METABOLIC AND THERMOREGULATORY CAPABILITIES OF JUVENILE STELLER SEA LIONS, *Eumetopias jubatus*

A Dissertation

by

LISA ANN HOOPES

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2007

Major Subject: Wildlife and Fisheries Sciences

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ABSTRACT

Metabolic and Thermoregulatory Capabilities of Juvenile Steller Sea Lions,
Eumetopias jubatus. (May 2007)

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Maintaining thermal balance is essential for all homeotherms but can be especially challenging for pinnipeds which must regulate over a variety of ambient temperatures and habitats as part of their life history. Young pinnipeds, with their immature physiology and inexperience, have the additional expense of needing to allocate energy for growth while still dealing with a thermally stressful aquatic environment. With the immense environmental and physiological pressures acting on juvenile age-classes, declines in prey resources would be particularly detrimental to survival. The goal of the present study was to examine the metabolic and thermoregulatory capabilities of juvenile Steller sea lions to better understand how changing prey resources indirectly impact juvenile age classes.

Data collected from captive Steller sea lions suggest that changes in body mass and body composition influence the thermoregulatory capabilities of smaller sea lions in stationary and flowing water. Serial thermal images taken of sea lions after emergence from the water show vasoconstriction of the flippers compared to the body trunk to help minimize heat loss. Despite this ability to vasoconstrict, sea lions in poor body condition displayed a reduced tolerance for colder water temperatures, suggesting that decreases in prey availability which affect insulation may limit survival in younger sea lions.

If reductions in prey availability (i.e., nutritional stress) were impacting western Alaskan populations, a reduction in energetic expenditures would be expected in these animals to cope. Measures of resting metabolism in juvenile free-ranging Steller sea lions across Alaska showed no differences between eastern and western capture locations, suggesting no evidence of metabolic depression in declining western stocks of sea lions.

Finally, thermal costs predicted by a thermal balance model were compared to actual costs measured in the present study. Model output reliably predicted thermoregulatory costs for juvenile Steller sea lions under certain environmental conditions. Basic physiological measurements combined with the predictive power of modeling will allow for greater exploration of the environmental constraints on juvenile Steller sea lions and identify directions of future study.

This work is dedicated to my parents, who taught me that I could be anything I wanted
to be when I grew up.

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CHAPTER I

INTRODUCTION

Metabolism is the process by which energy and materials are transformed within an organism and exchanged between the organism and its environment. Metabolic rates of organisms scale allometrically rather than isometrically with body mass and the relationship between basal metabolic rate (BMR) on body mass (M) is typically expressed as the well-known power function:

$$\mathrm{BMR} = \mathrm{a}\, M^{\mathrm{b}}$$

where *a* is a scaling constant, which is taxon dependent, and *b* is the scaling exponent. Given that heat produced through metabolic processes is lost through the body surface, it has been proposed that metabolic rate should be proportional to the organisms' surface area. This relationship was noted by Sarrus and Ramaeux in 1838 and tested by Max Rubner in 1883 (cited by White and Seymour, 2005) who suggested that the scaling exponent should have a value of ⅔ (0.67) as it was thought that this relationship could be described by geometric constraints due to the surface area to volume ratio of an organism. It was Max Kleiber who demonstrated an alternative scaling exponent of approximately ¾ for a variety of sized terrestrial organisms in his 1932 monograph (Kleiber, 1932). Kleiber's data set was expanded by both Benedict (1938, cited in White and Seymour, 2005) and later by Brody (1945, cited in White and Seymour, 2005) in his now famous mouse-to-elephant curve. This relationship has since been demonstrated in numerous organisms ranging in size over 20-fold including unicellular microorganisms,

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This thesis follows the style of *Journal of Experimental Biology*.

plants, ectothermic vertebrates and invertebrates, and endothermic birds and mammals (Hemmingsen, 1960; Peters, 1983; Schmidt-Nielsen, 1984; Niklas, 1994; Clarke and Johnston, 1999). When metabolic rate is expressed per unit body mass (i.e. mass specifically), the relationship is negative and b = -0.25, meaning that per unit of body mass, smaller animals (e.g., mouse) have a higher metabolic rate, than larger animals (e.g., elephant). Both the 0.75 and -0.25 slopes refer to what is known as the quarter power rule/law in biology (Hochachka et al., 2003).

Compelling evidence exists for quarter power scaling. Extensive research on allometry has shown that this is the case for not only BMR, but numerous other mechanisms of organism form, function, physiology, and life history (Peters, 1983). Not only does whole-organism metabolism scale to $M^{0.75}$, so does the size of biological structures (e.g., cross-sectional area of mammalian aorta and of tree trunks) (West et al., 1997). Mass specific metabolic rate and most other biological rates scale to the $M^{-0.25}$ (e.g., rates of cellular metabolism, circulatory and respiratory rates, stride frequencies) and most biological times scale as $M^{0.25}$ (e.g., life spans, gestation times, embryonic growth) (Lindstedt and Calder III, 1981; Peters, 1983; Schmidt-Nielsen, 1984; West et al., 1997; Savage et al., 2004).

Quarter power scaling is not universally accepted and debate still surrounds the use of slope of 0.75 as a generalization (Bokma, 2004). It has been demonstrated that individual processes that make up whole-animal metabolism (e.g., lung ventilation, cardiac output, circulation) have different scaling relationships (Darveau et al., 2002; Hochachka et al., 2003). Additionally there is some evidence supporting the use of the

⅔ scaling exponent in large mammals (≥ 10 kg) and in birds over the ¾ scaling exponent (Dodds et al., 2001; White and Seymour, 2003). Many proximate factors have been attributed to the differences in mammalian BMR including diet, geography, habitat productivity, and relative organ size, and it is likely that these factors are confounding the relationship of BMR to body mass (White and Seymour, 2003). Criticism leveled at the ¾ power scaling of metabolic rate also stems from the practice of many researchers to focus on interspecific data sets while ignoring the possibility for phylogenetic differences at the species level (Clarke and Johnston, 1999; Lovegrove, 2000; Bokma, 2004).

While it is generally agreed upon that the quarter power scaling rule explains most biological phenomena, the mechanism underlying this relationship has been the subject of much recent speculation and debate (West et al., 1997; Darveau et al., 2002; West et al., 2002; Banavar et al., 2003; Darveau et al., 2003; Hochachka et al., 2003). West et al. (1997) proposed a theoretical model to explain the scaling of metabolic rate based on the fractal-like nature of biological distribution systems, such as animal and plant vascular systems. The model assumes that metabolic rate equals the rate at which these networks deliver resources and that evolution has minimized the time and energy needed to transport substances inside the body. They assume that while organisms vary greatly in size, the terminal units in their distribution networks (e.g., blood capillaries) do not. Combined, the theory predicts that the number of terminal units in a network (which has a fractal structure at all scales) and the rate at which resources are delivered to cells is proportional to the ¾ power of body mass (West et al., 1997; West et al., 2002;

Bokma, 2004). An alternative theoretical model has been developed by Darveau et al. (2002) which attempts to predict the scaling of BMR and maximum metabolic rate from the scaling relationships of the individual processes that make up the overall rates. They have termed this multi-site concept an “allometic cascade” and suggest that each step in the pathway of ATP synthesis has its own scaling behavior with body mass and its own degree of control over whole-animal metabolic rates. Thus, both the scaling exponent and the control coefficient in the intracellular pathways of ATP cycling determine the overall scaling behavior of the whole organism (Darveau et al., 2002; Hochachka et al., 2003).

When analyzing data that vary in magnitude with the body size of the animals being studied, it is often desirable to remove the confounding effects of body mass. Physiological ecologists often use ratios (e.g. mass-specific metabolic rate) to increase the precision of the data and attempt to correct for body size differences among subjects (Packard and Boardman, 1988). Several papers have been published criticizing the wide use of ratios, percentages, and indices in physiological studies in this fashion (Packard and Boardman, 1988; Hayes and Shonkwiler, 1996; Packard and Boardman, 1999; Hayes, 2001). They contend that the problem lies with the fact that mass-specific data are not mass-independent unless the variable of interest varies proportionally (isometrically) with body size. Since most physiological variables change allometrically with body size, the use of ratios during data analysis could lead to erroneous conclusions (Packard and Boardman, 1988). Hayes (2001) suggests that the use of mass-specific data, especially as it relates to metabolism, should be avoided since mass-specific metabolism and whole-animal metabolism often do not measure the same biological concept. In contrast, while McNab (1999) states that total units of metabolism are the ecologically and evolutionary relevant units, he also maintains that the use of mass-specific rates of metabolism are acceptable and often preferred when examining the variation surrounding the scaling relationship of metabolism.

The regression line describing the relationship between metabolism and body mass (often referred to as the 'Kleiber line') is based on metabolism measurements of terrestrial mammals. So how do marine mammals compare? It has generally been assumed that the basal metabolic rate of marine mammals were elevated compared with terrestrial animals of the same size due to the need to maintain thermal balance in the high conductivity medium of water (Hart and Irving, 1959; South et al., 1976; Thompson et al., 1987; Whittow, 1987). Williams (1999) suggests that while marine mammals are more efficient locomotors compared to terrestrial mammals, a greater proportion of their total cost of locomotion is comprised of BMR and thermoregulatory functions.

Kleiber (1975) established criteria for measuring metabolic rate that required subjects to be adults, resting, post-absorptive, and thermoneutral. There is a potential problem in interpreting the published data for marine mammals in that many studies assessing metabolic rate in marine mammals failed to meet the criteria outlined by Kleiber (Lavigne et al., 1986). Juvenile mammals generally display elevated metabolic rates relative to adults, and this elevation is thought to be associated with extra energy required for growth. There is evidence that metabolic rates in marine mammals decline as animals mature (Matsuura and Whittow, 1973; Miller and Irving, 1975; Ashwell-Erickson and Elsner, 1981; Thompson et al., 1987; Rea and Costa, 1992; Donohue et al., 2000; Rutishauser et al., 2004). Comparisons of basal metabolism in marine to terrestrial mammals can be difficult due to several other factors, including differences in metabolic response across the different taxonomic groups of marine mammals, large changes in body composition (specifically body fat), and difficulty in defining "resting" in marine mammals (Costa and Williams, 1999).

Marine mammals spend all or some portion of their lives in water. It is no surprise that BMR increases exponentially with temperature in marine ectotherms due to the high thermal conductivity (25X) and specific heat loss (4000X) of water compared to air (Nadel, 1984). For marine endotherms maintaining a constant body core temperature is essential, heat input and heat loss must be balanced such that:

$$H_{total} = \pm H_k \pm H_c \pm H_r \pm H_e \pm H_s$$

where H_{total} is the rate of metabolic heat production, H_k is the conductive heat exchange, H_c is the convective heat exchange, H_r is the radiant heat exchange, H_e is the evaporative heat loss, and H_s is the rate of storage of heat in the body (Schmidt-Nielsen, 1983). The magnitude of heat loss from these processes (excluding evaporative heat loss) depends on the total surface area for transfer and the thermal gradient between the body surface and the environment. The balancing of this equation with body temperature, metabolic rate, and evaporative water loss determine the limits of extreme conditions which an animal can withstand.

Marine mammals are distributed across the world's oceans and can be found in variety of thermal environments, and as such, would be expected to deal with heat balance differently depending on whether they inhabit waters near the poles or the equator. In water, radiation and evaporation are considered relatively insignificant to heat balance, although, total evaporative heat loss from the lungs (via respiration) has been documented to be as much as 20% of the heat budget in California sea lions, *Zalophus californianus*, when exposed to warm conditions (Matsuura and Whittow, 1974). Evaporative heat losses are assumed to be minimal at cold temperatures. Alternately, convection is the primary mechanism for heat exchange, with some transfer through conduction (usually lumped with the convection term) (Schmidt-Nielsen, 1983). Preserving heat in cold water requires either a decrease in conductance (or the inverse, an increase in insulation) or an increase in metabolic heat through an increase in either metabolic rate and/or voluntary (exercise) or involuntary (shivering) muscular activity. For the large number of marine mammals distributed throughout cold waters, the main solution to preserving heat is effective insulation.

