration gradually increased throughout lactation from 59.69 ± 3.86 s in F_{VSP} to 66.88 ± 5.63 s in F_{LP} (Table 4.3). Maximum dive depth (Fig. 4.2C) and maximum dive duration (Fig. 4.2D) were reduced in all lactating female groups compared to F_{NP} . F_{VSP} exhibited the longest and most variable PDIs when compared with all other groups. Furthermore, F_{VSP} and F_{SP} had the fastest mean descent and ascent rates of all groups (Table 4.3).

Sub-adults made the greatest number of dives per bout (63.68 ± 9.11) of all groups (Fig. 4.2B). Along with F_{LP} , sub-adults exhibited the longest mean bout durations (SA: 138.60 ± 14.98 min; F_{LP} : 138.13 ± 11.27 min) of all groups (Fig. 4.2A). Sub-adults exhibited a deeper maximum dive depth (57.00 ± 3.37 m) and longer maximum dive duration (296.20 ± 25.26 s) than all adult female groups (Fig. 4.2C & 4.2D). Although males exhibited the deepest maximum dive depth of all groups (68.38 ± 4.35 m), they were second to sub-adults for the longest maximum dive duration (Table 4.3). Lastly, sub-adults exhibited relatively short mean PDIs in comparison to all other demographic groups, but were most comparable in PDI duration and variance to F_{NP} and F_{LP} (Table 4.3).

Distribution of Dive Depths

There was considerable overlap in the range of depths utilized by sea otters in this study, with the majority of dives occurring between 2-10 m for all groups; however, there were notable differences in the proportion of dives each demographic group made to specific depths (Fig. 4.3). In the first weeks postpartum, females reduced the relative proportion of foraging dives made between 2-10 m (F_{NP} : $74.07 \pm 5.13\%$; F_{VSP} : $61.89 \pm 7.57\%$), while greatly increasing the proportion of dives made to depths of 11-20 m (F_{NP} : $19.58 \pm 3.52\%$; F_{VSP} : $34.10 \pm 6.77\%$) (Fig. 4.3A). F_{VSP} also reduced the proportion of foraging dives that were deeper than 20 m. As dependent pups grew, females gradually reduced percent time foraging between 11-20 m and broadened the range of foraging depths they utilized (Fig. 4.3A). In fact, F_{SP} and F_{LP} exhibited a greater proportion of foraging dives to depths between 30-45 m when compared to females without pups. Sub-adults displayed a strong similarity in foraging dive depth proportions to adult females without pups (Fig. 4.3B). The distribution of foraging dives for adult males was right-shifted compared to adult females and sub-adults, with males diving to depths >20 m more frequently than all other groups (Fig. 4.3C).

Distribution of Dive Durations

All demographic groups exhibited considerable overlap in routine foraging dive durations (Fig. 4.4) with some exceptions. F_{VSP} displayed a narrower dive duration distribution than F_{NP} with the greatest proportion of dives between 21-60 s, and distinctly limited the proportion of dives they made of increasing duration (Fig. 4.4A). The dive duration distribution of F_{LP} was shifted to the right (Fig. 4.4A), with the greatest proportion of dives lasting between 40-80 s. Of all female groups, the greatest

proportion of dives lasting longer than 2 min were performed by F_{LP} (Fig. 4.4A); which informs why the mean dive duration of F_{LP} was longer than that of F_{NP} (Table 4.3), despite the fact that F_{NP} recorded the longest maximum dive duration of all adult female groups (Fig. 4.2D; Table 4.3).

Approximately 65% of sub-adult dives were 41-80 s in duration (Fig. 4.4B). Adult males exhibited the greatest range and variability in dive durations, and made more dives lasting ≥ 2 min than any other group (Fig. 4.4C). Across all study animals and demographic groups, individuals rarely exceeded ($>2\%$ of all dives) calculated aerobic dive limits as determined for sea otters in Chapter 3. Formally, an aerobic dive limit (ADL) is the amount of time an individual can remain underwater foraging before there is an increase in blood lactate above resting levels, indicating a shift to anaerobic metabolism, which is considered less efficient than aerobic diving (Kooyman et al., 1983). Aerobic dive limits are often estimated (cADL) by dividing total body oxygen stores by oxygen demand (Kooyman, 1989).

Activity Budgets

Activity budgets differed by demographic group (MANOVA: $F_{15,240} = 4.46$, $p < 0.0001$), but not by location ($F_{3,78} = 2.14$, $p < 0.1025$). Of the possible activity states, the amount of time individuals spent foraging (ANOVA: $F_{5,81} = 26.31$, $p < 0.0001$) and inactive (ANOVA: $F_{5,81} = 27.83$, $p < 0.0001$) differed by demographic group (Fig. 4.5). F_{LP} and sub-adults were the only groups that spent a greater proportion of each day foraging (F_{LP} : 49.6 ± 1.33 ; SA: 47.31 ± 1.80) rather than inactive (F_{LP} : 41.80 ± 1.40 ; SA: 44.61 ± 1.40). F_{VSP} showed significantly reduced foraging behavior (27.68 ± 1.99 , $p < 0.0001$) and increased percent time inactive (64.12 ± 2.20 , $p < 0.0001$) compared to all

other groups (Fig. 4.5). Percent time spent engaged in active-other behavior remained consistent (7-8%) across all demographic groups ($F_{5,81} = 0.1028$, $p = 0.9913$). It is important to note that because some active behaviors at the surface (i.e. swimming and grooming at the surface) are undetectable by TDRs, it is likely that for all demographic groups active-other behavior was underestimated and inactive behavior overestimated.

Discussion

A number of pinniped species, including Galapagos sea lions (Villegas-Amtmann et al., 2008), northern elephant seals (Field et al., 2005), and gray seals (Breed et al., 2006) exhibit spatial segregation in utilized foraging areas, which ostensibly reduces competition between individuals. Although male and female sea otters generally occupy different home-ranges (Loughlin, 1980b), our data show that adult male, adult female, and sub-adult sea otters display a high degree of overlap in utilized foraging depths (Fig. 4.3). For adult sea otters, diet specialization reduces competition between individuals foraging in the same area (Tinker et al., 2012); however, sub-adults may not yet be efficient foragers or prey-specialists (Chapter 3) and this high degree of overlap may make meeting daily energetic demands more challenging when competing with adults for limited resources.

Alternatively, although diet specialization may act to reduce competition between adults, male and female sea otters face very different energetic constraints. Lactation is the most energetically taxing life history period across mammalian species (Millar, 1977; Oftedal et al., 1987) and is known to be an exceptionally challenging life stage for female sea otters (Thometz et al., 2014). In a variety of species, lactating

females alter foraging behavior and employ a wide array of strategies to manage the increased energetic demands associated with pup rearing and lactation (Barclay, 1989; Page et al., 2005; Villegas-Amtmann et al., 2008). Our results show that female sea otters caring for dependent young exhibit changes in routine dive behavior in comparison to periods when they are without a pup (Fig. 4.2). Such differences influence not only a where and how a female finds prey, but also her ability to meet the increasing energetic demands of a growing pup over the course of lactation.

The Effects of Reproductive Stage on Foraging Behavior

Adult females exhibited marked changes in diving and foraging behavior over the course of pup dependency. Most notably, females with very small pups significantly reduced overall foraging effort (Fig. 4.2; Fig. 4.5). After giving birth to a pup, mean foraging bout duration decreased, number of dives per bout decreased (Table 4.3), and percent time foraging per day decreased from $41.05 \pm 0.86\%$ to $27.68 \pm 1.99\%$ (Fig. 4.5). Post dive intervals were longer and more variable due to the need to nurse, groom, and care for a highly dependent pup (Riedman et al., 1994). Females with very small pups increased the proportion of dives made to depths of 11-15 m, which may have reflected more frequent utilization of areas of canopy-forming giant kelp that occur over this depth range (Jackson, 1987; McLean, 1962). Staedler (2011) also found evidence suggesting that females with very small pups switch to foraging in areas of high kelp cover due to observed changes in adult female diet. Females may preferentially forage in areas of high kelp cover when pups are very small to minimize the chances of pups drifting away while females are underwater foraging. Very shallow dives (2-5 m) as well as dives deeper than 20 m were recorded less frequently for females with very

small pups compared to those with no pups, indicating a reduction in utilized foraging area for postpartum adult females. The overall reduction in foraging effort and utilized foraging area by postpartum females contributes to the depletion of their limited energy reserves (Monson et al., 2000; Thometz et al., 2014) and is thus only sustained for the first 3 weeks postpartum.

During late dependency, large pups increase daily energetic demands of adult females by an average of 80-90% (Thometz et al. 2014). To account for these heightened demands, females substantially increased foraging effort as dependency progressed. Ultimately, adult females with large pups were the only adult demographic group to spend more time foraging each day than inactive (Fig. 4.5). Females with large pups had significantly longer foraging bouts, made more dives per bout than females without pups, and exhibited the shortest post dive intervals of all demographic groups (Table 4.3). In addition, females with pups exhibited increased descent and ascent rates, and females with small and large pups had the greatest bottom time to dive time ratios (Table 4.3). The increased bottom time to dive time ratios of adult females with pups suggest an attempt by females to maximize bottom time while foraging during pup dependency, which may allow females to increase prey recovery rates on individual dives. Together, these results suggest that females during late-lactation are working harder than other demographic groups to maximize caloric intake.

Similar to other marine mammals such as South American fur seals (Thompson et al., 2003) and harbor seals (Thompson et al., 1994) which exhibit reduced foraging ranges at the beginning of lactation, postpartum female sea otters showed a reduction in foraging depths. This reduction was only apparent during the first few weeks

postpartum, after which females progressively broadened the range of foraging depths utilized (Fig. 4.3). Although dependent pups have limited diving capacities (Chapter 3), adult females may occasionally forage in deeper areas to exploit more energy-rich prey (Bodkin et al., 2004). Adult females did not appear to limit dive durations in relation to the age-specific cADLs of their dependent pups (Fig. 4.4A), however there were differences between females with and without pups in the likelihood of exceeding cADL. Among females without pups, 16 individuals made dives that surpassed predicted adult cADLs, compared to only two individuals among females with pups. Therefore, although there was not a direct correlation with age-specific cADLs of dependent young and the dive durations of adult females, there was a 1-1.5 min reduction in maximum dive durations (Fig. 4.3D) for females with pups, suggesting that dependent young do in fact constrain routine dive durations of adult females.

Development of Diving Ability and Foraging Behavior

The diving and foraging behavior of sub-adults was similar to adult females without pups (Table 4.2 & 4.3). For example, the distribution of foraging depths for sub-adults was nearly identical to adult females without pups (Fig. 4.4A & 4.4B). Both sub-adults and adult females without pups exhibited a narrow range of foraging dive depths, with over 70% of all dives occurring between 2-10 m. Given the similarities in diving behavior between sub-adults in this study (mean age 2.3 years) to adult females without pups, and the fact that sea otters do not have comparable diving abilities to adults throughout their first year post-weaning (Chapter 3), our data suggest that sea otters likely develop comparable diving abilities to adults by 2 years of age. The youngest study animal was estimated to be 1.1 years old when captured, and thus we

were unable to examine the development of diving behavior for juvenile sea otters (6 mo. – 1.5 yr). Future examination of the diving behavior of individuals during their first year post-weaning will provide valuable insight regarding this critical developmental stage.