Marine mammals that are strictly aquatic rely on a layer of subcutaneous adipose tissue known as blubber for insulation. This blubber layer can be a thick as 25 cm in Arctic bowhead whales, *Balaena mysticetes*, and can range in lipid content between 9 to 82% depending on the species and site (Lockyer et al., 1984; Ryg et al., 1988; Worthy and Edwards, 1990). Blubber can make up a substantial percentage of body mass, often in excess of 30%, and its insulative properties depends not only on thickness, but on lipid quality (Parry, 1949; Worthy and Edwards, 1990; Pabst et al., 1999; Dunkin et al., 2005). When ambient conditions are below core body temperatures, marine mammals can constrict blood flow to the highly vascularized blubber layer and the extremities, thus reducing the surface area available for heat loss. Measurements of skin temperature of submerged phocid seals support this idea for the temperature at the skin is close to that of the water (Irving, 1969).

Some marine mammals (pinnipeds, polar bears, sea otters) also use fur as an insulator. While the blubber layer in phocid seals (true or haired seals) is a sufficient insulator, otariid seal (eared seals) blubber tends to be thinner (Bryden and Molyneux, 1978) and their ability to thermoregulate is partially compensated by the pelage. To be an effective insulator, fur must decrease the skins' contact with the external environment. Dry fur is a better insulator than blubber; however, when submerged, fur losses most of its insulative properties (Scholander et al., 1950; Costa and Kooyman, 1982). Sea otters, *Enhydra lutris*, and northern fur seals, *Callorhinus ursinus*, have very dense pelts (60,000-130,000 hairs/cm^3) that trap a layer of air next to the skin surface, thus maintaining a dry skin surface when wet (Pabst et al., 1999). However, where the fur seal has thick fur and blubber and can spend more time submerged while foraging, sea otters have very little internal fat stores and are limited to short foraging dives since most of the air is compressed from the fur at depth (Costa and Kooyman, 1982).

A short-term option for balancing heat losses in a cold environment would be to increase metabolic heat production. Metabolic rate has been measured for a variety of marine mammals (e.g., Hart and Irving, 1959; Heath et al., 1977; Costa and Kooyman, 1982; Gallivan and Best, 1986; Thompson et al., 1987; Worthy and Lavigne, 1987;

Markussen et al., 1992; Rea and Costa, 1992; Renouf and Gales, 1994; e.g., Hansen et al., 1995; Boily and Lavigne, 1997; Rosen and Trites, 1999; Donohue et al., 2000; Arnould et al., 2001; Hurley and Costa, 2001; Trillmich and Kooyman, 2001; Sparling and Fedak, 2004), although as mentioned earlier, it is unclear whether the reported higher rates for marine mammals are due solely to the need to maintain thermal balance in water.

For marine mammals in tropical waters, the need to avoid overheating, especially during sustained swimming and or diving, is more pressing. Marine mammals are able to shunt heat to the surface of the skin where it can be lost through conductive processes to the water by bypassing the insulative layer. This is accomplished through vasodilation of the arterioles that run through the highly vascularized blubber layer that can carry blood (and heat) to the epidermal surface of the animal. Sparsely insulated appendages such as flippers, dorsal fins and flukes can also act as “thermal windows” to dissipate heat. Often these areas are also highly vascularized allowing warm blood to be carried to the skin surface where it can be cooled by exposure to ambient water (McGinnis et al., 1972; Hampton and Whittow, 1976; Pabst et al., 1999).

In air, radiant and evaporative heat exchange mechanisms become important for marine mammals along with conduction and convection. Similar to that in water, in order to maintain heat balance in air, marine mammals can alter their thermal balance through physiological modification of skin temperature via constriction or dilation of the blubber vasculature (i.e. changing insulation) and metabolic heat production. Behavioral thermoregulation is also a mechanism used by marine mammals to control the amount of surface area exposed to heat losses or gains (e.g., huddling, cooling off in water)(Odell, 1974; Limberger et al., 1986).

The range of temperatures within which regulatory metabolic heat production is unaffected by a temperature change is termed the animal's thermoneutral zone (TNZ). The limits of the TNZ are determined by an upper (T_{uc}) and lower (T_{lc}) critical temperature beyond which the animal increases metabolic heat production. While much research has been conducted on determining components of the TNZ in air (e.g., Heath et al., 1977; Hansen et al., 1995; Hansen and Lavigne, 1997b; Donohue et al., 2000; e.g., Arnould et al., 2001) surprisingly few studies of the TNZ in marine mammals have been conducted in water.

Thermoneutral zone studies in marine mammals have focused on phocid seals, which tend to have a broad TNZ compared with the narrow TNZ in air of most terrestrial mammals. Studies with harbor seals, *Phoca vitulina*, suggest that the T_{lc} in water was higher (near 20 ºC) in the summer than when measured in the winter (13 ºC). This seasonal change in the T_{lc} was attributed to a 30% increase in insulation during the winter (Hart and Irving, 1959). Harp seals, *Phoca groenlandica*, appear to have a broader TNZ than harbor seals of at least 28 ºC and the T_{lc} for this species is below 0 ºC in water (Gallivan and Ronald, 1979). The T_{uc} for these species are not known. Northern elephant seal, *Mirounga angustirostris*, pups with sufficient lipid stores at the end of the post-weaning fast were shown to be able to remain thermally neutral in 4 ºC water; however, water temperatures of approximately 14 ºC and warmer may be outside their TNZ (Noren, 2002).

Little is known of the TNZ in otariids. Sea lions are generally temperate water otariids and are considered to be leaner animals than Arctic phocids of the same size. Liao (1990) examined the metabolic response of sub-adult California sea lions to changing water temperatures (range 5-30 ºC). The TNZ was approximately 20 ºC and the T_{lc} was about 14 ºC, which is close to ambient water conditions within the home range of this species. This suggests that California sea lions in the wild live at the edge of their TNZ, implying an increased reliance on physiological mechanisms to maintain thermoneutrality or increased levels of activity to offset thermal costs. Rutishauser et al. (2004) estimated the T_{lc} of young Antarctic fur seals, *Arctocephalus gazella*, at about 14 ºC, which is 10-15 ºC above water temperatures normally experienced by these otariids, suggesting they too might have a high metabolic overhead living outside their TNZ. Although T_{lc} was not determined for post-molt northern fur seal pups, Donohue et al. (2000) noticed no differences in metabolic rate when pups were exposed to 5, 10, and 20 ºC water suggesting that this species may have a greater thermal tolerance to cold than Antarctic fur seals. While no studies have examined the T_{uc} of otariids in water, limited work with California sea lions suggests the T_{uc} in air is between 22 and 30 ºC, with smaller sea lions being able to tolerate higher temperatures (Matsuura and Whittow, 1973; Matsuura and Whittow, 1975).

Little work has been done to examine the TNZ of cetaceans (whales, dolphins, porpoises) due to the logistics involved in working with such large, solely aquatic animals and due to the difficulty in being able to thermally manipulate such large quantities of water in a captive setting. Costa and Williams (1999) report that captive bottlenose dolphins, *Tursiops truncatus*, in 28 ºC water have a TNZ of at least 15 ºC and the T_{lc} depended on the size of the animal along with the quality and thickness of the blubber layer. They also note the TNZ of bottlenose dolphins can be shifted depending on the temperature of water to which they are acclimated (Costa and Williams, 1999). Studies of temperature regulation in small odontocetes (toothed whales) have largely focused on changes in core body temperature, heat production, and heat flux across the body surface (McGinnis et al., 1972; Hampton and Whittow, 1976; Whittow, 1987; Worthy and Edwards, 1990; Heath and Ridgway, 1999; Noren et al., 1999; Williams et al., 1999; Meagher et al., 2002). Studies with captive Pacific bottlenose dolphins, *Tursiops gilli*, and Hawaiian spinner dolphins, *Stenella longirostris*, suggest that these species displayed elevated body temperatures and insulation while at rest in warm tropical water (24 ºC) in order to conserve body heat (McGinnis et al., 1972; Hampton and Whittow, 1976). These species exist near their T_{lc} and may rely on heat generated from swimming to maintain thermal balance (Hampton and Whittow, 1976).

Given that experimental determination of TNZ is not feasible for mysticete (baleen) whales, attempts at theoretical calculations of T_{lc} have been made using various heat-flow models (Hokkanen, 1990; Lavigne et al., 1990; Watts et al., 1993). Although there is some level of uncertainty associated with modeling the thermal environment of a living organism (Kvadsheim et al., 1997), these studies suggest that blue whales, *Balaenoptera musculus*, should have little trouble maintaining thermal balance in the coldest of waters (-2 ºC) and estimated T_{lc} was below the lowest recorded sea surface temperatures (Lavigne et al., 1990; Watts et al., 1993).

Unlike other marine mammals, manatees, *Trichechus* sp., and sea otters exhibit atypical metabolic rates compared with terrestrial mammals of a similar size. Manatees exhibit body temperatures near the lower end of the normal mammalian range and relatively high T_{lc} (20-23 ºC) compared with other marine mammals (Gallivan et al., 1983; Irvine, 1983; Miculka and Worthy, 1995). Combined with a lower than predicted metabolic rate based on size alone, these physiological limits constrain the tolerance of these animals to changing water temperature. In contrast, sea otters maintain a higher than normal metabolic rate (2.5X) than would be predicted by body size. Thermoneutral zone studies suggest sea otters tolerate a broader range of temperatures in air (-20 to 21 ºC) than in water (20 to 33 ºC) (Morrison et al., 1974).

The 14 extant members of the Family Otariidae are widely distributed from the equator (e.g., Galapagos fur seal, *Arctocephalus galapagoensis*) to the poles (e.g., northern fur seal) and hence are subject to a wide variety of environmental conditions. Summer water temperatures near the Galapagos Islands can reach 35 ºC, while winter water temperatures in the Bering Sea can reach near freezing temperatures (0 ºC). While otariids must deal with the thermal challenges of swimming and diving in water, they also have the dual challenge of coping with terrestrial conditions for reproduction, parturition, and nursing. For example, female Galapagos fur seals exhibit lower field metabolic rates than Antarctic fur seals, implying a lower metabolic overhead while onshore due to the need to reduce thermal stress on land in the warmer environment (Costa and Trillmich, 1988). Of the pinnipeds, otariids are considered to be the most terrestrial, spending upwards of 30% of their lives on shore (Gentry, 1970).

In order to maintain thermal equilibrium animals should theoretically show a preference for a habitat that keeps them within their TNZ (Trites, 1990). This is not always possible, and as already discussed, many marine mammals exist near their critical temperature limits. In these situations, animals usually initially rely on behavioral mechanisms as they are less of a drain on energy stores and later on physiological mechanisms as previously discussed. For example, a sea lion on land in the winter might compensate for heat loss by increasing metabolism and/or muscular activity to produce heat. This energy would come either from recently ingested food, or through mobilization of lipid stores. Active foraging in water requires additional energy expenditure in a medium that promotes heat loss, while mobilization of fat stores reduces valuable blubber thickness needed to thermoregulate. It has been suggested that the heat generated through locomotion or as a result of digestion may be used by animals to offset heat loss; however, experimental results are mixed (Costa and Kooyman, 1984; Feldkamp, 1987; Klassen et al., 1989; Wilson and Culik, 1991; MacArthur and Campbell, 1994; Chappell et al., 1997; Jenson et al., 1999; Campbell et al., 2000; Hindle et al., 2003; Rosen and Trites, 2003; Bech and Praesteng, 2004; Williams et al., 2004b), and in marine mammals the use of heat generated through activity and feeding to offset an increase in metabolism for thermoregulation has only been observed in sea otters (Costa and Kooyman, 1984). Collectively, thermoregulation is series of complex behavioral and physiological energetic tradeoffs employed by an animal to prevent thermal imbalance in a given environment (Rosen et al., 2007). These thermal challenges are further magnified when resources are limited.

Steller Sea Lion

Steller sea lions, *Eumetopias jubatus,* are the largest of the otariids and are distributed throughout the northern Pacific Rim from California north through the Gulf of Alaska and the Aleutian Islands, including the waters near the Pribilof Islands over to the Kamchatka Peninsula and the Sea of Okhotsk. Stellers are considered ‘lean’ animals with an average blubber layer less than 2-3 cm thick (Pitcher et al., 2000) and forage in water that can reach near-freezing temperatures (Jefferson et al., 1993).