Sub-adults exhibited long foraging bout durations that were comparable to adult females with large pups (Fig. 4.2A), however sub-adults made more dives per foraging bout than all other demographic groups (Fig. 4.2B; Table 4.3). We attribute the larger number of dives per foraging bout to the shallower average dives of sub-adults in comparison to females with large pups. However, this may also indicate lower foraging efficiency of sub-adults compared to adult conspecifics. Reduced foraging efficiency of young and inexperienced individuals has been documented in a variety of species, including brown pelicans (Brandt, 1984), Galapagos fur seals (Horning and Trillmich, 1999), Galapagos sea lions (Jeglinski et al., 2012), and grey seals (Breed et al., 2011). Likewise, sub-adult sea otters exhibited the second highest percent time foraging ($47.31 \pm 1.80\%$) of all demographic groups, second only to adult females with large pups ($49.60 \pm 1.33\%$). Sub-adults were also the only group, besides females with large pups, to spend significantly more time per day foraging than inactive (Fig. 4.5), which emphasizes the high degree of foraging effort they expend each day.

Demographic Consequences

Resource limitation is a central issue related to the population recovery of the southern sea otter (Estes et al., 2003; Johnson et al., 2009; Tinker et al., 2008). In central California, resource limitation results in overall increased rates of foraging at the population level and diet specialization at the individual level. Furthermore, food

intake rates are lower in areas of limited resources in comparison to areas where resources are abundant (Tinker et al., 2008). Together, these factors indicate that individuals are expending a greater amount of energy per day searching for prey, while getting proportionally less calories per unit effort. As a result, body condition of sea otters is typically reduced in resource limited areas (Monson et al., 2000).

Pup rearing is an energetically expensive process for female sea otters (Thometz et al., 2014) and in resource limited areas female sea otters must accomplish this task with reduced body condition and limited energetic reserves (Monson et al., 2000). Despite this, birth rates are fairly consistent across locations (Riedman et al., 1994) and females utilize a “bet-hedging” strategy, deciding to keep or abandon a pup shortly after parturition depending on environmental and physiological factors (Monson et al., 2000; Stearns, 1992). Abandonment appears to be the leading cause of pup mortality during the first weeks postpartum (Garshelis and Garshelis, 1987; Thometz et al., 2014). For females that keep a pup, daily energetic demands increase steadily throughout lactation (Thometz et al., 2014) and females with large pups must forage nearly 50% of the day to bring in sufficient calories (Fig. 4.5). Sub-adults in central California also spend nearly half of each day foraging to support high energy demands (Fig. 4.5). Because time must also be allocated to other essential behaviors such as grooming (self and pup), nursing, and resting (Kenyon, 1969; Yeates, 2006), there is likely a limit to the amount of time individuals are capable of foraging per day before they can no longer remain energetically balanced. As sea otters in Monterey and Big Sur, CA are thought to be at carrying capacity and thus subject to density-dependent resource limitation (Tinker et al., 2008), females with large pups and sub-adults from

our study area may be approaching their upper sustainable limit for percent time foraging.

Population regulation has been shown to result from variation in a number of life history traits and vital rates, including increasing mortality of specific age-classes, increasing length of dependency, increasing age at sexual maturity, and reducing birth rates (Fowler, 1981). For southern sea otters, the highest rates of mortality occur in pups shortly after birth and in juveniles during their first year post-weaning, both of which are known to vary in response to density-dependent factors such as resource abundance (Estes, 1990). In addition, southern sea otters are experiencing a disproportionately high amount of prime-age female mortality (Tinker et al., 2006). Although density-dependent increases in mortality are traditionally thought to most strongly impact the youngest and oldest age-classes (Clutton-Brock et al., 1983), for southern sea otters, density-dependent forces may also be acting on prime-age females. Our results support the hypothesis that variation in per-capita prey availability coupled with high basal metabolic rates and a high cost of pup rearing may largely explain the disproportionately high rates of prime-age female mortality observed in central California (Thometz et al., 2014; Tinker et al., 2006).

Conclusions

Unlike other benthic foragers, such as New Zealand sea lions (Costa et al., 2001) and Australian sea lions (Crocker et al., 2001), our data suggest that sea otters do not respond to resource limitation by regularly exceeding cADL (Fig. 4.4). Rather, individuals increase foraging effort and percent time foraging per day. Reproductive stage, along with the physiological and behavioral development of dependent young,

markedly influence the foraging behavior of adult females. The high energetic cost (Thometz et al., 2014) and maternal behaviors (Jameson and Johnson, 1993) associated with rearing young are reflected in the changes in diving and foraging behavior of adult females throughout dependency. Females with large pups and sub-adults display the greatest foraging effort of all groups, providing additional evidence that the first years post-weaning and late-lactation are the most energetically and physiologically challenging life stages for sea otters. Ultimately, it appears that sub-adults and females with large pups in the central portion of the current southern sea otter range may have little flexibility to adapt to further reductions in prey distribution and abundance.

Table 4.1: Summary information on TDR deployments for study animals.

Location	Otter ID	Sex	Age	Age Class	Implant Date	TDR Stop Date	Total TDR Days	Pup Birth?	Pup Success?
Big Sur	5-093	F	8	A	30-Mar-09	13-Sep-09	167	Y	N
Big Sur	5-028	F	10	A	22-Mar-09	16-Jun-09	86	Y	N
Big Sur	6-043	F	9	A	12-Nov-08	9-Jun-09	209	Y	Y
Big Sur	6-209	F	6	A	30-Nov-08	5-Feb-10	432	Y	N
Big Sur	6-370	M	8	A	10-Nov-08	3-Nov-09	358	n/a	n/a
Big Sur	6-409	M	9	A	9-Nov-08	4-Nov-09	360	n/a	n/a
Big Sur	6-436	F	5	A	11-Nov-08	24-Sep-10	682	Y	Y
Big Sur	7-660	F	9	A	11-Nov-08	10-Nov-11	1094	Y	Y
Big Sur	6-067	F	4	A	3-Nov-09	9-Nov-11	736	N	n/a
Big Sur	6-553	F	2.5	SA	4-Nov-09	24-Sep-10	324	N	n/a
Big Sur	6-514	F	4	A	3-Nov-09	4-May-11	547	Y	N
Monterey	7-649	F	4	A	28-Sep-07	6-Oct-08	374	Y	Y
Monterey	7-609	F	4	A	26-Sep-07	19-Aug-08	328	N	n/a
Monterey	7-828	F	6	A	27-Sep-07	10-Sep-08	349	Y	N
Monterey	5-117	F	5.5	A	1-Feb-10	23-Jun-10	142	Y	Y
Monterey	6-765	F	8	A	7-Jun-10	16-Jun-11	374	Y	Y
Monterey	Jack	M	6	A	2-Feb-10	9-Jan-11	341	n/a	n/a
Monterey	ORWH	M	10	A	8-Jun-10	5-Feb-11	242	n/a	n/a
Monterey	5-217	F	6	A	2-Feb-10	12-Jun-10	130	N	n/a
Monterey	5-349	F	7	A	22-Apr-09	8-Jun-10	412	Y	Y
Monterey	7-722	F	5	A	30-Apr-09	24-Oct-10	542	Y	Y
Monterey	6-381	F	6	A	16-Jun-09	15-Aug-10	425	Y	Y
Monterey	1030-06	M	9	A	23-Apr-09	25-Aug-10	489	n/a	n/a
Monterey	1037-07	F	7	A	23-Apr-09	22-Jun-10	425	Y	Y
Monterey	7-633	F	5.5	A	17-Jun-09	28-Jul-10	406	N	n/a
Monterey	5-296	F	5.5	A	23-Apr-09	27-Oct-10	552	Y	Y
Monterey	6-485	M	6	A	30-Apr-09	27-Apr-10	362	n/a	n/a
Monterey	6-493	F	4	A	11-Jun-09	4-Sept-11	815	Y	Y
Monterey	7-747	M	10	A	22-Apr-09	8-Jun-10	412	n/a	n/a
Monterey	5-069	F	2.5	SA	28-May-09	28-Oct-10	518	N	n/a
Monterey	6-268	F	7.0	A	17-Jun-09	7-Jun-10	355	Y	Y
Monterey	Marigold	F	1.1	SA	24-Aug-09	29-Oct-09	66	N	n/a
Monterey	George	M	11	A	9-Jun-09	31-Mar-10	295	n/a	n/a
Monterey	6-131	F	9	A	16-Jun-09	6-Jun-10	355	Y	N
Monterey	Lola	F	3.1	SA	16-Dec-10	2-Sept-11	260	N	n/a
Monterey	Blanca	F	2.3	SA	3-Dec-09	26-Jun-11	570	Y	N
Monterey	1000-05	F	8	A	17-Jun-09	3-Oct-10	473	Y	Y
Monterey	1038-07	F	5	A	29-Jul-10	7-Aug-11	374	Y	Y

Table 4.2: Matrix of PERMANOVA and SIMPER results for all pairwise comparisons between demographic groups. Significant pairwise comparisons are displayed with associated t- and p-values. Non-significant results are distinguished (n.s.) along with their associated p-values. The top four dive attributes that contributed the most to the variance observed between each pair are displayed for all significant pairwise comparisons. Percent contribution of each dive attribute to significant pairwise comparisons between demographic groups is displayed in parentheses.

	F _{NP}	F _{VSP}	F _{SP}	F _{LP}	SA	M
F _{NP}	---					
F _{VSP}	t = 3.28, p = 0.001 Variance PDI (12.60) Ascent Rate (12.29) Descent Rate (10.01) Maximum Duration (9.96)	---				
F _{SP}	t = 2.47, p = 0.004 Ascent Rate (11.67) Mean Dive Depth (11.54) Descent Rate (11.17) Mean Dive Duration (10.76)	n.s. p = 0.13	---			
F _{LP}	t = 1.99, p = 0.015 Bottom Time (12.72) Mean Dive Duration (12.45) Mean Dive Depth (11.17) Maximum Duration (9.66)	t = 2.83, p = 0.001 Variance PDI (15.61) Mean PDI (11.84) Mean Bout Duration (10.23) # Dives per Bout (10.09)	n.s. p = 0.097	---		
SA	n.s. p = 0.247	t = 2.34, p = 0.001 Maximum Duration (12.71) Maximum Depth (12.13) # Dives per Bout (11.48) Mean Bout Duration (10.37)	t = 1.84, p = 0.023 Maximum Duration (15.80) Maximum Depth (12.43) Mean Dive Depth (8.97) # Dives per Bout (8.49)	n.s. p = 0.109	---	
M	t = 2.21, p = 0.002 Maximum Depth (18.35) Maximum Duration (13.29) Mean Dive Duration (9.36) Mean Dive Depth (9.22)	t = 2.39, p = 0.001 Maximum Depth (23.81) Maximum Duration (15.98) Variance PDI (9.48) Mean PDI (8.13)	t = 2.09, p = 0.007 Maximum Depth (21.10) Maximum Duration (16.91) Mean Dive Depth (9.02) BT/DT (8.42)	t = 2.41, p = 0.004 Maximum Duration (15.04) Maximum Depth (14.90) # Dives per Bout (9.81) Mean Dive Duration (7.82)	n.s. p = 0.062	---

Table 4.3: Summary of 12 foraging dive attributes of southern sea otters by demographic group (mean \pm SE).

Group	Mean Bout Duration (min)	Mean Dives Per Bout (#)	Mean Dive Depth (m)	Mean Dive Duration (s)	Maximum Dive Depth (m)	Maximum Dive Duration (s)	Mean PDI (s)	Variance PDI (s)	Descent Rate (m/s)	Mean Bottom Time (s)	Ascent Rate (m/s)	Bottom Time/ Dive Time
M (n=5)	101.96 \pm 10.07	34.62 \pm 4.13	10.35 \pm 3.75	66.86 \pm 5.07	68.38 \pm 4.35	280.50 \pm 29.66	110.66 \pm 6.26	97.47 \pm 4.30	0.79 \pm 0.08	41.69 \pm 3.54	0.83 \pm 0.05	0.58 \pm 0.01
F _{NP} (n=25)	119.79 \pm 7.25	50.01 \pm 3.71	7.65 \pm 0.89	59.96 \pm 3.77	41.84 \pm 2.26	258.40 \pm 11.15	90.44 \pm 3.88	84.02 \pm 2.32	0.69 \pm 0.02	36.40 \pm 2.62	0.68 \pm 0.04	0.58 \pm 0.01
F _{VSP} (n=19)	73.29 \pm 5.16	26.17 \pm 2.51	9.84 \pm 1.20	59.69 \pm 3.86	25.53 \pm 2.39	174.00 \pm 10.49	119.10 \pm 9.82	112.75 \pm 7.41	0.80 \pm 0.05	37.77 \pm 3.05	1.02 \pm 0.08	0.60 \pm 0.02
F _{SP} (n=15)	104.63 \pm 9.49	40.08 \pm 4.77	10.44 \pm 1.80	67.89 \pm 6.41	29.27 \pm 3.28	171.73 \pm 9.43	100.37 \pm 9.98	92.17 \pm 5.38	0.83 \pm 0.05	45.16 \pm 4.59	0.99 \pm 0.08	0.63 \pm 0.01
F _{LP} (n=15)	138.13 \pm 11.27	59.29 \pm 6.86	9.19 \pm 1.50	66.88 \pm 5.63	38.33 \pm 3.29	192.67 \pm 10.22	78.03 \pm 6.23	72.30 \pm 3.35	0.75 \pm 0.04	44.38 \pm 4.13	0.83 \pm 0.06	0.63 \pm 0.01
SA (n = 5)	138.60 \pm 14.98	63.68 \pm 9.11	7.87 \pm 1.74	62.25 \pm 7.72	57.00 \pm 3.37	295.20 \pm 25.26	83.43 \pm 5.97	84.65 \pm 4.12	0.73 \pm 0.05	36.93 \pm 5.41	0.70 \pm 0.07	0.58 \pm 0.01

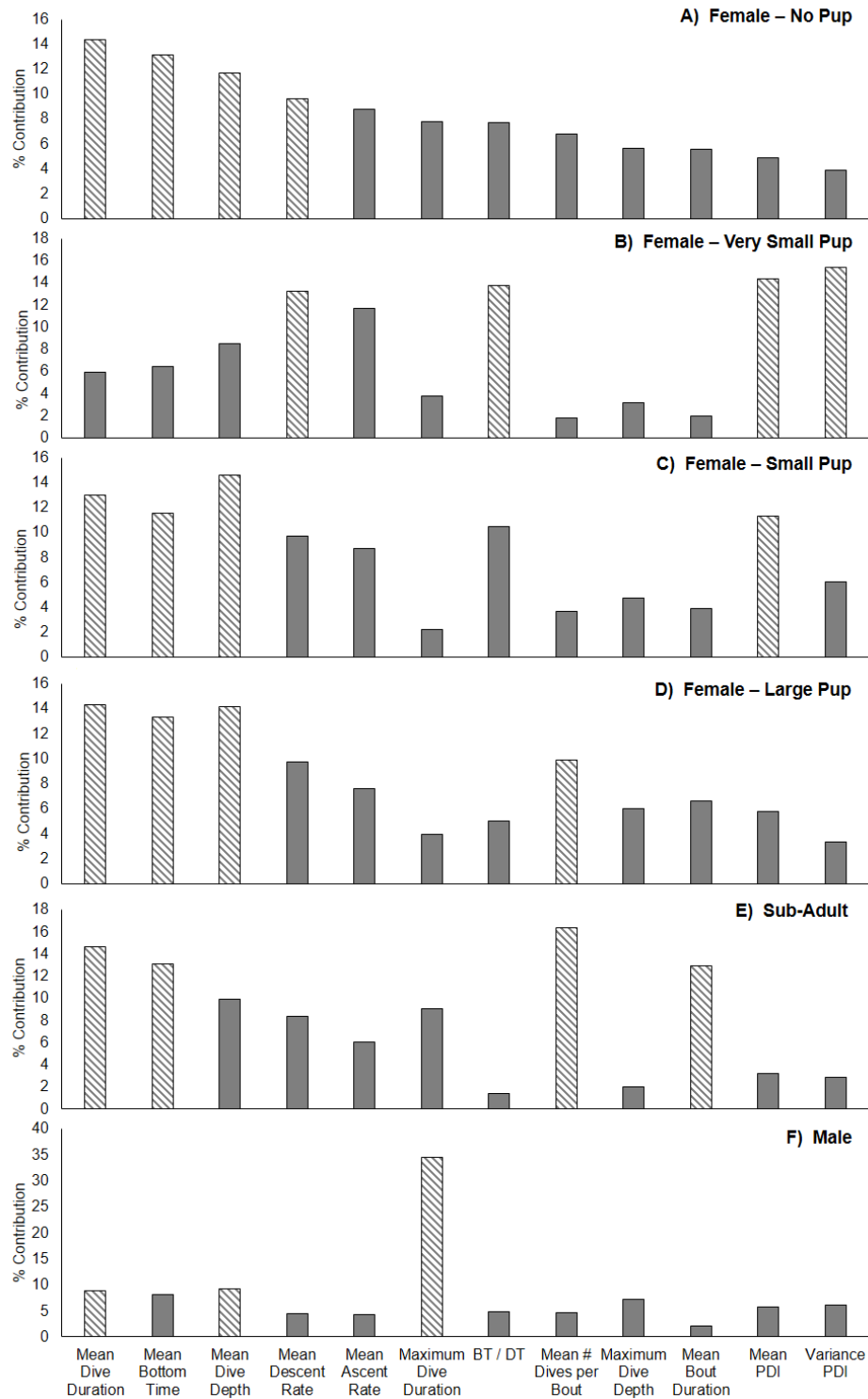


Figure 4.1: Histograms display the proportional contribution of each dive attribute to the characterization of each demographic group (Panels A-F) across all dive attributes from PERMANOVA and SIMPER analyses. Grey striped bars denote the dive attributes that cumulatively contribute approximately 50% to the characterization of each demographic group.

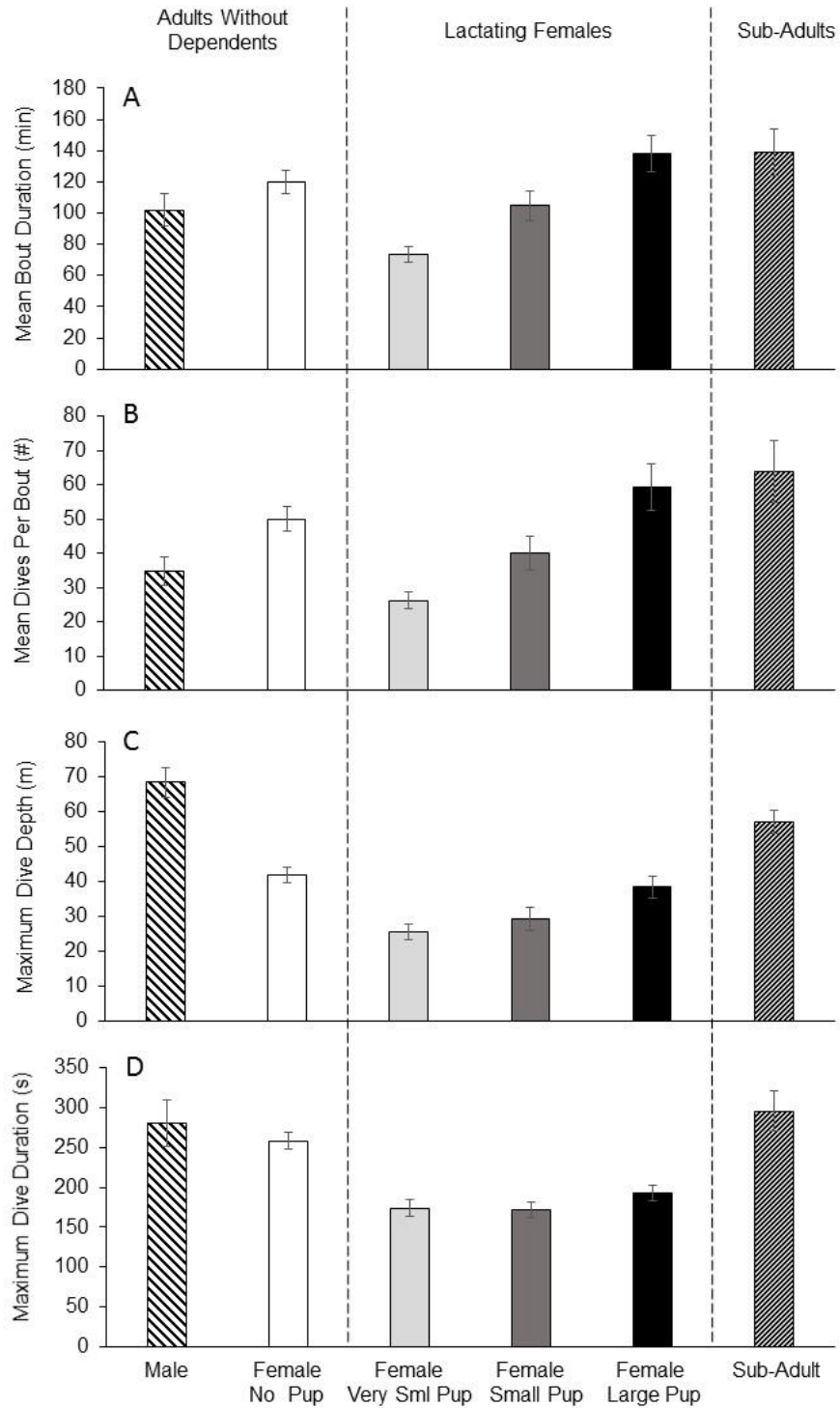


Figure 4.2: Stacked histograms display (A) mean bout duration, (B) mean number of dives per bout, (C) maximum dive depth, and (D) maximum dive duration, for southern sea otters across six demographic groups. Dashed lines in each panel differentiate adults without dependent young, lactating females, and sub-adults. Vertical bars display means (\pm SE).