Stellers exhibit a high degree of sexual dimorphism with males reaching an average body mass of 1100 kg compared to females which reach an average size of 270 kg. This size difference has allowed a polygynous breeding system to develop on established rookeries and rocky outcroppings. Males arrive in May to compete for territories, fasting the duration of the breeding season to maintain their territories. Females arrive by late May to give birth to a single pup usually in June and averaging approximately 22 kg in size. Mating occurs about 10 days after parturition, after which males depart the rookery. Females remain on land with their pups for 11-14 days after birth, and then begin to make brief foraging trips alternating with periods of nursing their new pup (Gentry, 1970). Pups are completely terrestrial for the first 2-3 weeks and then transition rapidly to exploring the near shore waters. By a few months of age they are well adapted to aquatic life and have been observed dispersing long distances from their natal rookery (Raum-Suryan et al., 2004). Pups are typically weaned within the first year, although observations of suckling juveniles two and three years old have been reported (Pitcher and Calkins, 1981; Trites and Porter, 2002).

The development of diving plays a major role in determining what age a pinnipeds can successfully forage and survive independently (Horning and Trillmich, 1997). This is likely constrained by the ontogeny of thermoregulatory capabilities in young pups entering the water for the first time. Development of thermoregulatory capabilities may limit the age at which pups begin to enter the water due to the need for pups to allocate energy between maintenance, growth and activity (Donohue et al., 2000).

Steller sea lions in the U.S. are currently listed as threatened within their eastern Alaskan range (east of Cape Suckling, 144º W) and endangered in their western Alaskan range (west of 144º W). Population levels have declined over 85% of peak levels during the past 30 years (Braham et al., 1980; Loughlin et al., 1984; Merrick et al., 1987; Loughlin et al., 1992; Trites and Larkin, 1996; Loughlin, 1998; Calkins et al., 1999). Several mechanisms have been proposed to account for the decline, including commercial and subsistence hunting (e.g., Pascual and Adkinson, 1994), disease (e.g., Burek et al., 2005; Bowen et al., 2006), pollutants (e.g., Beckmen et al., 2004), killer whale predation (e.g., Springer et al., 2003; Williams et al., 2004a), ecosystem-wide ocean climate changes (e.g., Guénette et al., 2006; e.g., Trites et al., 2007), malnutrition (e.g., Merrick et al., 1997; Rosen and Trites, 2000; Trites and Donnelly, 2003), and competition with commercial fisheries (e.g., Cornick et al., 2006; Guénette et al., 2006). The leading, but unconfirmed, hypothesis for the decline is reduced juvenile survival resulting from a historical shift in the quality and/or quantity of prey available. This is referred to as the nutritional stress hypothesis in the literature (York, 1994; Merrick and Loughlin, 1997; Calkins et al., 1998; Trites and Donnelly, 2003). If the energetic demands of young sea lions are not being met by energy input (i.e., nutritional stress), these animals might be forced to tap into their insulatory stores, thus comprising their ability to thermoregulate and increasing the metabolic costs associated with staying warm. The smaller size of these juvenile sea lions means increased heat loss due to a smaller surface area to volume ratio and their immaturity and inexperience are suggestive of limited diving abilities. Thus, declining blubber stores could also impact foraging by limiting how far the sea lion can range to productive feeding locations, how deep they could dive, and how long they have at depth to search for prey. If this continues over the long term, lipid stores would continue to be depleted until body condition deteriorated to such a state where thermal balance could not longer be maintained, ultimately leading to death.

Scope of the Project

The goal of the present investigation was to examine the thermal energetics of juvenile Steller sea lions. Currently, no information is available about the thermal limits for young Steller sea lions in air or in water. Steller sea lions are a model species for undertaking energetic studies due to the need for a greater understanding of their general physiology and energetic requirements. Additionally, there are a modest number of Steller sea lions of varying age in captivity available for research purposes.

Because nutritional deficiencies may impact thermoregulatory abilities in young Steller sea lions, the influence of varying body condition on thermoregulatory ability in cold water was examined in captive animals at the Vancouver Aquarium. How these sea lions responded to a range of cold water temperatures in stationary and flowing water was also assessed. The results of this study are presented in Chapter II.

After emergence from thermal trials in cold water, serial thermal images were taken of captive sea lions held at ambient air conditions to assess the spatial and temporal distribution of thermal windows on the trunk and appendages. The results of this study are presented in Chapter III.

Current estimates of Steller sea lions in Alaska suggest that the western population is continuing to decline, while the eastern population has remained stable or has slightly increased. If nutritional stress is still impacting sea lions within the western Alaskan range, these animals would be expected to limit energy expenditures in order to cope. One of the most common ways to limit energy expenditures is through a lowering of metabolic rate, known as metabolic depression. Metabolic studies were conducted on free-ranging juvenile Steller sea lions captured from populations in the region of decline (Aleutian Islands, Prince William Sound) and the region of stability (Southeast Alaska) and compared to determine evidence of metabolic depression. The results of this study are presented in Chapter IV.

In order to better understand the relationship between Steller sea lions and their thermal environment, modifications were made to a predictive energetics-based model using metabolic data presented in the previous chapters. One of the goals of this model was to determine a threshold of body condition, below which thermal costs in water would be incurred in varying sized sea lions. The results of this effort are presented in Chapter V and the project overview and conclusions are presented in Chapter VI.

CHAPTER II

INFLUENCE OF BODY CONDITION ON METABOLISM AND THERMOREGULATORY CAPABILITIES OF JUVENILE STELLER SEA LIONS

Introduction

Pinnipeds face unique thermoregulatory challenges among marine mammals in that while they lead a primarily aquatic existence they also reproduce on land. Foraging at sea can be energetically costly given the high thermal conductivity and specific heat of water which can draw heat away from an animal 25 times faster than air (Schmidt-Nielsen, 1983; Nadel, 1984). This is especially true for younger, smaller animals which may lose more heat as a result of a smaller surface area to volume ratio compared to that of larger animals. Being able to balance, or counter, these thermal losses is critical for juvenile pinnipeds to master as they can impact survival at independence when young animals transition to the water to begin foraging and diving (Baker and Donohue, 2000; Donohue et al., 2000).

Thermoregulatory challenges in water can potentially be overcome by increasing insulation through the addition of lipid to the subcutaneous blubber layer. However, the blubber layer serves multiple, often conflicting, roles including acting both as insulator and energy store. This is certainly the case for ice-breeding phocids such as harp seals, where pups enter the water shortly after weaning and must rely primarily on blubber stores as both an energy source and insulator during the 6 week fast until food resources become available (Worthy and Lavigne, 1983b). Other physiological mechanisms that can compensate for elevated heat losses over the short term include increasing metabolic rate, lowering core body temperature, and restricting blood flow or modifying blood flow through heat exchangers. Additionally, heat losses may be compensated for through metabolic increases due to activity and/or digestion (Costa and Williams, 1999; Rosen et al., 2007). In the short term, animals typically opt to cope with thermal stress by increasing metabolic rates through increased activity, shivering, and non-shivering thermogenesis (Schmidt-Nielsen, 1983). This energy comes either from the ingestion of food, or through mobilization of lipid stores. Foraging requires increased energy expenditures in a medium that promotes heat loss, while depleting blubber stores for energy needs reduces insulation and ultimately threatens thermal stability. The latter can be particularly costly when resources are limited and ultimately this imbalance could produce a situation where body condition deteriorates and energy deficits increase until thermal balance can no longer be supported. It should be noted that all of these mechanisms are likely employed and that thermoregulation is a series of complex energetic tradeoffs employed by an animal to balance thermal stability within its environment (Rosen et al., 2007).

Juvenile marine mammals have been shown to have elevated metabolic rates, a trait usually associated with the energetic cost of growth (Matsuura and Whittow, 1973; Miller and Irving, 1975; Ashwell-Erickson and Elsner, 1981; Thompson et al., 1987; Rea and Costa, 1992; Hansen and Lavigne, 1997a; Donohue et al., 2000; Rutishauser et al., 2004). These rates decline as animals mature, complicating the relationship between metabolic rate and thermoregulation. The interpretation of metabolic rate in marine mammals is further complicated by the large changes in body composition (usually lipid) that some species undergo as part of their natural life history. It is generally agreed upon that adipose tissue is metabolically inert relative to lean tissue, thus making a minimal contribution to an animals overall metabolism (Rea and Costa, 1992). This has been demonstrated in several pinniped species (Rea and Costa, 1992; Aarseth et al., 1999; Donohue et al., 2000; Noren, 2002; Rutishauser et al., 2004), however, changes in lipid mass could influence metabolism if decreases in blubber stores impacted an animal's ability to thermoregulate. Thus, animals that undergo extended fasting events, should mobilize blubber and core reserves equally to maintain thermal balance (Ryg et al., 1988; Rea and Costa, 1992).

Several studies have examined the thermoregulatory response in pinnipeds concurrent with natural changes in body composition (e.g., post-weaning and/or molting) (Hart and Irving, 1959; Miller and Irving, 1975; Worthy and Lavigne, 1983a; Worthy and Lavigne, 1987; Costa and Trillmich, 1988; Worthy, 1991; Hansen and Lavigne, 1997b; Hedd et al., 1997; Donohue et al., 2000; Noren, 2002; Rutishauser et al., 2004; Harding et al., 2005). Few of these studies examine the thermoregulatory response with changing body composition in otariids. Donohue et al. (2000) found that post-molt northern fur seal pups had lower mass-specific metabolic rates than pre-molt pups, suggesting increased thermoregulatory capacity due, in part, to increased lipid stores. Similar increases in body lipid reserves were measured in pre-and post-molt Subantarctic fur seal pups and suggest a thermoregulatory benefit (Beauplet et al., 2003). Similarly, the interaction of body composition and body size appears to impact thermoregulation in young Antarctic fur seals (Rutishauser et al., 2004).

Steller sea lions (*Eumetopias jubatus*) are the largest of the otariids, inhabiting the near-freezing waters of Alaska and the Bering Sea. While other sympatric pinnipeds rely on dense pelage (e.g., northern fur seal) or thick blubber layers (e.g., phocids, walrus), Steller sea lions are considered to be 'lean' animals with relatively thin blubber layers (Pitcher et al., 2000). Declines in western Steller sea lion populations have been attributed to reduced juvenile survival and nutritional limitations (York, 1994; Merrick et al., 1995; Trites and Donnelly, 2003), although supporting data are equivocal. If energy deficits are not met by energy intake, as a result of nutritional stress, then juvenile sea lions would be expected to tap into their energy (lipid) stores, thus reducing valuable insulation needed to prevent heat losses.

The present study quantifies the thermoregulatory response of juvenile Steller sea lions in cold water and examines the influence of varying body condition on the thermoregulatory response. Specific goals of the study were to (1) investigate the thermal response of captive juvenile Steller sea lions to a range of cold water temperatures, (2) assess the effect of varying body condition on thermoregulatory capabilities, (3) examine differences in thermal response to stationary versus flowing water, and (4) compare metabolic capacity of sea lions in an air and water medium.

Materials and Methods

Study Local and Subjects

Experiments were conducted with a group of Steller sea lions that had been captured as pups and raised at the Vancouver Aquarium Marine Science Centre (Vancouver, BC, Canada). Nine female Steller sea lions – eight juveniles, 2-3.5 years old; and one sub-adult, 5.5 years old – participated in experimental trials between 2003 and 2005, with some individuals being measured multiple times across years. Additionally, limited measurements were opportunistically collected from four juvenile female pups (8 mo. old). Individual sea lions sampled were of varying age, body size, and body condition (Table 1). Sea lions were held in an outdoor compound with access to ambient seawater and haul out space. Animals were generally fed a daily diet of Pacific herring (*Clupea pallasi*), occasionally with other fish species, equivalent to approximately 5-6% of their body mass and supplemented with vitamins. All experimental protocols were conducted under Texas A&M University Laboratory Animal Care Committee Animal Use Protocol number 2001-319, University of Central Florida Institutional Animal Care and Use Committee number 02-07W and the University of British Columbia Animal Care Committee.