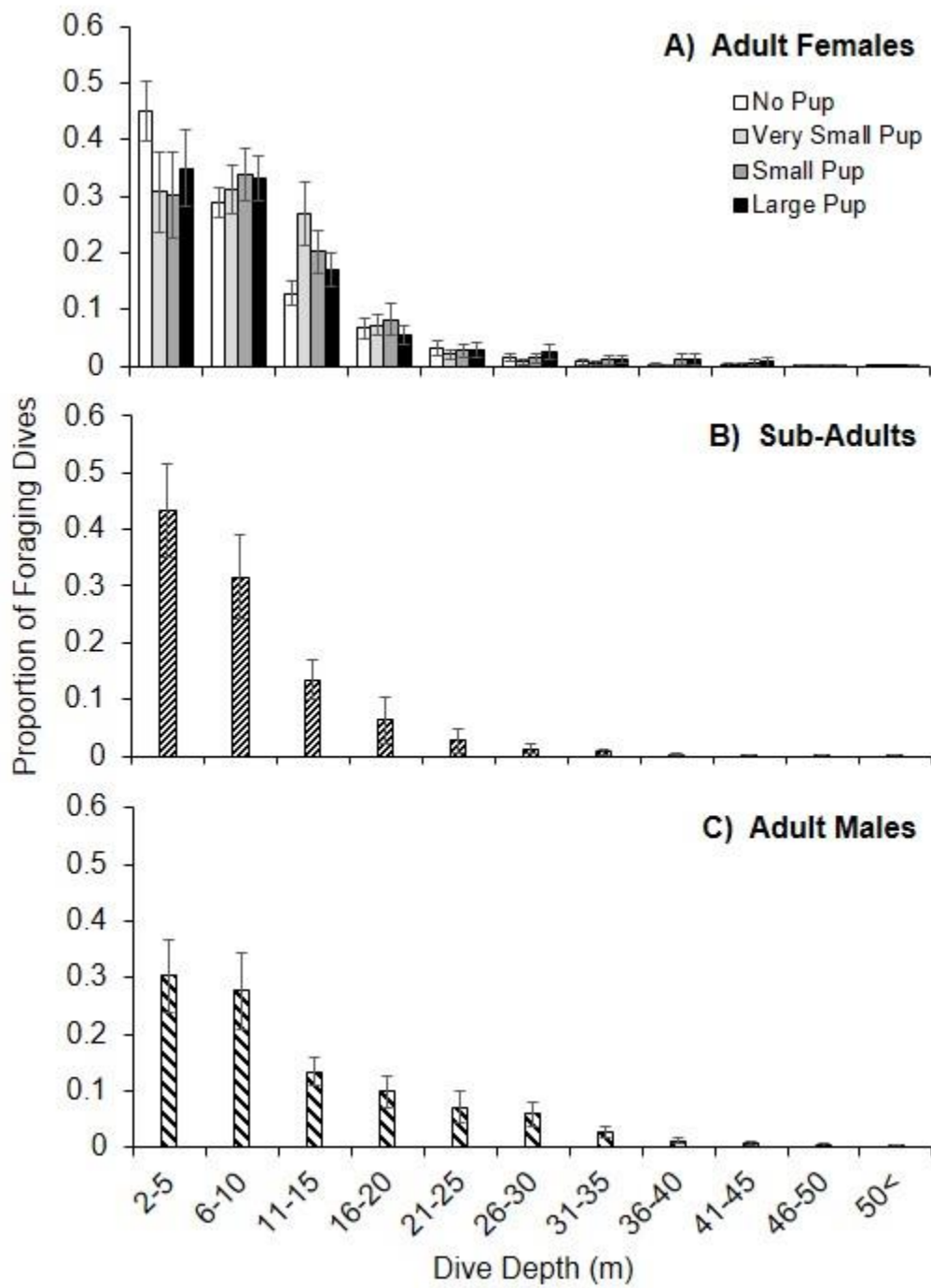


Figure 4.3: Proportional distribution of foraging dive depths for (A) adult female, (B) sub-adult, and (C) adult male sea otters. Vertical bars display the mean proportion (\pm SE) of all recorded foraging dives across a range of depths. Data for adult females (A) are differentiated by reproductive stage.

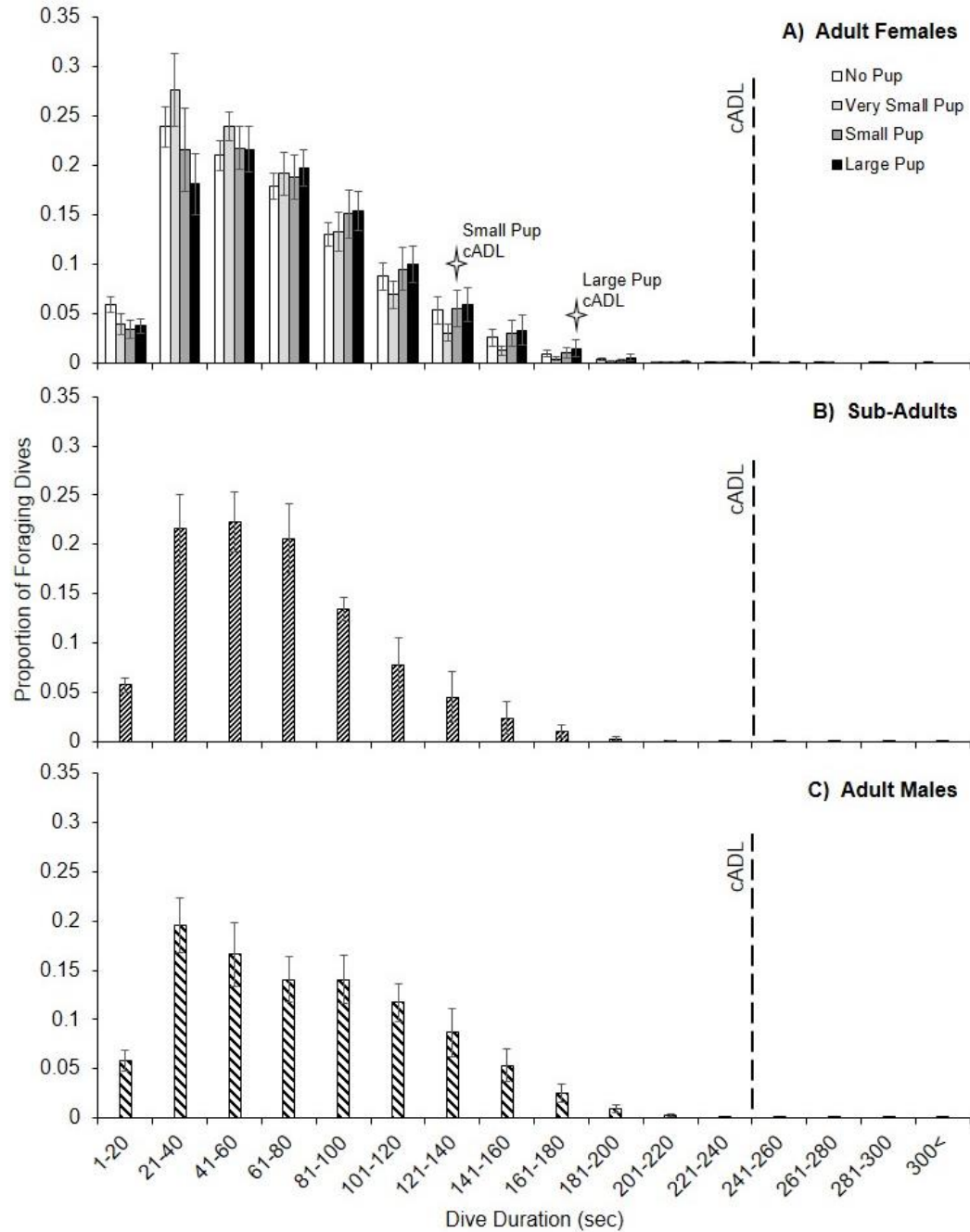


Figure 4.4: Proportional distribution of foraging dive durations for (A) adult female, (B) sub-adult, and (C) adult male sea otters. Vertical bars display the mean proportion (\pm SE) of all recorded foraging dives across a range of dive durations. Data for adult females (A) are differentiated by reproductive stage. The dashed vertical line in all three panels denotes the approximate cADL for adult sea otters adapted from Chapter 3, assuming the same diving metabolic rate for female and male sea otters. Age-specific cADLs of small and large pups adapted from Chapter 3 are denoted in panel A.

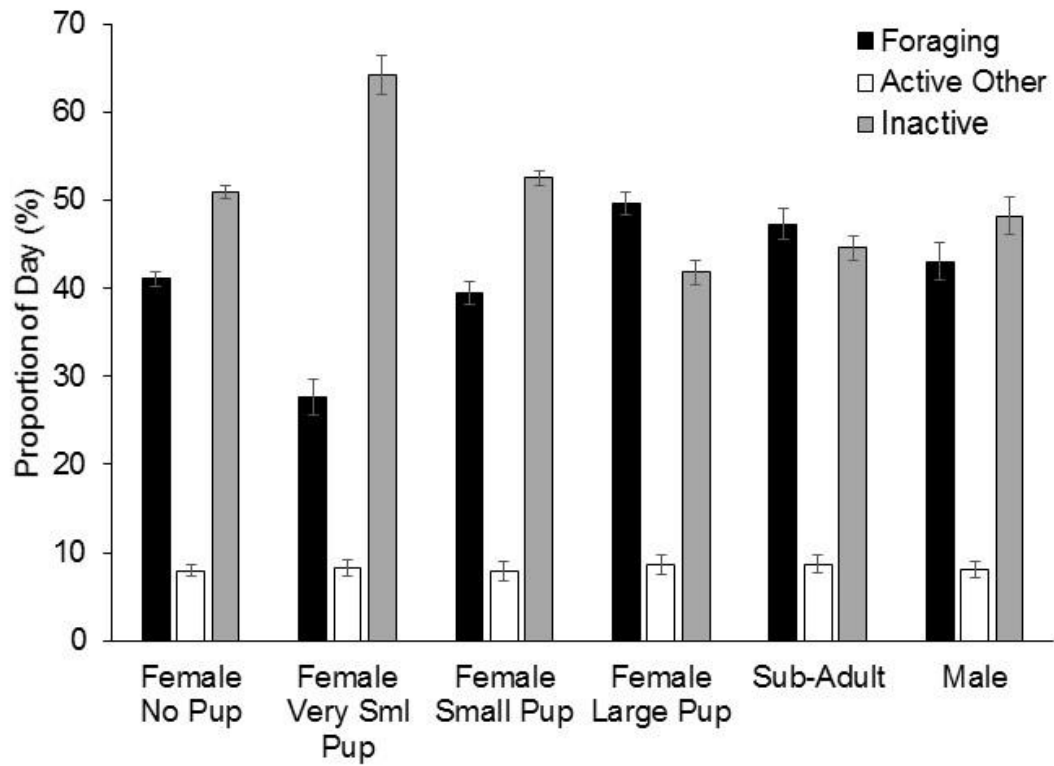


Figure 4.5: Daily activity budgets of sea otters across demographic groups. Behaviors of interest include foraging (black), active-other (white), and inactive (grey). Values (mean \pm SE) are grand means of all individuals in each group.

CHAPTER 5

Synthesis

Physiologically Challenging Life Stages

Sea otters have the highest mass-specific resting metabolic rate of any marine mammal and consequently must consume an average of 20-25% of their body weight in food each day (Costa and Kooyman, 1982; Costa and Williams, 1999; Kenyon, 1969; Morrison et al., 1974; Williams, 1989; Yeates et al., 2007). The high cost of living for sea otters, however, is not equal across life stages. My dissertation has provided a variety of evidence identifying the most energetically and physiologically challenging life stages for sea otters and discusses how these groups are differentially sensitive to disturbance within their environment.

Upon reaching sexual maturity, female sea otters must support the increasing demands of a dependent pup for an average of six months every year (Kenyon, 1969; Riedman and Estes, 1990; Riedman et al., 1994). Thus, a particularly important component of my thesis was quantifying the energetic constraints of adult females during this key life stage. I found that lactation greatly influences the energetic demands of an adult female which progressively increase throughout lactation. Around the average age of weaning, daily energetic demands of an adult female are increased

96% above pre-pregnancy levels. These metabolic demands are quite high among marine mammals, as female sea otters invest 128% and 28% more energy per kilogram into their young than would a similarly sized phocid or otariid, respectively. Ultimately, the high cost of pup rearing in combination with resource limitation appears to influence female body condition, parental provisioning strategies, pup abandonment and subsequent mortality during the first weeks postpartum, and end-lactation adult female mortality.

For juveniles and sub-adults in their first years post-weaning, both energetic and behavioral challenges must be overcome in order to maintain positive energy balance. Although mass-specific resting metabolic rates begin to approach adult levels around the age of weaning, increased time spent engaged in highly active behaviors elevates daily mass-specific energetic requirements of young individuals above average adult levels. Young sea otters are also physiologically limited. Blood and muscle oxygen stores do not reach adult levels until after the first year post-weaning and, consequently, young sea otters do not have comparable cADLs to adults until approximately 2 years of age. In addition, large mass-specific lung capacities result in high positive buoyancy and increased foraging costs for younger and smaller individuals. Collectively, physiological limitations and behavioral naiveté result in increased energetic demands and foraging effort for sea otters during their first years post-weaning.

What Does This Mean for Population Recovery?

For many years, the slow population recovery of the southern sea otter has been attributed to high rates of mortality associated with pollution and disease (Gerber et al.,

2004; Jessup et al., 2007; Johnson et al., 2009; Miller et al., 2004). Although pollution and disease are important sources of mortality for southern sea otters, the data from my dissertation suggest that sea otters in the central portion of their current range are likely at carrying capacity and experiencing density-dependent resource limitation and associated mortality (Tinker et al., 2006; Tinker et al., 2008). Thus, in order for the southern sea otter population to reach pre-exploitation levels, it is critical that individuals be allowed to expand from their current range along the California coastline and recolonize their historic range. The recent removal of the “no otter zone” (77 FR 75266, December 19, 2012) in southern California has been an important and critical step for enabling natural range expansion of sea otters into historically occupied habitats. Although there will likely be continued conflict with commercial fisheries as sea otters expand their current range (Fanshawe et al., 2003; Riedman and Estes, 1990), sea otters will bring about ecosystem-wide changes and associated benefits (Estes and Palmisano, 1974). Nearshore ecosystems both north and south of the current southern sea otter range will regain their historic apex predator, which will exert top-down control and have important indirect effects that may result in increased biodiversity and ecosystem resilience (Estes and Duggins, 1995; Estes and Palmisano, 1974; Estes et al., 1978; Estes et al., 2004; Hughes et al., 2013; Kvitek et al., 1992; Reisewitz et al., 2006; Weitzman, 2013).

Future Directions

Cooke et al. (2013) discussed the benefits of combining physiological and behavioral research to better understand and address conservation problems. They stated that, “conservation practitioners need predictive demographic models that have

specific mechanistic levers identified through physiology and parameterized with behavioral information.” My thesis has been a crucial step in the ability to create such predictive models for sea otters. We now have the necessary pieces to produce robust predictive models and this would be a natural extension of my research. These models would be highly useful to management and conservation agencies as sea otters recolonize their historic range. I intend to continue working collaboratively with both governmental and non-governmental organizations to aid in the creation of predictive models which will help managers and conservationists make informed decisions regarding the protection and management of this threatened species. Ultimately, I would like to continue conducting similar physiological and behavioral research on other threatened and endangered species, with a focus on research that is relevant to current conservation concerns.

Bibliography

- Altmann, J.** (1974). Observational Study of Behavior: Sampling Methods. *Behaviour* **49**, 227–267.
- Ames, J., Hardy, R. A. and Wendell, F. E.** (1986). A simulated translocation of sea otters, *Enhydra lutris*, with a review of capture, transport and holding techniques. *Mar. Resour. Tech. Rep.* **52**, 1–17.
- Andersen, R., Gaillard, J., John, D., Linnell, C. and Duncanx, P.** (2000). Factors affecting maternal care in an income breeder, the European roe deer. *J. Anim. Ecol.* **69**, 672–682.
- Anderson, M. J., Gorley, R. N. and Clarke, K. R.** (2008). PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. 2008 214.
- Barclay, R. M. R.** (1989). The effect of reproductive condition on the foraging behavior of female hoary bats, *Lasiurus cinereus*. *Behav. Ecol. Sociobiol.* **24**, 31–37.
- Bartlett, D. and Areson, J. G.** (1977). Quantitative lung morphology in newborn mammals. *Respir. Physiol.* **29**, 193–200.
- Bauplet, G., Barbraud, C., Chambellant, M. and Guinet, C.** (2005). Interannual variation in the post-weaning and juvenile survival of subantarctic fur seals: influence of pup sex, growth rate and oceanographic conditions. *J. Anim. Ecol.* **74**, 1160–1172.
- Bodkin, J. L., Esslinger, G. G. and Monson, D. H.** (2004). Foraging Depths of Sea Otters and Implications to Coastal Marine Communities. *Mar. Mammal Sci.* **20**, 305–321.
- Boness, D. J. and Bowen, W. D.** (1996). The Evolution of in Maternal Care Pinnipeds. *Bioscience* **46**, 645–654.
- Boness, D. J., Clapham, P. J. and Mesnick, S. L.** (2002). Life history and reproductive strategies. In *Marine Mammal Biology an Evolutionary Approach* (ed. Hoelzel, A. R.), pp. 278–324. Malden, MA: Blackwell Science Ltd.
- Bowen, W. D., Iverson, S. J., Boness, D. J. and Oftedal, O. T.** (2001). Foraging effort, food intake and lactation performance depend on maternal mass in a small phocid seal. *Funct. Ecol.* **15**, 325–334.
- Brandt, C. A.** (1984). Age and hunting success in the brown pelican: influences of skill and patch choice on foraging efficiency. *Oecologia* **62**, 132–137.

- Breed, G. a, Bowen, W. D., McMillan, J. I. and Leonard, M. L.** (2006). Sexual segregation of seasonal foraging habitats in a non-migratory marine mammal. *Proc. R. Soc. London B* **273**, 2319–26.
- Breed, G., Bowen, W. and Leonard, M.** (2011). Development of foraging strategies with age in a long-lived marine predator. *Mar. Ecol. Prog. Ser.* **431**, 267–279.
- Brody, S.** (1945). *Bioenergetics and Growth*. New York: Reinhold.
- Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M. and West, G. B.** (2004). Toward a Metabolic Theory of Ecology. *Ecology* **85**, 1771–1789.
- Buckley, F. G. and Buckley, P. A.** (1974). Comparative Feeding Ecology of Wintering Adult and Juvenile Royal Terns (Aves: Laridae, Sterninae). *Ecology* **55**, 1053–1063.
- Burns, J. M.** (1999). The development of diving behavior in juvenile Weddell seals: pushing physiological limits in order to survive. *Can. J. Zool.* **77**, 737–747.
- Burns, J. M. and Castellini, M. A.** (1996). Physiological and behavioral determinants of the aerobic dive limit in Weddell seal (*Leptonychotes weddellii*) Pups. *J. Comp. Physiol. B* **166**, 473–483.
- Burns, J. M., Clark, C. A. and Richmond, J. P.** (2004). The impact of lactation strategy on physiological development of juvenile marine mammals: implications for the transition to independent foraging. *Int. Congr. Ser.* **1275**, 341–350.
- Burns, J. M., Costa, D. P., Frost, K. and Harvey, J. T.** (2005). Development of body oxygen stores in harbor seals: effects of age, mass, and body composition. *Physiol. Biochem. Zool.* **78**, 1057–1068.
- Burns, J. M., Lestyk, K. C., Folkow, L. P., Hammill, M. O. and Blix, A. S.** (2007). Size and distribution of oxygen stores in harp and hooded seals from birth to maturity. *J. Comp. Physiol. B* **177**, 687–700.
- Cashman, M. E.** (2002). Diving in the southern sea otter, *enhydra lutris nereis*: morphometrics, buoyancy, and locomotion. Masters thesis, University of California Santa Cruz, USA.
- Castellini, M. A. and Somero, G. N.** (1981). Buffering Capacity of Vertebrate Muscle: Correlations with Potentials for Anaerobic Function. *J. Comp. Physiol.* **143**, 191–198.