Experimental Design

Sea lions began each experimental trial in a state of reduced body mass (10-15% reduction), and body condition, as a result of being fed a sub-maintenance diet as part of other studies (Kumagai, 2004; Rosen and Trites, 2005; Kumagai et al., 2006; T. Dudot,

Table 1. *Summary of captive Steller sea lion experimental trials conducted between 2003 and 2005*

Animal	Trial Date	Age at Trial (yr)	Trial Duration (d)	Initial Mass (kg)
F97HA	Spring 2003	5.5	17	131.6
	Fall 2003	6.0	36	136.4
F00ED	Spring 2003	2.5	18	101.2
	Fall 2003	3.0	37	95.1
F00NU	Spring 2003	2.5	17	113.0
	Fall 2003	3.0	37	112.0
	Spring 2004	3.5	31	145.0
F00TS	Spring 2003	2.5	18	124.4
	Fall 2003	3.0	36	121.3
F00YA	Spring 2004	3.5	31	128.7
F03AS	Spring 2004	0.7	-	56.9
	Summer 2005	2.0	65	81.7
F03IZ	Spring 2004	0.7	-	55.4
	Summer 2005	2.0	72	77.0
F03MA	Spring 2004	0.7	-	57.7
	Summer 2005	2.0	68	79.6
F03WI	Spring 2004	0.7	-	54.3
	Summer 2005	2.0	75	79.4

personal communication). Throughout the course of each trial in the present study (ranging from 17-75 d in length) animals were fed at a daily rate comparable to 6-7% of their body mass, resulting in a steady gain of mass. Most sea lions reached pre-weight loss body masses by the end of each experimental period. Metabolic measurements were collected from each sea lion on three separate occasions through the course of the experiment; an initial set when sea lion body condition was in a reduced state, the second mid-way through the experiment at some transitional state, and the last set after the sea lion had recovered lost body mass. Body condition was assessed at the start and end of each experiment through deuterium isotope dilution techniques and ultrasound measurements of blubber depth. Additional ultrasound measurements were taken throughout the course of the experiment when the opportunity arose. Body mass was recorded daily, and other morphometrics were measured at least once a week.

Metabolic Measurements

Standard metabolic rate (SMR) was measured in water, and for a subset of animals, in air, via open flow respirometry. Standard metabolic rate measurements were made when the animal was post-absorptive, quiescent but awake, and non-pregnant comparable to the conditions set by Kleiber (1975), but measurements were performed beyond the thermoneutral zone. Water trials were conducted in a temperature-controlled seawater swim flume as described by Rosen and Trites (2002b). The swim flume was equipped with a 120-l lexan dome under which the sea lions could surface to breathe. Water flow was generated by two turbines and flow rates were controlled by individual rheostats. Actual water speed was determined through calibration with a low-flow water velocity meter (Flow-Mate 2000, Marsh-McBirny Inc., MD, USA). Air was drawn through the dome at a constant rate (140-200 l min^{-1}) and was regulated by a flow meter (King Instrument Company, CA, USA) in series with a vacuum pump. In later experiments, the flow meter and vacuum were replaced with a flow generator and controller (500H Flow Kit, Sable Systems International, NV, USA). Oxygen and carbon dioxide concentrations, within a desiccated subsample of expired air, were determined by an oxide cell analyzer (FC-1B, Sable Systems International, NV, USA) and an infrared gas analyzer (CA-1B, Sable Systems International, NV, USA), respectively.

The system was baselined to known ambient air concentrations before and after each trial and N_2 recovery rates at each flume speed were measured at the end of the study to correct for mass flow. The amount of oxygen consumed during the trial was calculated from the difference in oxygen concentration between air entering and leaving the chamber, with flow corrected to standard temperature and pressure, dry (STPD). Carbon dioxide was not removed from the sampled air stream, therefore, oxygen consumption rates were determined by Datacan V software (Sable Systems International, NV, USA) using equation 3b from Withers (1977) to remove the affect of carbon dioxide on oxygen readings. The entire system was calibrated through the course of the experiments using gases of known concentration and a standard nitrogen dilution technique (Fedak et al., 1981). Theoretical and measured changes in oxygen consumption inside the chamber agreed to within 0.01%-0.04%.

Sea lions had previous experience with the swim flume and were trained to enter the flume willingly. Sea lions were usually tested in the morning, a minimum of 16 h after their last meal. Metabolic rate was determined over a combination of water temperatures (2, 4, and 8 °C) and water flow speeds (0, 0.5, 1.0, and 1.2 m s^{-1}) to examine the potential interaction of temperature and convective heat loss as a function of water flow past the animal. It should be noted that sea lions were not actively swimming in the flume; rather, the animals remained stationary under the dome while water flow speed was altered to estimate the heat generated due to convective loss without the added cost of swimming. The four flow speeds were measured in succession for 25 minutes each at a given temperature within a single day. Water temperature in the flume was monitored with a thermocouple attached to a digital thermometer (Model #61220-670, VWR International, Ontario, Canada). For each animal, SMR was determined from the lowest rate of oxygen consumption recorded during a minimum 15 min period. For all trials, sea lion activity was recorded every 5 min. and whenever activity changed. Oxygen consumption rates were converted to energy consumption assuming that 1 l O_2 = 20.1 kJ.

In-water metabolic measurements were made opportunistically on four juvenile female pups (8 mo. old). For these measurements, sea lions were tested in the swim flume at the same three water temperatures listed above but only at flume speeds of 0 and 0.5 m s^{-1}. Trials were 20 min in duration at each speed and each pup was restricted to measurements at a single temperature per day. Body mass was recorded daily; however, mass was not experimentally modified in these animals (beyond natural growth) nor was body condition assessed. For each animal, SMR was determined from the lowest rate of oxygen consumption recorded during (at minimum) a 10 min period. For all trials, pup sea lion activity was recorded every 5 min and whenever activity changed.

In-air measurements were conducted on four of the same juvenile animals used in the in-water trials. Instrumentation and methodology were similar to that described above and as described in Rosen and Trites (1999). Sea lions were trained to enter a dry metabolic chamber (approximately 1050 l), equipped with a fan to circulate air and a video camera to monitor activity. Metabolism was monitored over a 50-min period, following a 5-10 min acclimation period. For all trials, sea lion activity was recorded every 5 min and whenever activity changed. SMR was determined from the lowest rate of oxygen consumption during a minimum 15 min period where the animal was inactive. Both ambient and chamber air temperatures, atmospheric pressure, and humidity were recorded with a digital weather station (WS-7014U, Springfield Instrument Canada, Ontario, Canada).

Body Condition

Body condition was assessed in sea lions by measuring the thickness of the subcutaneous blubber layer, and by estimating body composition calculated from total body water (TBW). Blubber thickness was measured using ultrasound (either Scanoprobe II, Scanco Inc., NY, USA or SonoSite 180PLUS, SonoSite Inc., WA, USA) at five locations along the body the sea lion: neck, dorsal and laterally along the axillary girth, and dorsal and laterally along the hip girth (Fig. 1).

Total body water pool was determined using deuterium isotope (^2H_2O) dilution. An initial serum sample was collected from the caudal gluteal vein of the sea lion to determine background isotope levels. Deuterium oxide was then injected intramuscularly at a dose of 0.10-0.15 mg kg^{-1} and allowed to equilibrate with body water for 120 min (D. Rosen, personal communication), after which, a second serum sample was obtained. Sera and dosage samples were analyzed by Metabolic Solutions Inc. (Nashua, NH, USA) and calculated TBW was converted to total body lipid (TBL) using predictive equations derived for adult Antarctic fur seals (Arnould et al., 1996) and correcting for the approximate 4% overestimation of TBW (Bowen and Iverson, 1998). Lean body mass (LBM) was calculated as the difference between total body mass (TBM) and TBL and then expressed as percentage of total mass (%TBL, %LBM).

Morphometrics

Body mass was measured by using having the sea lions hold position on a platform scale (±0.1 kg, Pacific Industrial Scale Company Ltd.). Standard pinniped morphometric measurements, including standard length were taken (American Society of Mammalogists, 1967). Girths were measured at three locations (shoulder, axilla and hips) along the axis of the sea lion.

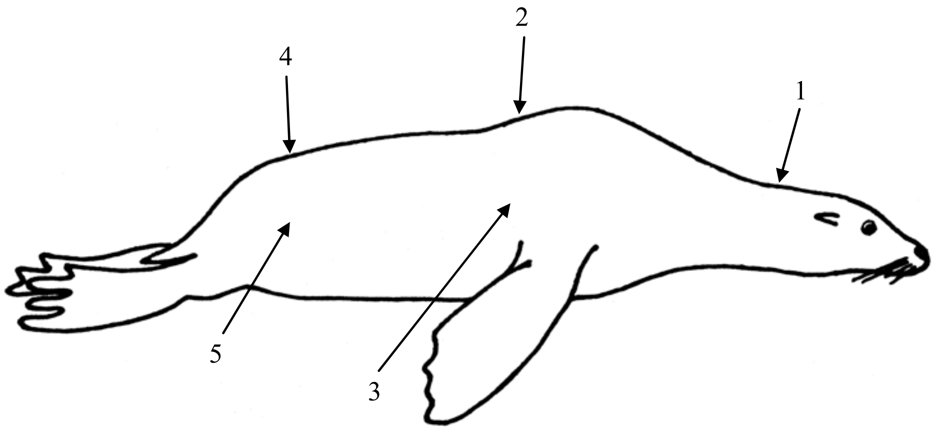

Figure 1. Locations along the trunk of the sea lion where blubber depth was measured via ultrasound: neck (1), dorsal axillary (2), lateral axillary (3), dorsal hips (4) and lateral hips (5).

Statistical Analyses

SAS (version 9.1) and JMP (SAS Institute, version 6.0) software were used for statistical analyses. Repeated measures analysis of covariance (RM ANCOVA) was used to examine the response of metabolic rate at each of the sampling points (initial, mid, final) to water temperature and flow speed using a mixed model (PROC MIXED) to account for the non-independence of longitudinal data and a covariate (body mass) that changed over time. Significant treatment effects in the mixed model were further explored using repeated measures analysis of variance (RM ANOVA) and paired t-tests. A Bonferroni correction was applied to multiple pairwise comparisons. Least-squares linear regression was used when indicated. All data were explored for sphericity, normality and equality of variances where appropriate using Mauchley's, Shaprio-Wilk and Levene's F tests, respectively. For non-normal distributions or those with unequal variances, a Mann-Whitney rank sum test or a Kruskal-Wallis one-way ANOVA on ranks was used. Means are reported with ±1 standard error of the mean (SEM). Results were considered significant at $P<0.05$, unless otherwise noted.

Results

Body Condition

Sea lions lost an average of 11.8 ± 0.8 kg of body mass (9.8%, range 7.1-13.0%) and 15.1% of TBL (range 5.5-40.7%) prior to the start of the present study. Over the course of the experimental trials, sea lions experienced a mean increase in body mass of 12.7 ± 1.4 kg (11.7%), although body mass gain varied across individuals (Table 2). In all but one trial (Spring 2003), sea lions had returned to or exceeded original body mass levels recorded prior to the start of the reduction in energy intake. Mean daily rates of total body mass gain (kg d^{-1}) did not differ between trials; with the exception of the sea lions sampled in Summer 2005, which had significantly lower rates of mass gain ($F_{3,10}$=13.61, all $P \leq 0.007$, Table 3).