- Clark, C. A., Burns, J. M., Schreer, J. F. and Hammill, M. O.** (2007). A longitudinal and cross-sectional analysis of total body oxygen store development in nursing harbor seals (*Phoca vitulina*). *J. Comp. Physiol. B* **177**, 217–27.
- Clutton-Brock, T. H., Guinness, F. E. and Albon, S. D.** (1983). The Cost of Reproduction to Red Deer Hinds. *J. Anim. Ecol.* **52**, 367–383.
- Congdon, J. D., Dunham, A. E. and van Loben Sels, R. C.** (1994). Demographics of Common Snapping Turtles (*Chelydra Serpentina*): Implications for Conservation and Management of Long-Lived Organisms. *Am. Zool.* **34**, 397–408.
- Cooke, S. J., Blumstein, D. T., Buchholz, R., Caro, T., Fernández-Juricic, E., Franklin, C. E., Metcalfe, J., O'Connor, C. M., St. Clair, C. C., Sutherland, W. J., et al.** (2013). Physiology, Behavior, and Conservation*. *Physiol. Biochem. Zool.* **87**, 000–000.
- Costa, D. P. and Kooyman, G. L.** (1982). Oxygen consumption, thermoregulation, and the effect of fur oiling and washing on the sea otter, *Enhydra lutris*. *Can. J. Zool.* **60**, 2761–2767.
- Costa, D. P. and Kooyman, G. L.** (1984). Contribution of Specific Dynamic Action to Heat Balance and Thermoregulation in the Sea Otter *Enhydra lutris*. *Physiol. Zool.* **57**, 199–203.
- Costa, D. P. and Williams, T. M.** (1999). Marine Mammal Energetics. In *Biology of Marine Mammals* (ed. Reynolds, J. E. and Rommel, S.), pp. 176–217. Washington, DC: Smithsonian Institution Press.
- Costa, D. P., Gales, N. J. and Crocker, D. E.** (1998). Blood volume and diving ability of the New Zealand sea lion, *Phocarctos hookeri*. *Physiol. Zool.* **71**, 208–213.
- Costa, D. P., Gales, N. J. and Goebel, M. E.** (2001). Aerobic dive limit: how often does it occur in nature? *Comp. Biochem. Physiol.* **129**, 771–83.
- Crocker, D. E., Gales, N. J. and Costa, D. P.** (2001). Swimming speed and foraging strategies of New Zealand sea lions (*Phocarctos hookeri*). *J. Zool.* **254**, 267–277.
- Croll, D. A., Gaston, A. J., Burger, A. E. and Konnoff, D.** (1992). Foraging Behavior and Physiological Adaptation for Diving in Thick-Billed Murres. *Ecology* **73**, 344–356.
- Crouse, D. T., Crowder, L. B. and Caswell, H.** (1987). A Stage-Based Population Model for Loggerhead Sea Turtles and Implications for Conservation. *Ecology* **68**, 1412–1423.

- De Kroon, H., van Groenendael, J. and Ehrlen, J.** (2000). Elasticities: A Review of Methods and Model Limitations. *Ecology* **81**, 607–618.
- Dejours, P.** (1987). Water and air physical characteristics and their physiological consequences. In *Comparative physiology: life in water and on land* (ed. Dejours, P., Bolis, L., Taylor, C. R., and Weibel, E.), pp. 3–11. Springer-Verlag.
- Denison, D. M. and Kooyman, G. L.** (1973). The structure and function of the small airways in pinniped and sea otter lungs. *Respir. Physiol.* **17**, 1–10.
- El-Sayed, H., Goodall, S. R. and Hainsworth, R.** (1995). Re-evaluation of Evans blue dye dilution method of plasma volume measurement. *Clin. Lab. Haematol.* **17**, 189–194.
- Esslinger, G. G., Bodkin, J. L., Breton, A. R., Burns, J. M. and Monson, D. H.** (2014). Temporal patterns in the foraging behavior of sea otters in Alaska. *J. Wildl. Manage.* **78**, 689–700.
- Estes, J. A.** (1990). Growth and Equilibrium in Sea Otter Populations. *J. Anim. Ecol.* **59**, 385–401.
- Estes, J. A. and Duggins, D. O.** (1995). Sea Otters and Kelp Forests in Alaska: Generality and Variation in a Community Ecological Paradigm. *Ecol. Monogr.* **65**, 75–100.
- Estes, J. A. and Palmisano, J. F.** (1974). Sea Otters: Their Role in Structuring Nearshore Communities. *Science (80-)*. **185**, 1058–1060.
- Estes, J. A., Smith, N. S. and Palmisano, J. F.** (1978). Sea Otter Predation and Community Organization in the Western Aleutian Islands, Alaska. *Ecology* **59**, 822–833.
- Estes, J. A., Jameson, R. J. and Rhode, E. B.** (1982). Activity and Prey Election in the Sea Otter: Influence of Population Status on Community Structure. *Am. Nat.* **120**, 242–258.
- Estes, J. A., Underwood, K. E. and Karmann, M. J.** (1986). Activity-Time Budgets of Sea Otters in California. *J. Wildl. Manage.* **50**, 626–636.
- Estes, J. A., Hatfield, B. B., Ralls, K. and Ames, J.** (2003). Causes of mortality in California sea otters during periods of population growth and decline. *Mar. Mammal Sci.* **19**, 198–216.

- Estes, J. A., Danner, E. M., Doak, D. F., Konar, B., Springer, A. M., Steinberg, P. D., Tinker, M. T. and Williams, T. M.** (2004). Complex Trophic Interactions in Kelp Forest Ecosystems. *Bull. Mar. Sci.* **74**, 621–638.
- Fanshawe, S., Vanblaricom, G. R. and Shelly, A. A.** (2003). Restored Top Carnivores as Detriments to the Performance of Marine Protected Areas Intended for Fishery Sustainability: a Case Study with Red Abalones and Sea Otters. *Conserv. Biol.* **17**, 273–283.
- Fedak, M. A., Rome, L. and Seeherman, H. J.** (1981). One-step N₂-dilution technique for calibrating open-circuit VO₂ measuring systems One-step Na-dilution technique for calibrating VO₂ measuring systems. *J. Appl. Physiol.* **51**, 772–776.
- Field, I. C., Bradshaw, C. J. a, Burton, H. R., Sumner, M. D. and Hindell, M. A.** (2005). Resource partitioning through oceanic segregation of foraging juvenile southern elephant seals (*Mirounga leonina*). *Oecologia* **142**, 127–35.
- Fish, F. E., Smelstoys, J., Baudinette, R. V and Reynolds, P. S.** (2002). Fur does not fly, it floats: buoyancy of pelage in semi-aquatic mammals. *Aquat. Mamm.* **28.2**, 103–112.
- Fisher, E. M.** (1941). Notes on the Teeth of the Sea Otter. *J. Mammal.* **22**, 428–433.
- Foldager, N. and Blomqvist, C. G.** (1991). Repeated plasma volume determination with the Evans Blue dye dilution technique: the method and a computer program. *Comput. Biol. Med.* **21**, 35–41.
- Fowler, C. W.** (1981). Density Dependence as Related to Life History Strategy. *Ecology* **62**, 602–610.
- Fowler, S. L., Costa, D. P., Arnould, J. P. Y., Gales, N. J. and Burns, J. M.** (2007). Ontogeny of oxygen stores and physiological diving capability in Australian sea lions. *Funct. Ecol.* **21**, 922–935.
- Garshelis, D. L.** (1984). Age Estimation of Living Sea Otters. *J. Wildl. Manage.* **48**, 456–463.
- Garshelis, D. L. and Garshelis, J. A.** (1987). Atypical pup rearing strategies by sea otters. *Mar. Mammal Sci.* **3**, 263–270.
- Gelatt, T. S., Siniff, D. B. and Estes, J. A.** (2002). Activity Patterns and Time Budgets of the Declining Sea Otter Population at Amchitka Island, Alaska. *J. Wildl. Manage.* **66**, 29–39.

- Georges, J.-Y. and Guinet, C.** (2000). Maternal Care in the Subantarctic Fur Seals on Amsterdam Island. *Ecology* **81**, 295–308.
- Gerber, L. R., Tinker, M. T., Doak, D. F., Estes, J. A. and Jessup, D. A.** (2004). Mortality Sensitivity in Life-Stage simulation Analysis: A Case Study of Southern Sea Otters. *Ecol. Appl.* **14**, 1554–1565.
- Haley, M. P.** (1994). Resource-holding power asymmetries, the prior residence effect, and reproductive payoffs in male northern elephant seal fights. *Behav. Ecol. Sociobiol.* **34**, 427–434.
- Horning, M. and Trillmich, F.** (1997). Ontogeny of Diving Behaviour in the Galápagos Fur Seal. *Behaviour* **134**, 1211–1257.
- Horning, M. and Trillmich, F.** (1999). Lunar cycles in diel prey migrations exert a stronger effect on the diving of juveniles than adult Galapagos fur seals. *Proc. R. Soc. London B* **266**, 1127–1132.
- Huang, S., Chou, L.-S. and Ni, I.-H.** (2009). Comparable length at weaning in cetaceans. *Mar. Mammal Sci.* **25**, 875–887.
- Hughes, B. B., Eby, R., Van Dyke, E., Tinker, M. T., Marks, C. I., Johnson, K. S. and Wasson, K.** (2013). Recovery of a top predator mediates negative eutrophic effects on seagrass. *Proc. Natl. Acad. Sci. U. S. A.* **110**, 15313–8.
- Jackson, G. A.** (1987). Modelling the Growth and Harvest Yield of the Giant Kelp *Macrocystis pyrifera*. *Mar. Biol.* **95**, 611–624.
- Jameson, J. and Johnson, A. M.** (1993). Reproductive Characteristics of Female Sea Otters. *Mar. Mammal Sci.* **9**, 156–167.
- Jeglinski, J., Werner, C., Robinson, P., Costa, D. and Trillmich, F.** (2012). Age, body mass and environmental variation shape the foraging ontogeny of Galapagos sea lions. *Mar. Ecol. Prog. Ser.* **453**, 279–296.
- Jessup, D. A., Miller, M. A., Kreuder-Johnson, C., Conrad, P. A., Tinker, M. T., Estes, J. and Mazet, J. A. K.** (2007). Sea otters in a dirty ocean. *J. Am. Vet. Med. Assoc.* **231**, 1648–1652.
- Johnson, C. K., Tinker, M. T., Estes, J. A., Conrad, P. A., Staedler, M., Miller, M. A., Jessup, D. A. and Mazet, J. A. K.** (2009). Prey choice and habitat use drive sea otter pathogen exposure in a resource-limited coastal system. *Proc. Natl. Acad. Sci. U. S. A.* **106**, 2242–7.
- Jonsson, K. I.** (1997). Capital and Income Breeding as Alternative Tactics of Resource Use in Reproduction. *Oikos* **78**, 57–66.

- Kanatous, S. B., Hawke, T. J., Trumble, S. J., Pearson, L. E., Watson, R. R., Garry, D. J., Williams, T. M. and Davis, R. W.** (2008). The ontogeny of aerobic and diving capacity in the skeletal muscles of Weddell seals. *J. Exp. Biol.* **211**, 2559–65.
- Kenyon, K. W.** (1969). The Sea Otter *Enhydra lutris* in the Eastern Pacific Ocean. *North Am. Fauna* 1–352.
- Kleiber, M.** (1947). Body Size and Metabolic Rate. *Physiol. Rev.* **27**, 511–541.
- Kooyman, G. L.** (1989). *Diverse Divers*. New York: Springer-Verlag.
- Kooyman, G. L. and Ponganis, P. J.** (1998). The physiological basis of diving to depth: birds and mammals. *Annu. Rev. Physiol.* **60**, 19–32.
- Kooyman, G. L., Wahrenbrock, E. A., Castellini, M. A., Davis, R. W. and Sinnott, E. E.** (1980). Aerobic and Anaerobic Metabolism During Voluntary Diving in Weddell Seals: Evidence of Preferred Pathways from Blood Chemistry and Behavior. *J. Comp. Physiol. B* **138**, 335–346.
- Kooyman, G. L., Castellini, M. A., Davis, R. W. and Maue, R. A.** (1983). Aerobic Diving Limits of Immature Weddell Seals. *J. Comp. Physiol.* **151**, 171–174.
- Kvitek, R. G., Oliver, J. S., DeGange, A. R. and Anderson, B. S.** (1992). Changes in Alaskan Soft-Bottom Prey Communities Along a Gradient in Sea Otter Predation. *Ecology* **73**, 413–428.
- Lenfant, C., Johansen, K. and Torrance, J. D.** (1970). Gas transport and oxygen storage capacity in some pinnipeds and the sea otter. *Respir. Physiol.* **9**, 277–286.
- Lockyer, C.** (1984). Review of baleen whale (Mysticeti) reproduction and implications for management. *Reports Int. Whal. Com.* 27–48.
- Lockyer, C.** (2007). All creatures great and smaller: a study in cetacean life history energetics. *J. Mar. Biol. Assoc. UK* **87**, 1035–1045.
- Loughlin, T. R.** (1980a). Radio telemetric determination of 24-hour feeding activities of sea otters, *Enhydra lutris*. In *A Handbook on Biotelemetry and Radio Tracking* (ed. Amlaner, C. J. and MacDonald, D. W.), pp. 717–724. Oxford: Pergamon Press.
- Loughlin, T. R.** (1980b). Home Range and Territoriality of Sea Otters Near Monterey, California. *J. Wildl. Manage.* **44**, 576–582.

- Lydersen, C., Ryg, M. S., Hammill, M. O. and O'Brien, P. J.** (1992). Oxygen stores and aerobic dive limit of ringed seals (*Phoca hispida*). *Can. J. Zool.* **70**, 458–461.
- MacArthur, R. A., Humphries, M. M., Fines, G. A. and Campbell, K. L.** (2001). Body oxygen stores, aerobic dive limits, and the diving abilities of juvenile and adult muskrats (*Ondatra zibethicus*). *Physiol. Biochem. Zool.* **74**, 178–90.
- McIntyre, I. W., Campbell, K. L. and MacArthur, R. A.** (2002). Body oxygen stores, aerobic dive limits and diving behaviour of the star-nosed mole (*Condylura cristata*) and comparisons with non-aquatic talpids. *J. Exp. Biol.* **205**, 45–54.
- McLean, J.** (1962). Sublittoral Ecology of Kelp Beds of the Open Coast Area near Carmel, California. *Biol. Bull.* **122**, 95–114.
- McLellan, W. A., Koopman, H. N., Rommel, S. A., Read, A. J., Potter, C. W., Nicolas, J. R., Westgate, A. J. and Pabst, D. A.** (2002). Ontogenetic allometry and body composition of harbour porpoises (*Phocoena phocoena*, L.) from the western North Atlantic. *J. Zool.* **257**, 457–471.
- Millar, J. S.** (1975). Tactics of energy partitioning in breeding *Peromyscus*. *Can. J. Zool.* **53**, 967–76.
- Millar, J. S.** (1977). Adaptive Features of Mammalian Reproduction. *Evolution* (N. Y). **31**, 370–386.
- Miller, M. A., Grigg, M. E., Kreuder, C., James, E. R., Melli, A. C., Crosbie, P. R., Jessup, D. A., Boothroyd, J. C., Brownstein, D. and Conrad, P. A.** (2004). An unusual genotype of *Toxoplasma gondii* is common in California sea otters (*Enhydra lutris nereis*) and is a cause of mortality. *Int. J. Parasitol.* **34**, 275–84.
- Mitchell, B., McCowan, D. and Nicholson, I. A.** (1976). Annual cycles of body weight and condition in Scottish Red deer, *Cervus elaphus*. *J. Zool. London* **180**, 107–127.
- Mohri, M., Sharifi, K. and Eidi, S.** (2007). Hematology and serum biochemistry of Holstein dairy calves: age related changes and comparison with blood composition in adults. *Res. Vet. Sci.* **83**, 30–9.
- Monson, D. H., Estes, J. A., Bodkin, J. L. and Siniff, D. B.** (2000). Life history plasticity and population regulation in sea otters. *Oikos* **90**, 457–468.

- Monson, D. H., McCormick, C. and Ballachey, B. E.** (2001). Chemical Anesthesia of Northern Sea Otters (*Enhydra lutris*): Results of Past Field Studies. *J. Zoo Wildl. Med.* **32**, 181–189.
- Morrison, P., Rosenmann, M. and Estes, J. A.** (1974). Metabolism and Thermoregulation in the Sea Otter. *Physiol. Zool.* **47**, 218–229.
- Nagy, K. A.** (2005). Field metabolic rate and body size. *J. Exp. Biol.* **208**, 1621–5.
- Noren, S. R., Williams, T. M., Pabst, D. A., McLellan, W. A. and Dearolf, J. L.** (2001). The development of diving in marine endotherms: preparing the skeletal muscles of dolphins, penguins, and seals for activity during submergence. *J. Comp. Physiol. B* **171**, 127–134.
- Noren, S. R., Lacave, G., Wells, R. S. and Williams, T. M.** (2002). The development of blood oxygen stores in bottlenose dolphins (*Tursiops truncatus*): implications for diving capacity. *J. Zool.* **258**, 105–113.
- Noren, S. R., Iverson, S. J. and Boness, D. J.** (2005). Development of the blood and muscle oxygen stores in gray seals (*Halichoerus grypus*): implications for juvenile diving capacity and the necessity of a terrestrial postweaning fast. *Physiol. Biochem. Zool.* **78**, 482–490.
- Oftedal, O. T., Boness, D. J. and Tedman, R. A.** (1987). The behavior, physiology, and anatomy of lactation in the pinnipedia. In *Current Mammalogy* (ed. Genoways, H. H.), pp. 175–245. Plenum Publishing Corporation.
- Page, B., McKenzie, J. and Goldsworthy, S.** (2005). Inter-sexual differences in New Zealand fur seal diving behaviour. *Mar. Ecol. Prog. Ser.* **304**, 249–264.
- Parworth, L. P., Frost, D. E., Zuniga, J. R. and Bennett, T.** (1998). Propofol and fentanyl compared with midazolam and fentanyl during third molar surgery. *J. Oral Maxillofac. Surg.* **56**, 447–53; discussion 453–4.
- Payne, S. F. and Jameson, R. J.** (1984). Early Behavioral Development of the Sea Otter, *Enhydra lutris*. *J. Mammal.* **65**, 527–531.
- Perrin, W. F. and Reilly, S. B.** (1984). Reproductive Parameters of Dolphins and Small Whales of the Family Delphinidae. *Reports Int. Whal. Com.* 97–133.
- Ponganis, P. J.** (2011). Diving Mammals. *Compr. Physiol.* **1**, 517–535.
- Ponganis, P. J. and Kooyman, G. L.** (2000). Diving physiology of birds: a history of studies on polar species. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* **126**, 143–51.

- Ponganis, P. J., Kooyman, G. L. and Castellini, M. A.** (1993). Determinants of the Aerobic Dive Limit of Weddell Seals: Analysis of Diving Metabolic Rates, Postdive End Tidal Po₂ ' s, and Blood and Muscle Oxygen Stores. *Physiol. Zool.* **66**, 732–749.
- Ponganis, P. J., Costello, M. L., Starke, L. N., Mathieu-Costello, O. and Kooyman, G. L.** (1997). Structural and biochemical characteristics of locomotory muscles of emperor penguins, *Aptenodytes forsteri*. *Respir. Physiol.* **109**, 73–80.
- Ponganis, P. J., Starke, L. N., Horning, M. and Kooyman, G. L.** (1999). Development of diving capacity in emperor penguins. *J. Exp. Biol.* **202**, 781–786.
- Pontier, D., Gaillard, J.-M. and Allaine, D.** (1993). Maternal investment per offspring and demographic tactics in placental mammals. *Oikos* **66**, 424–430.
- Quinn, G. P. and Keough, M. J.** (2003). *Experimental Design and Data Analysis for Biologists*. Cambridge, UK: Cambridge University Press.
- Ralls, K. and Siniff, D. B.** (1990). Time Budgets and Activity Patterns in California Sea Otters. *J. Wildl. Manage.* **54**, 251–259.
- Rasmussen, J. P., Dauchot, P. J., DePalma, R. G., Sorensen, B., Regula, G., Anton, a H. and Gravenstein, J. S.** (1978). Cardiac Function and Hypercarbia. *Arch. Surg.* **113**, 1196–200.
- Reisewitz, S. E., Estes, J. A. and Simenstad, C. A.** (2006). Indirect food web interactions: sea otters and kelp forest fishes in the Aleutian archipelago. *Oecologia* **146**, 623–31.
- Reynafarje, B.** (1963). Simplified method for the determination of myoglobin. *J. Lab. Clin. Med.* **61**, 138–145.
- Reynolds, J. E. and Rommel, S. A. eds.** (1999). *The Biology of Marine Mammals*. Washington, DC: Smithsonian Institution Press.
- Richmond, J. P., Burns, J. M., Rea, L. D. and Mashburn, K. L.** (2005). Postnatal ontogeny of erythropoietin and hematology in free-ranging Steller sea lions (*Eumetopias jubatus*). *Gen. Comp. Endocrinol.* **141**, 240–7.
- Richmond, J. P., Burns, J. M. and Rea, L. D.** (2006). Ontogeny of total body oxygen stores and aerobic dive potential in Steller sea lions (*Eumetopias jubatus*). *J. Comp. Physiol. B* **176**, 535–545.