Along with increases in body mass, sea lions generally exhibited increases in mean total body lipid (3.71 ± 1.68 kg, 40.8%) and lean mass (8.38 ± 1.06 kg, 9.1%). However, two sea lions exhibited decreases in TBL despite increases in total body mass (Table 2). Sub-adult sea lion F97HA lost 2.3 kg (16.4%) of fat mass, while juvenile sea lion F00TS lost 11.2 kg (54%). Conversely, two juvenile sea lions (F00ED, F00NU) experienced large gains in TBL as a percentage of their starting body mass (142.6% and 136.0%, respectively, Table 2). Mean daily rates of lipid and lean mass gain did not differ between sampling trips (TBL: $F_{3,10}$=0.653, P=0.60, LBM: $F_{3,10}$=0.666, P=0.59). Overall, both TBL and LBM increased over the course of the experiment, however,

Table 2. *Initial and final values of body mass (kg), total body lipid (%), and standard metabolic rate (MJ d^{-1}) of Steller sea lions in still water (2, 4, 8 ºC)*

			2 °C		4 °C		8 °C	
Animal	Age class		Initial	Final	Initial	Final	Initial	Final
F03AS	Juvenile	BM	81.7					90.8
		TBL	11.8					13.7
		SMR	33.9	38.2			30.7	29.2
F03IZ	Juvenile	BM	77.0					90.8
		TBL	14.0					12.7
		SMR	31.0	36.8			28.9	26.0
F03MA	Juvenile	BM	79.6					92.0
		TBL	14.1					13.9
		SMR	22.0	28.4			22.0	25.5
F03WI	Juvenile	BM	79.4					85.6
		TBL	13.0					15.6
		SMR	31.0	26.3			26.3	26.3
F00ED	Juvenile	BM	101.2					107.7
		TBL	14.8					16.6
		SMR	33.6	29.8	31.0	27.8	30.1	24.0
	Juvenile	BM	95.1					112.4
		TBL	6.1					14.8
		SMR	89.1	42.0	64.0	45.7	71.5	37.9
F00NU	Juvenile	BM	113.0					120.8
		TBL	15.0					15.4
		SMR	35.6	31.5	29.8	39.7	24.9	26.9
	Juvenile	BM	112.0					131.6
		TBL	7.5					17.7
		SMR	48.3	33.3	70.0	35.9	46.3	32.1
	Juvenile	BM	145.0					160.2
		TBL	20.3					20.5
		SMR	35.6	39.1	36.5	38.2	33.3	37.3

Table 2. *Continued*

			2 °C		4 °C		8 °C	
Animal	Age class		Initial	Final	Initial	Final	Initial	Final
F00TS	Juvenile	BM	124.4					130.5
		TBL	17.3					7.9
		SMR	30.7	25.8	26.6	23.2	23.7	26.9
	Juvenile	BM	121.3					138.8
		TBL	12.3					16.9
		SMR	60.8	41.7	46.3	42.5	46.3	38.2
F00YA	Juvenile	BM	128.7					145.4
		TBL	11.7					16.7
		SMR	58.8	37.0	55.0	38.2	52.7	36.5
F97HA	Sub-adult	BM	131.6					136.4
		TBL	13.4					11.2
		SMR	42.0	45.2	39.9	37.3	44.6	39.7
	Sub-adult	BM	136.4					152.7
		TBL	12.2					14.8
		SMR	50.4	56.7	46.9	47.2	50.4	50.4

BM, body mass; TBL, total body lipid; SMR, standard metabolic rate.

Table 3. *Daily body mass loss and subsequent gain (kg d^{-1}) for captive Steller sea lions sampled between 2003 and 2005*

	Spring 2003		Fall 2003		Spring 2004		Summer 2005	
Animal	Loss	Gain	Loss	Gain	Loss	Gain	Loss	Gain
F97HA	1.27	0.42	1.27	0.47				
F00ED	1.20	0.35	1.75	0.48				
F00NU	0.98	0.56	1.86	0.54	0.44	0.51		
F00TS	1.23	0.31	1.90	0.50				
F00YA					0.57	0.56		
F03AS							0.16	0.14
F03IZ							0.22	0.24
F03MA							0.22	0.24
F03WI							0.15	0.15

Body mass loss occurred during sub-maitenance feeding as part of other experiments (Kumagai, 2004; Rosen and Trites, 2005; Kumagai et al., 2006; T. Dudot, personal communication). Rates of body mass gain occurred in the present study.

when expressed as a percentage of total body mass (%TBL, %LBM) this relationship was not significant (*t*=1.40, d.f.=13, *P*=0.186, Fig. 2).

Ultrasound measurements of blubber depth varied with individual animal and measurement location, but were generally 2.0 cm or less (Table 4). Comparisons of initial and final blubber depth measurements at each of the locations along the body were not significantly different (paired t-tests, all P>0.05) except that initial blubber depths at the lateral axillary site were significantly thicker compared to final measurements (*t*=2.287, d.f.=13, *P*=0.040). Initial and final girth measurements are listed in Table 5. Comparisons of initial and final girth measurements at the shoulder and hips were not significantly different (paired t-tests, all P>0.05); however, final axillary girth measurements were significantly larger than initial values (*t*=-2.903, d.f.=13, *P*=0.012).

Blubber depths at the axillary girth (dorsal) position were used to calculate the blubber volume (and subsequently blubber mass) changes during the course of the trials using a dual cone approximation of a sea lion body (Feldkamp, 1987; Gales and Burton, 1987; Castellini and Calkins, 1993; Stelle et al., 2000). Briefly, the body of a sea lion was represented as two unequal length cones joined at their bases, with the ends representing the head and the tail of the sea lion. An identical, smaller set of dual cones placed inside the larger can be used to distinguish between body core tissues (within inner cones) and subcutaneous blubber (space between inner and outer cones). Thus, total blubber volume is represented by a layer that is thickest at the axillary girth.

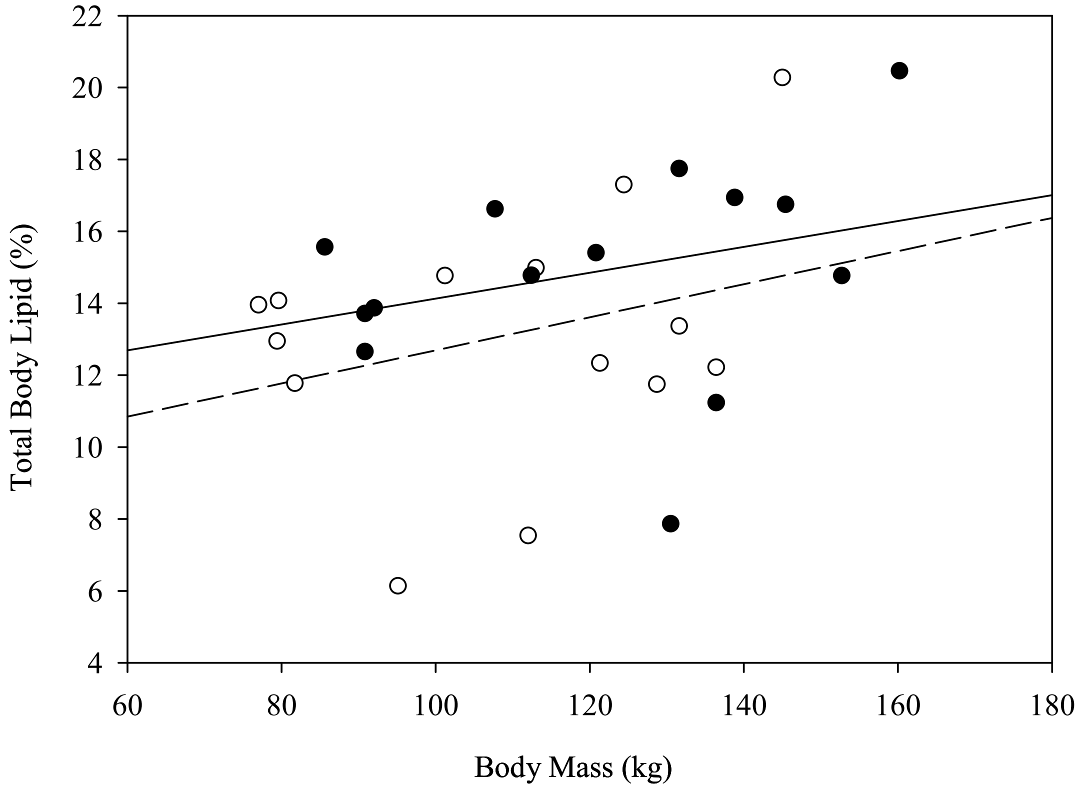

Figure 2. The relationship between body mass (kg) and total body lipid (TBL, % of total mass) for sea lions at the start (open circles, dashed line) and end of the experiment (closed circles, solid line). Although end %TBL values were higher than initial levels, values were not significantly different (*t*=1.40, d.f.=13, *P*=0.186).

Table 4. *Initial and final blubber depth measurements (mm) at the neck, axilla (dorsal and lateral), and hip (dorsal and lateral) locations along the sea lion trunk*

Animal	Age class		Initial	Final
F03AS	Juvenile	Neck	5	4
		Axillary-dorsal	8	10
		Axillary-lateral	14	15
		Hip-dorsal	7	7
		Hip-lateral	-	-
F03IZ	Juvenile	Neck	-	-
		Axillary-dorsal	6	8
		Axillary-lateral	12	12
		Hip-dorsal	7	8
		Hip-lateral	-	-
F03MA	Juvenile	Neck	6	7
		Axillary-dorsal	7	8
		Axillary-lateral	14	13
		Hip-dorsal	4	5
		Hip-lateral	-	-
F03WI	Juvenile	Neck	7	7
		Axillary-dorsal	8	8
		Axillary-lateral	14	18
		Hip-dorsal	8	6
		Hip-lateral	-	-
F00ED	Juvenile	Neck	8	15
		Axillary-dorsal	11	14
		Axillary-lateral	14	11
		Hip-dorsal	7	14
		Hip-lateral	9	10
	Juvenile	Neck	6	7
		Axillary-dorsal	13	9
		Axillary-lateral	11	6
		Hip-dorsal	-	-
		Hip-lateral	7	6
F00NU	Juvenile	Neck	11	9
		Axillary-dorsal	17	13
		Axillary-lateral	14	17
		Hip-dorsal	13	7
		Hip-lateral	7	10

Table 4. *Continued*

Animal	Age class		Initial	Final
	Juvenile	Neck	12	10
		Axillary-dorsal	14	19
		Axillary-lateral	10	7
		Hip-dorsal	9	12
		Hip-lateral	10	9
	Juvenile	Neck	10	12
		Axillary-dorsal	22	19
		Axillary-lateral	26	13
		Hip-dorsal	14	12
		Hip-lateral	18	10
F00TS	Juvenile	Neck	14	14
		Axillary-dorsal	14	22
		Axillary-lateral	21	13
		Hip-dorsal	8	12
		Hip-lateral	9	9
	Juvenile	Neck	10	8
		Axillary-dorsal	11	13
		Axillary-lateral	9	10
		Hip-dorsal	6	16
		Hip-lateral	4	12
F00YA	Juvenile	Neck	8	11
		Axillary-dorsal	14	20
		Axillary-lateral	22	17
		Hip-dorsal	11	11
		Hip-lateral	9	11
F97HA	Sub-adult	Neck	14	10
		Axillary-dorsal	17	12
		Axillary-lateral	23	13
		Hip-dorsal	17	8
		Hip-lateral	19	11
	Sub-adult	Neck	12	8
		Axillary-dorsal	15	18
		Axillary-lateral	11	7
		Hip-dorsal	12	18
		Hip-lateral	6	11

Table 5. *Initial and final girth (shoulder, axillary, hips) measurements (mm)*

Animal	Age class		Initial	Final
F03AS	Juvenile	Shoulder	112	118
		Axillary	105	112
		Hip	72	73
F03IZ	Juvenile	Shoulder	115	121
		Axillary	102	108
		Hip	72	74
F03MA	Juvenile	Shoulder	115	121
		Axillary	101	109
		Hip	67	73
F03WI	Juvenile	Shoulder	112	116
		Axillary	108	111
		Hip	72	73
F00ED	Juvenile	Shoulder	118	122
		Axillary	117	111
		Hip	74	69
	Juvenile	Shoulder	119	122
		Axillary	105	114
		Hip	71	75
F00NU	Juvenile	Shoulder	125	124
		Axillary	126	125
		Hip	79	70
	Juvenile	Shoulder	127	127
		Axillary	125	126
		Hip	73	82
	Juvenile	Shoulder	141	146
		Axillary	135	140
		Hip	89	91
F00TS	Juvenile	Shoulder	134	129
		Axillary	125	123
		Hip	79	74
	Juvenile	Shoulder	135	128
		Axillary	114	133
		Hip	78	82

Table 5. *Continued*

Animal	Age class		Initial	Final
F00YA	Juvenile	Shoulder	135	137
		Axillary	122	126
		Hip	78	82
F97HA	Sub-adult	Shoulder	133	132
		Axillary	123	128
		Hip	88	87
	Sub-adult	Shoulder	137	134.5
		Axillary	126	132
		Hip	85	91

Using the equation for the volume of a cone, the total blubber volume can be calculated as the difference between the inset cones. The radius of the sea lion was calculated as girth/2π. Girth and ultrasound blubber depth measurements taken at the axillary position at the start and end of the thermal trials were used in the calculations to approximate the change in total blubber. Estimates of blubber volume were converted to blubber mass based on blubber density values of 0.94 g cm^{-3} for pinniped blubber (G. Worthy, personal communication).

The change in blubber mass, coinciding with body mass gain during the trials, varied across individual sea lions and ranged from a loss of 0.8 kg to a gain of 1.68 kg of blubber (Table 6). Knowing total mass gained for each animal during each trial and the change in TBF from deuterium isotope estimates, a clearer picture of the changes in body composition were evident. For example, sea lion F97HA gained 5.9 kg of mass during Spring 2003 trials while losing 2.3 kg of fat, 1 kg of which, was lost from blubber stores. Similarly, this same animal gained 16.3 kg in Fall 2003, 5.9 kg of which was fat (10.4 kg lean mass), and roughly 1 kg of this fat was stored as blubber (leaving 4.9 kg of fat stored as visceral tissue) (Table 6). Most animals stored a fraction (8-63%) of their fat gain as blubber, but a few animals lost blubber despite increasing overall fat mass, which suggests fat storage in other locations. Total body fat values for sea lion F00TS are not physiologically possible with the measured mass gain and likely an error in deuterium sampling occurred.

Table 6. *Change in body mass (kg), total lipid mass (kg), and blubber mass (kg) for individual Steller sea lions*

Animal	Trial Dates	BM Gain (kg)	Change in TBL (kg)	Change in Blubber (kg)
F97HA	Spring 2003	5.9	-2.26	-0.98
	Fall 2003	16.3	5.88	0.94
F00ED	Spring 2003	5.9	2.97	0.41
	Fall 2003	17.3	10.77	-0.64
F00NU	Spring 2003	7.8	1.68	-0.97
	Fall 2003	19.6	14.91	1.28
	Spring 2004	15.2	3.38	-0.58
F00TS	Spring 2003	5.3	-11.25*	1.68*
	Fall 2003	17.5	8.55	0.91
F00YA	Spring 2004	16.7	9.23	1.66
F03AS	Summer 2005	8.1	2.84	0.53
F03IZ	Summer 2005	15.5	0.75	0.48
F03MA	Summer 2005	15.5	1.55	0.28
F03WI	Summer 2005	10.8	3.04	-0.08

BM, body mass; TBL, total body lipid.

Positive values indicate and increase, negative values represent a decrease.

Total body lipid was determined by isotope dilution and blubber mass was estimated using the dual cone approximation to calculate blubber volume.

*Values are suspect since they suggest animal was losing more fat mass than can be accounted for by total mass gain.

Metabolic Rates

A total of 504 individual measurements of metabolism were collected over the duration of the study. Repeated measures ANCOVA model results show that water temperature ($F_{2,162}$=7.67, P=0.0007) and water flow speed ($F_{3,162}$=16.67, P<0.0001) had significant effects on SMR at each sampling point in the trial. Repeated samplings across a trial (representing changing body condition) also significantly influenced SMR ($F_{2,297}$=9.90, P<0.0001).

Temperature

Overall, SMR (MJ d^{-1}) generally increased with decreasing water temperatures (Figs. 3, 4, 5). At all measurement intervals (initial, mid, final), SMRs at 2 ºC were significantly higher than at 8 ºC (paired t-tests, all P<0.05), with levels at 4 ºC being intermediary and not significantly different (all P>0.05) from either.

Sea Lion Condition

Sea lions demonstrated individual patterns of change in total body mass and %TBL over the course of the experiments (Table 2). While final total body mass values were significantly higher than initial levels (t=8.76, d.f.=13, P<0.0001), %TBL values remained similar (t=1.39, d.f.=13, P=0.187). While mean SMR decreased over the course of the experiment with initial values (just after food restriction) being higher than final (recovered) values, this trend was not significant (RM ANOVA, all P>0.05).

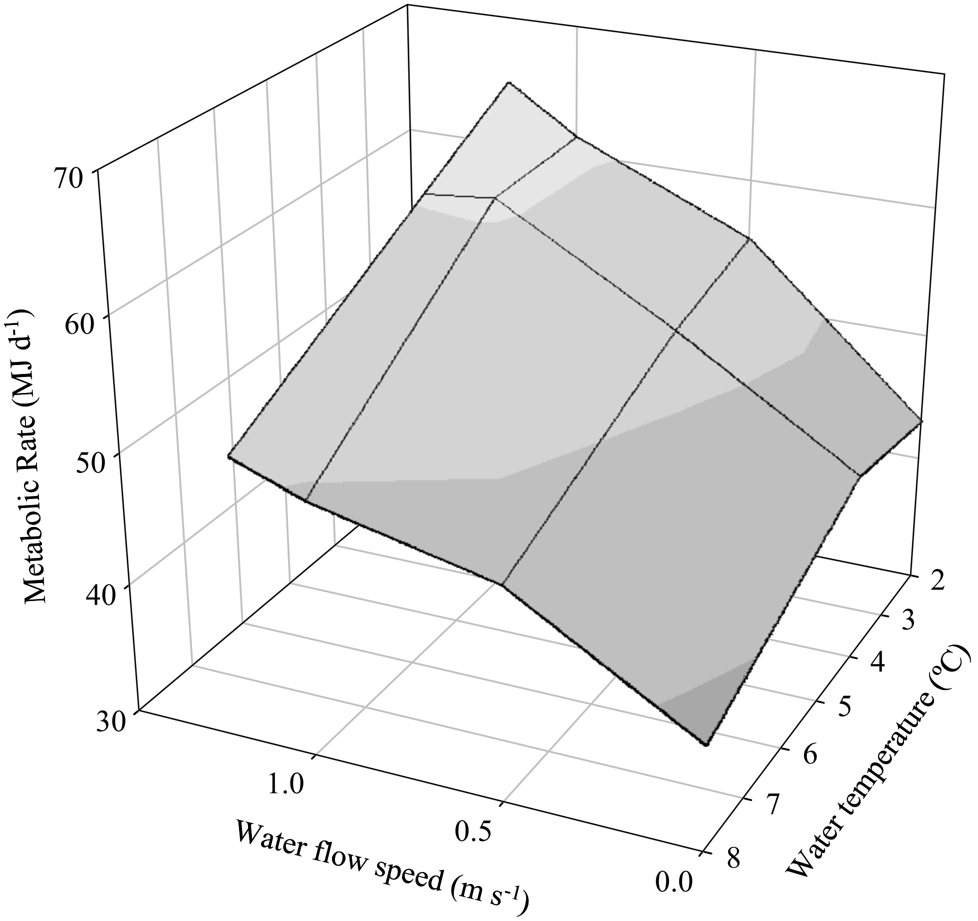

Figure 3. Three dimensional plot of mean SMR (MJ d^{-1}) as a function of both water temperature (2, 4, 8 ºC) and water flow speed (0, 0.5, 1.0. 1.2 m s^{-1}) for sea lions at the start of the experiment (reduced body condition).

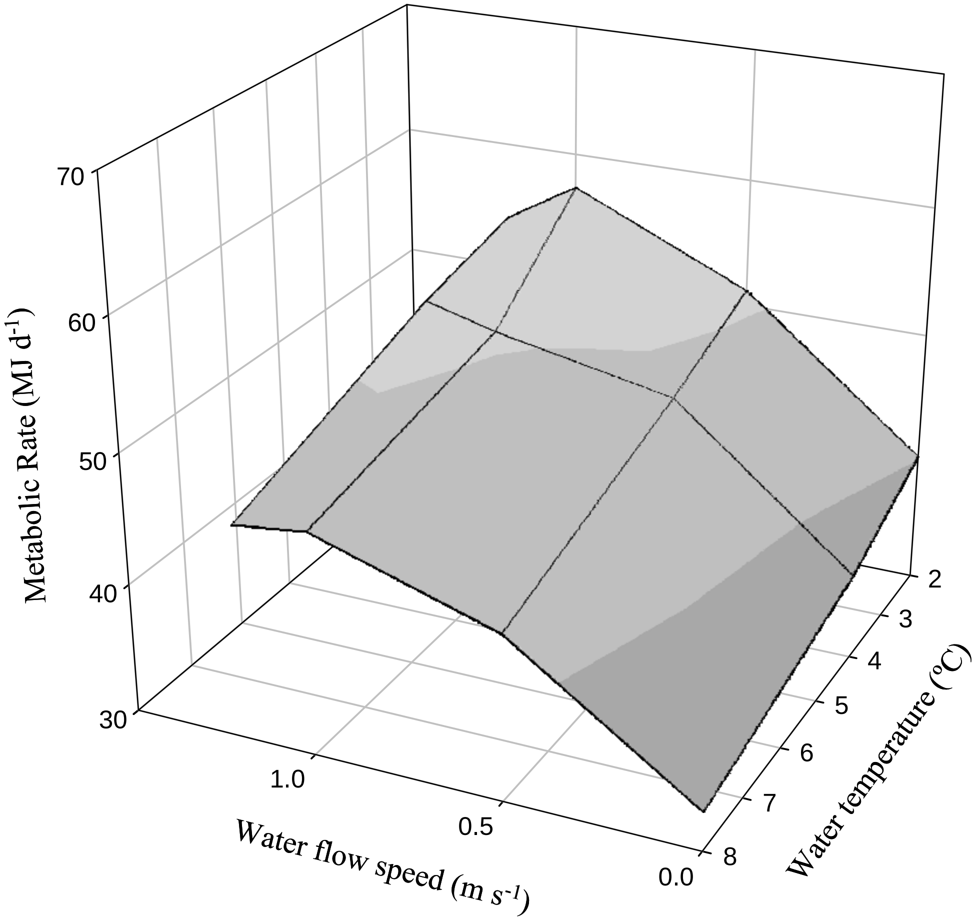

Figure 4. Three dimensional plot of mean SMR (MJ d^{-1}) as a function of both water temperature (2, 4, 8 ºC) and water flow speed (0, 0.5, 1.0. 1.2 m s^{-1}) for sea lions mid-way through the experiment (transitional body condition).

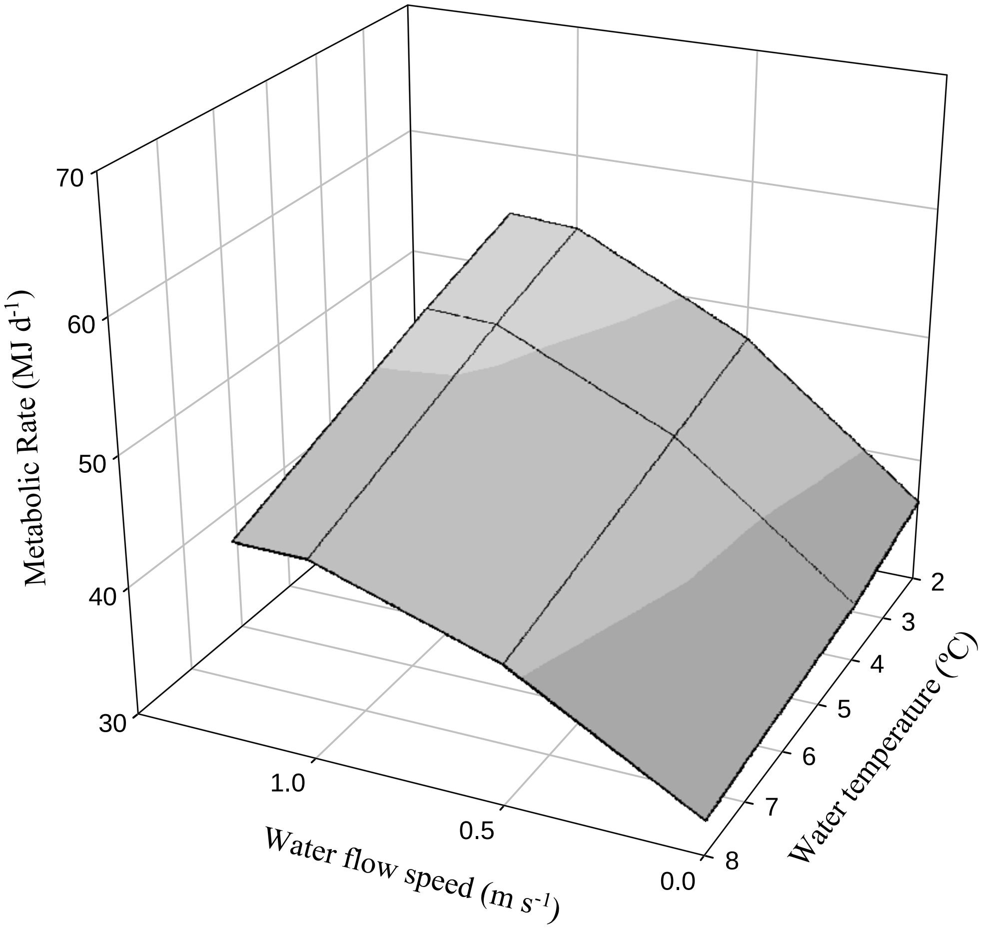

Figure 5. Three dimensional plot of mean SMR (MJ d^{-1}) as a function of both water temperature (2, 4, 8 ºC) and water flow speed (0, 0.5, 1.0. 1.2 m s^{-1}) for sea lions at the end of the experiment (recovered body condition).