- Riedman, M. L. and Estes, J. A.** (1990). The Sea Otter (*Enhydra lutris*): Behavior, Ecology, and Natural History. Biological Report 90 (14). *U.S. Fish Wildl.* 126.
- Riedman, M. L., Estes, J. A., Staedler, M. M., Giles, A. A. and David, R.** (1994). Breeding Patterns and Reproductive Success of California Sea Otters. *J. Wildl. Manage.* **58**, 391–399.
- Ronchetti, E.** (1985). Robust Model Selection in Regression. *Stat. Probab. Lett.* **3**, 21–23.
- Sato, K., Naito, Y., Kato, A., Niizuma, Y., Watanuki, Y., Charrassin, J. B., Bost, C., Handrich, Y. and Le Maho, Y.** (2002). Buoyancy and maximal diving depth in penguins: do they control inhaling air volume? *J. Exp. Biol.* **205**, 1189–97.
- Scharf, F. S., Juanes, F. and Rountree, R. A.** (2000). Predator size - prey size relationships of marine fish predators: interspecific variation and effects of ontogeny and body size on trophic-niche breadth. **208**, 229–248.
- Schmidt-Nielsen, K.** (1997). *Animal Physiology: adaptation and environment*. 5th ed. New York: Cambridge University Press.
- Seal, U. S., Swaim, W. R. and Erickson, A. W.** (1967). Hematology of the ursidae. *Comp. Biochem. Physiol.* **22**, 451–60.
- Shaffer, S. A., Costa, D. P., Williams, T. M. and Ridgway, S. H.** (1997). Diving and swimming performance of white whales, *Delphinapterus leucas*: an assessment of plasma lactate and blood gas levels and respiratory rates. *J. Exp. Biol.* **200**, 3091–9.
- Shero, M. R., Andrews, R. D., Lestyk, K. C. and Burns, J. M.** (2012). Development of the aerobic dive limit and muscular efficiency in northern fur seals (*Callorhinus ursinus*). *J. Comp. Physiol. B* **182**, 425–36.
- Skrovan, R. C., Williams, T. M., Berry, P. S., Moore, P. W. and Davis, R. W.** (1999). The Diving Physiology of Bottlenose Dolphins (*Tursiops truncatus*) II. biomechanics and changes in buoyancy at depth. *J. Exp. Biol.* **202**, 2749–2761.
- Snyder, G. K.** (1983). Respiratory adaptations in diving mammals. *Respir. Physiol.* **54**, 269–294.
- Spence-Bailey, L. M., Verrier, D. and Arnould, J. P. Y.** (2007). The physiological and behavioural development of diving in Australian fur seal (*Arctocephalus pusillus doriferus*) pups. *J. Comp. Physiol. B* **177**, 483–94.

- Staedler, M. M.** (2011). Maternal care and provisioning in the southern sea otter (*Enhydra lutris nereis*): reproductive consequences of diet specialization in an apex predator. Masters thesis, University of California Santa Cruz, USA. 108.
- Stearns, S. C.** (1992). *The evolution of life histories*. New York: Oxford University Press.
- Stephenson, R.** (1995). Respiratory and plumage gas volumes in unrestrained diving ducks (*Aythya affinis*). *Respir. Physiol.* **100**, 129–37.
- Tarasoff, F. J. and Kooyman, G. L.** (1973). Observations on the anatomy of the respiratory system of the river otter, sea otter, and harp seal. I. The topography, weight, and measurements of the lungs. *Can. J. Zool.* **51**, 163–170.
- Thometz, N. M., Tinker, M. T., Staedler, M. M., Mayer, K. A. and Williams, T. M.** (2014). Energetic Demands of Immature Sea Otters from Birth to Weaning: Implications for maternal costs, reproductive behavior, and population level trends. *J. Exp. Biol.* In Press.
- Thompson, P. M., Miller, D., Cooper, R. and Hammond, P. S.** (1994). Changes in the Distribution and Activity of Female Harbour Seals During the Breeding Season: implications for their lactation and mating patterns. *J. Anim. Ecol.* **63**, 24–30.
- Thompson, P. M., Mackay, A., Tollit, D. J., Enderby, S. and Hammond, P. S.** (1998). The influence of body size and sex on the characteristics of harbour seal foraging trips. *Can. J. Zool.* **76**, 1044–1053.
- Thompson, D., Moss, S. and Lovell, P.** (2003). Foraging behaviour of South American fur seals *Arctocephalus australis*: extracting fine scale foraging behaviour from satellite tracks. *Mar. Ecol. Prog. Ser.* **260**, 285–296.
- Tinker, M. T., Doak, D. F., Estes, J. A., Hatfield, B. B., Staedler, M. M. and Bodkin, J. L.** (2006). Incorporating diverse data and realistic complexity into demographic estimation procedures for sea otters. *Ecol. Appl.* **16**, 2293–312.
- Tinker, M. T., Costa, D. P., Estes, J. A. and Wieringa, N.** (2007). Individual dietary specialization and dive behaviour in the California sea otter: Using archival time–depth data to detect alternative foraging strategies. *Deep Sea Res. Part II Top. Stud. Oceanogr.* **54**, 330–342.
- Tinker, M. T., Bentall, G. and Estes, J. A.** (2008). Food limitation leads to behavioral diversification and dietary specialization in sea otters. *Proc. Natl. Acad. Sci.* **105**, 560–565.

- Tinker, M. T., Guimarães, P. R., Novak, M., Marquitti, F. M. D., Bodkin, J. L., Staedler, M., Bentall, G. and Estes, J. A.** (2012). Structure and mechanism of diet specialisation: testing models of individual variation in resource use with sea otters. *Ecol. Lett.* **15**, 475–83.
- Toms, J. D. and Lesperance, M. L.** (2003). Piecewise Regression: a tool for identifying ecological thresholds. *Ecology* **84**, 2034–2041.
- Trillmich, F.** (1996). Parental Investment in Pinnipeds. *Adv. Study Behav.* **25**, 533–577.
- Trillmich, F. and Dellinger, T.** (1991). The Effects of El Nino on Galapagos Pinnipeds. In *Pinnipeds and El Nino* (ed. Trillmich, F.), pp. 66–74. Springer.
- Vaillant, M., Bohec, C., Prud'Homme, O., Wienecke, B., Maho, Y., Kato, A. and Ropert-Coudert, Y.** (2013). How age and sex drive the foraging behaviour in the king penguin. *Mar. Biol.* **160**, 1147–1156.
- Verrier, D., Groscolas, R., Guinet, C. and Arnould, J. P. Y.** (2011a). Development of fasting abilities in subantarctic fur seal pups: balancing the demands of growth under extreme nutritional restrictions. *Funct. Ecol.* **25**, 704–717.
- Verrier, D., Guinet, C., Authier, M., Tremblay, Y., Shaffer, S., Costa, D. P., Groscolas, R. and Arnould, J. P. Y.** (2011b). The ontogeny of diving abilities in subantarctic fur seal pups: developmental trade-off in response to extreme fasting? *Funct. Ecol.* **25**, 818–828.
- Villegas-Amtmann, S., Costa, D., Tremblay, Y., Salazar, S. and Aurióles-Gamboa, D.** (2008). Multiple foraging strategies in a marine apex predator, the Galapagos sea lion *Zalophus wollebaeki*. *Mar. Ecol. Prog. Ser.* **363**, 299–309.
- Weise, M. J. and Costa, D. P.** (2007). Total body oxygen stores and physiological diving capacity of California sea lions as a function of sex and age. *J. Exp. Biol.* **210**, 278–89.
- Weitzman, B. P.** (2013). Effects of Sea Otter Colonization on Soft-Sediment Intertidal Prey Assemblages in Glacier Bay, Alaska. Masters thesis, University of California Santa Cruz, USA. 78.
- Wikelski, M. and Cooke, S. J.** (2006). Conservation physiology. *Trends Ecol. Evol.* **21**, 38–46.
- Williams, T. M.** (1989). Swimming by sea otters: adaptations for low energetic cost locomotion. *J. Comp. Physiol. A* **164**, 815–24.

- Williams, T. M.** (2000). Sink or Swim: Strategies for Cost-Efficient Diving by Marine Mammals. *Science* (80-.). **288**, 133–136.
- Williams, T. D. and Pulley, L. T.** (1983). Hematology and Blood Chemistry in the Sea Otter (*Enhydra lutris*). *J. Wildl. Dis.* **19**, 1–4.
- Williams, T. D. and Siniff, D. B.** (1983). Surgical implantation of radio telemetry devices in the sea otter. *J. Am. Vet. Med. Assoc.* **183**, 1290–1291.
- Williams, T. M. and Worthy, G. A. J.** (2002). Anatomy and Physiology: the challenge of aquatic living. In *Marine Mammal Biology an Evolutionary Approach* (ed. Hoelzel, A. R.), pp. 73–97. Malden, MA: Blackwell Science Ltd.
- Williams, T. D., Allen, D. D., Groff, J. M. and Glass, R. L.** (1992). An analysis of California sea otter (*Enhydra lutris*) pelage and integument. *Mar. Mammal Sci.* **8**, 1–18.
- Williams, T. M., Fuiman, L. A., Horning, M. and Davis, R. W.** (2004). The cost of foraging by a marine predator, the Weddell seal *Leptonychotes weddellii*: pricing by the stroke. *J. Exp. Biol.* **207**, 973–982.
- Williams, T. M., Rutishauser, M., Long, B., Fink, T., Gafney, J. and Casper, D.** (2007). Seasonal Variability in Otariid Energetics : Implications for the Effects of Predators on Localized Prey Resources. *Physiol. Biochem. Zool.* **80**, 433–443.
- Williams, T. M., Noren, S. R. and Glenn, M.** (2011). Extreme physiological adaptations as predictors of climate-change sensitivity in the narwhal, *Monodon monoceros*. *Mar. Mammal Sci.* **27**, 334–349.
- Wilson, R. and Zimmer, I.** (2004). Inspiration by Magellanic penguins: reduced swimming effort when under pressure. *Mar. Ecol. Prog. Ser.* **278**, 303–307.
- Wilson, R. P., Hustler, K., Ryan, P. G., Burger, A. E. and Christian, E.** (1992). Diving Birds in Cold Water: Do Archimedes and Boyle Determine Energetic Costs ? *Am. Nat.* **140**, 179–200.
- Withers, P. C.** (1977). Measurement of VO₂, VCO₂, and evaporative water loss with a flow-through mask. *J. Appl. Physiol.* **42**, 120–123.
- Yaster, M., Nichols, D. G., Deshpande, J. K. and Wetzel, R. C.** (1990). Midazolam-Fentanyl Intravenous Sedation in Children: Case Report of Respiratory Arrest. *Pediatrics* **86**, 463–467.
- Yeates, L. C.** (2006). Physiological Capabilities and Behavioral Strategies for Marine Living by the Smallest Marine Mammal, the Sea Otter (*Enhydra lutris*). PhD thesis, University of California Santa Cruz, USA. 136.

- Yeates, L. C., Williams, T. M. and Fink, T. L.** (2007). Diving and foraging energetics of the smallest marine mammal, the sea otter (*Enhydra lutris*). *J. Exp. Biol.* **210**, 1960–70.
- Zimmer, I., Ropert-Coudert, Y., Kato, A., Ancel, A. and Chiaradia, A.** (2011). Does foraging performance change with age in female little penguins (*Eudyptula minor*)? *PLoS One* **6**, e16098.