Log-log regression of SMR plotted as a function of body mass for all sea lions measured within their thermoneutral zone (TNZ, T_w=8 ºC) at the end of trial (recovered) yielded a significant positive relationship (r^2=0.70, $F_{1,17}$=38.14, P<0.0001, Fig. 6): SMR=2.95 $M^{0.63}$, where SMR is expressed as MJ d^{-1} and M represents body mass in kg. These values were 2.4 to 4.0 times the predicted levels for adult terrestrial mammals of the same size (Kleiber, 1975).

In-water SMRs of sea lions increased with mass at each water temperature measurement over the duration of each experiment (Fig. 7). While, mean SMR increased with decreasing water temperature and decreased with improving total body mass, individual animals displayed a varied response (Figs. 8, 9, Table 2). Figures 8 and 9 show SMR as a function of temperature and varying body condition for a selection of sea lions in the study. Those sea lions experiencing the greatest SMR under lean conditions (Fig. 8 A1) tended to be smaller in size (body mass), and had lost greater amounts of TBL prior to start of trials. Conversely, those sea lions with the lowest SMR were larger animals.

For example, sea lion F00ED generally displayed the highest SMR at each water temperature when thin (Fig. 8 A1). This sea lion started the experiment at 95.1 kg after losing 14.0 kg (12.7%) mass and 5.6 kg (49.1%) of TBL. SMR at 2 ºC under these conditions was 28.2 % higher than the rate measure at 4 ºC for this sea lion, and 19.8% higher than the rate measured at 8 ºC (Fig. 8 A1). Conversely, sea lion F00NU displayed the lowest SMR at each water temperature during 2004. This sea lion weighted 145.0 kg at the start of the trials and had lost 12.3 kg (7.8%) mass and 5.6 kg

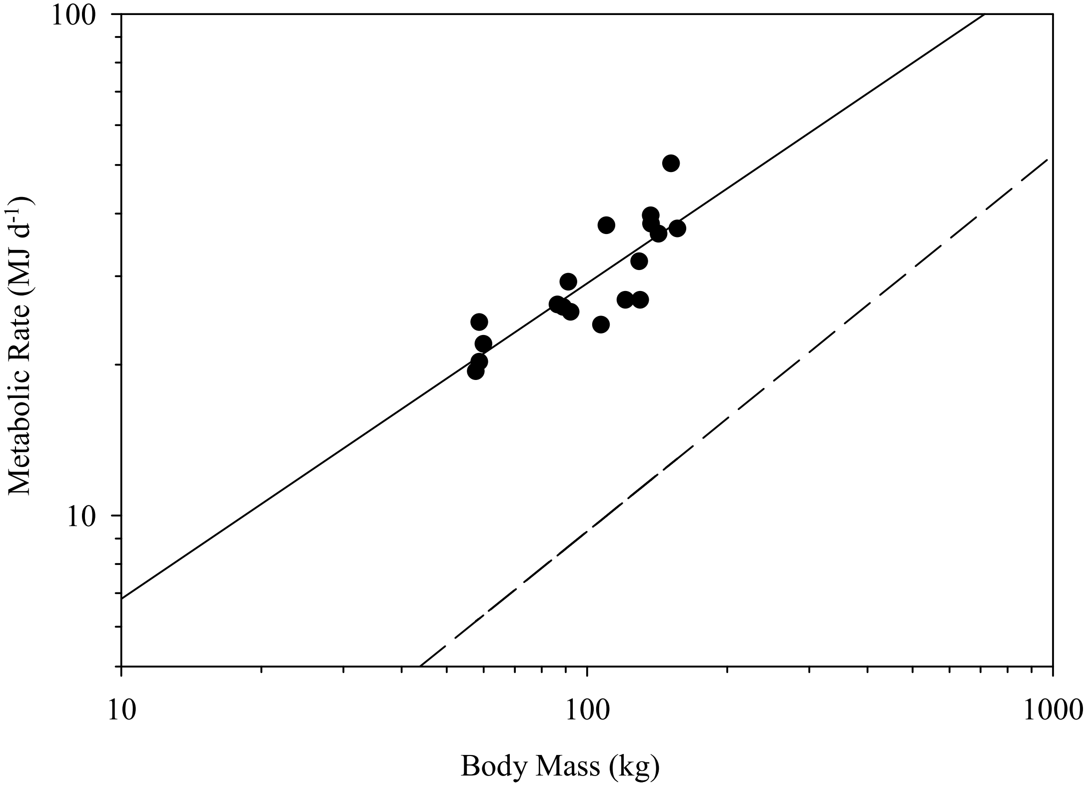

Figure 6. Log-log plot of whole-body metabolism (MJ d^{-1}) as function of body mass (kg) for all sea lions (*N*=18, including 8 mo. old pups) within their TNZ (8 ºC water, recovered body mass). The linear fit of the line is expressed at SMR (MJ d^{-1})=2.95 *M*0.63 (r^2=0.70), where *M* represents body mass in kg. Kleiber (1975) line (dashed line) of predicted metabolic rates for adult terrestrial mammals of similar size is plotted for comparison.

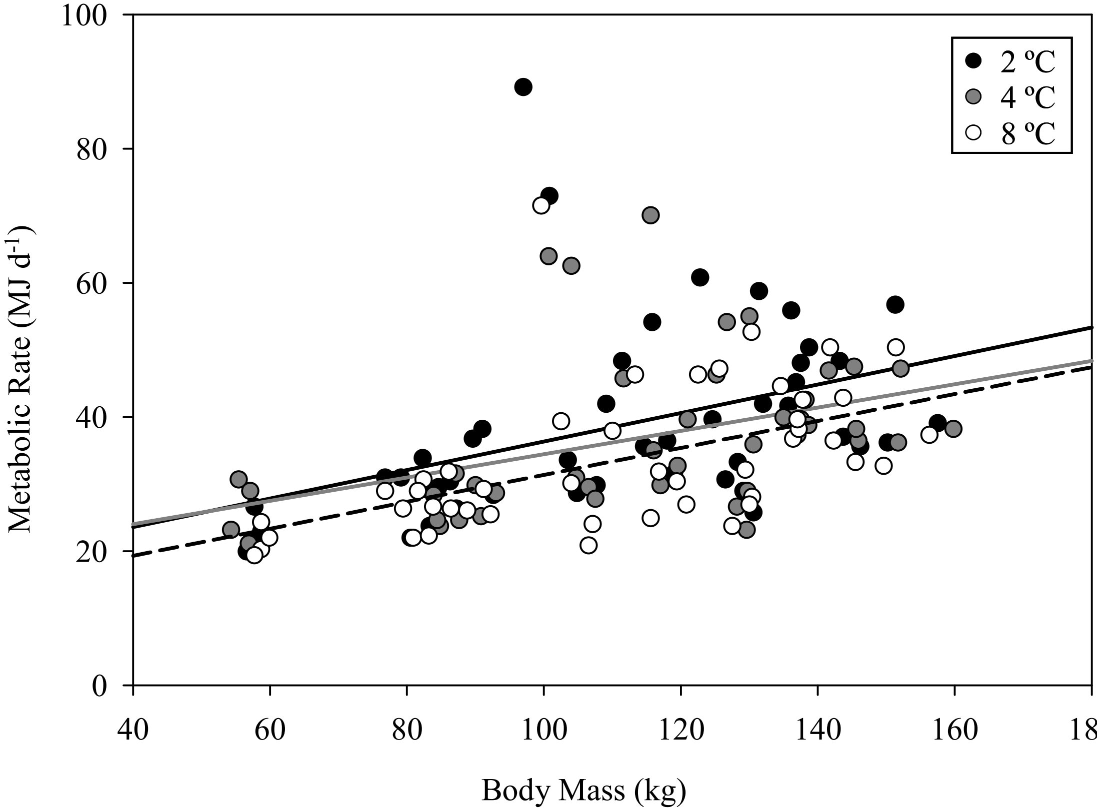

Figure 7. Whole body metabolic rate (MJ d^{-1}) as a function of body mass (kg) for sea lions measured at 2, 4, and 8 ºC water temperatures (still water). All body condition states were included. The regression equation for sea lions at 2 ºC is y=15.09+0.21x (r^2=0.18, sold black line, P=0.0016), at 4 ºC is y=17.04+0.17x (r^2=0.18, solid grey line, P=0.0002) and at 8 ºC is y=11.28+0.20x (r^2=0.28, dashed line, P<0.0001). Metabolic data for 8 mo. pups were included. Each point represents the lowest rate of oxygen consumption for an individual sea lion during at least a 15 min. period and includes values for all animals at all replicates within a experiment.

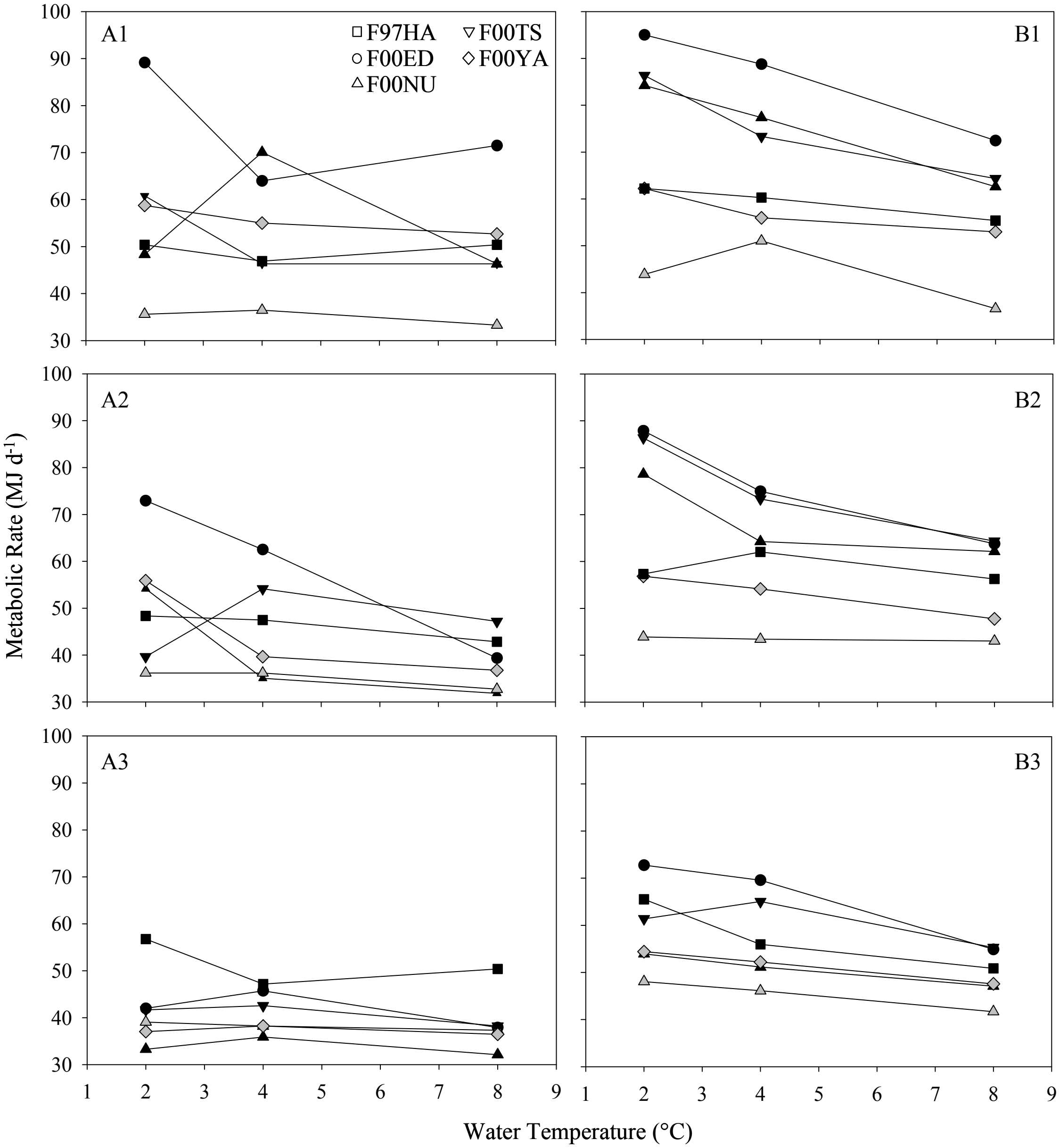

Figure 8. Metabolic rate (MJ d^{-1}) as a function of water temperature (ºC) for juvenile and sub-adult sea lions (F97HA, F00ED, F00NU, F00TS, F00YA) in still (A) and moving (B) water and at reduced (1), transitional (2), and recovered (3) body condition states. Black and grey symbols represent measurements from Fall 2003 and Spring 2004, respectively.

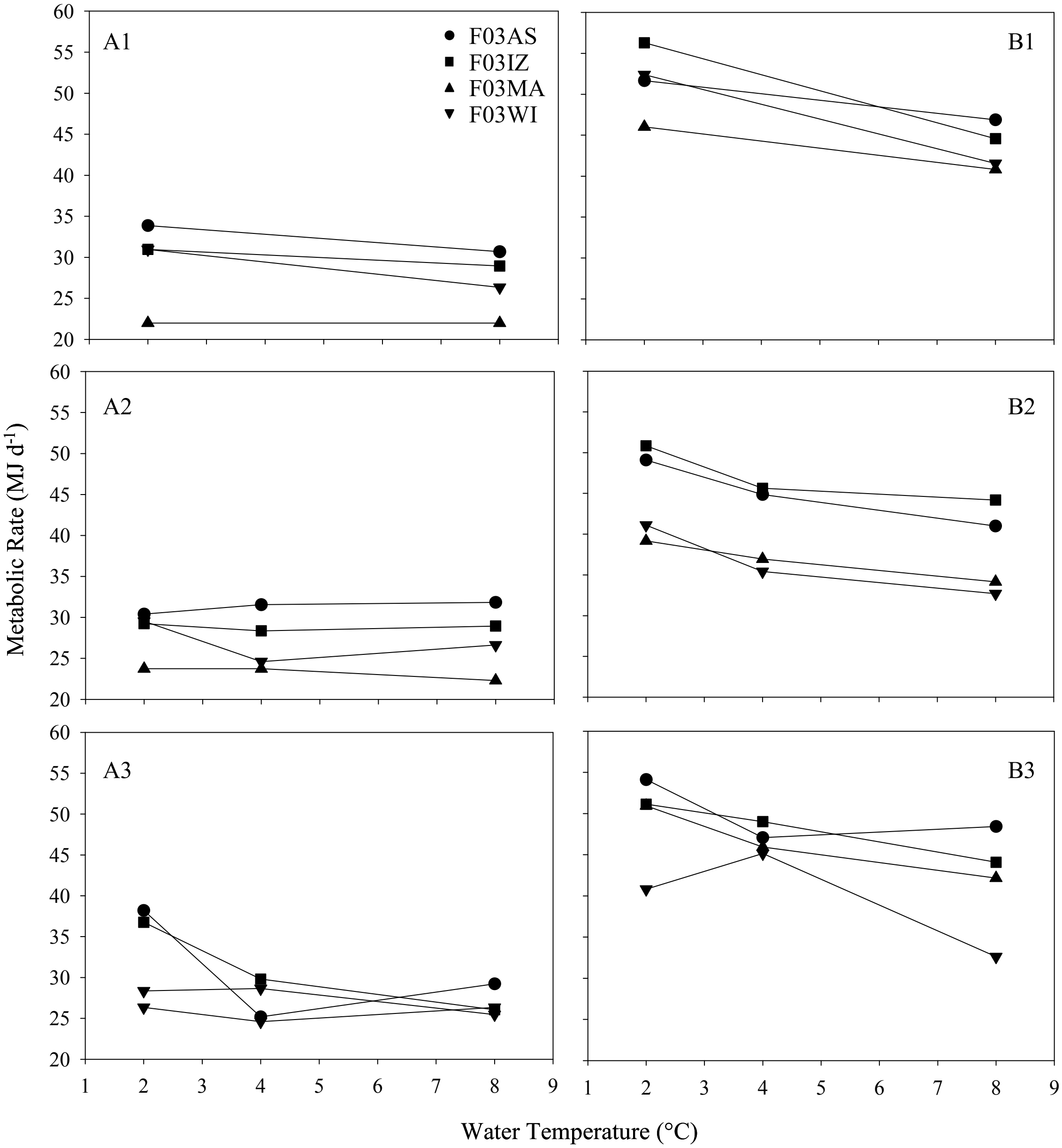

Figure 9. Metabolic rate (MJ d^{-1}) as a function of water temperature for juvenile sea lions (F03AS, F03IZ, F03MA, F03WI) in still (A) and moving water (B) and at reduced (1), transitional (2), and recovered (3) body condition states.

(16.0%) of TBL. This sea lion showed little change (6.5% drop) in SMR across varying water temperatures under these conditions (Fig. 8 A1). At the end of the trial, sea lion F00ED had regained 17.3 kg (18.2%) and 10.8 kg (186.2%) of TBL and reduced her SMR by 52.9% at 2 ºC, 28.5% at 4 ºC and 47.0% at 8 ºC (Fig. 8 A3). Sea lion F00NU gained 15.2 kg (10.5%) of body mass and 3.4 kg of TBL (11.6%) and showed little change in SMR (12.2% increase) from initial conditions (Fig. 8 A3). Interestingly in Fall 2003 trials, sea lion F00NU gained comparable amounts of body mass (19.6 kg, 17.5%) but much more TBL (14.9 kg, 176.6%) at her starting body mass of 113.0 kg. This resulted in as much as a 25 fold difference in the reduction of mass specific SMR for this sea lion (Table 7).

When oxygen consumption rate are monitored over a range of water temperatures, an inflection in the data is usually suggestive of a critical temperature, beyond which an individual animal must increase metabolic costs to remain thermally neutral. While the response to varying water temperature was not consistent across all sea lions, an inflection at 4 ºC was evident for two sea lions in reduced body condition (F00ED, F00TS Fig. 8 A1). This inflection at 4 ºC (suggesting a lower critical temperature, T_{lc}) was no longer evident once body mass and/or TBL were regained in these individual sea lions (Fig. 8 A3).

Surprisingly, the group of youngest sea lions (F03AS, F03IZ, F03MA, F03WI, 77-81 kg) showed little change in SMR across water temperatures and across varying body condition states (Fig. 9 A1-A3). These sea lions had lost an average of 9.3 kg of body mass (10.5%) and 3.7 kg of TBL (24.3%, range 5.1-41.5%) prior to the start of

Table 7. *Percent body mass, total body lipid, and mass specific standard metabolic rate change over the course of the trials at each water temperature*

Animal	Sampling Date	Tw (ºC)	BM gain (%)	TBL change (%)	msSMR change (%)
F97HA	Spring 2003	2	4.1	-12.8	3.8
		4			-7.8
		8			-12.7
	Fall 2003	2	12.0	35.3	3.3
		4			-6.3
		8			-6.2
F00ED	Spring 2003	2	5.8	19.9	-14.5
		4			-12.8
		8			-22.5
	Fall 2003	2	18.2	184.6	-58.1
		4			-35.4
		8			-51.9
F00NU	Spring 2003	2	7.1	9.9	-14.8
		4			28.6
		8			3.7
	Fall 2003	2	17.5	176.6	-40.3
		4			-54.6
		8			-39.4
	Spring 2004	2	10.5	11.5	1.6
		4			-4.4
		8			4.4
F00TS	Spring 2003	2	4.2	-52.3	-18.9
		4			-13.9
		8			11.3
	Fall 2003	2	14.4	57.1	-38.0
		4			-16.8
		8			-26.5
F00YA	Spring 2004	2	13.0	61.1	-42.3
		4			-38.1
		8			-36.6
F03AS	Summer 2005	2	11.1	29.5	2.2
		8			-13.7
F03IZ	Summer 2005	2	18.2	7.0	1.7
		8			-22.3

Table 7. *Continued*

Animal	Sampling Date	Tw (ºC)	BM gain (%)	TBL change (%)	msSMR change (%)
F03MA	Summer 2005	2	16.8	13.9	12.1
		8			1.5
F03WI	Summer 2005	2	10.3	29.6	-23.0
		8			-8.1

Tw, water temperature; BM, body mass; TBL, total body lipid; msSMR, mass specific standard metabolic rate.

experimental trials and gained an average of 12.5 kg of body mass (14.1%) and 2.1 kg of TBL (20.1%, range 7.5-30.2%) during the experimental trials. Metabolic rate data was not collected at 4 ºC in these sea lions when body condition was reduced, but there was some evidence of an inflection point at 4ºC at other body condition states (Fig. 9, A2-A3). On a mass specific basis, SMR for these younger sea lions was 32-34% higher than rates measured in sea lion F00NU (juvenile, 145 kg) under reduced body conditions, and 20-31% higher in the same animal once body condition had returned.

Water Flow

Within each water temperature, SMR increased as water flow increased from 0 to 1.2 m s^{-1} (Figs. 3, 4, 5). At 2 ºC, SMR measured in stationary water (0 m s^{-1}) was significantly lower (RM ANOVA, *P*<0.05) than rates measured at higher water flow speeds (1.0, 1.2 m s^{-1}), with intermediary flow (0.5) grouping either with stationary water or flow speeds. Similar patterns were observed at all stages of body condition and at 4 and 8 ºC. When SMR was compared across sampling intervals, no differences were evident (RM ANOVA, *P*>0.05) with the exception of significantly higher initial SMRs compared to mid-experiment levels (*P*=0.002). Since there were no statistical differences between the three flow velocities (0.5, 1.0, 1.2 m s^{-1}) in the majority of comparisons, data were pooled (averaged) for each animal to provide a single estimate of metabolism in moving water.

In comparisons of stationary (0 m s^{-1}) versus moving (0.5, 1.0, 1.2 m s^{-1} pooled) water, SMR was significantly higher in moving water at all temperatures (paired t-tests, all P<0.001). Sea lions were grouped by size (70-99 kg, 100-130 kg, >130 kg) to compare mean SMR in stationary and moving water (Table 8). Smaller sea lions incurred larger increases in SMR in moving water compared with larger sea lions (Table 8). Additionally, smaller sea lions had higher mean SMR levels at colder temperatures, and when in a state of reduced body condition compared with the levels in the larger sub-adult animal (Table 8).

Air Versus Water Comparison

In-air SMRs for juvenile sea lions (T_a=12.6 ºC, *N*=4, age 2 yr) ranged from 23.2 to 39.1 MJ d^{-1} at the start of the experiment under reduced body conditions, 29.2 to 46.9 MJ d^{-1} mid-way through, and 24.3 to 60.8 MJ d^{-1} by the end of the study, when an improved body condition was achieved. With improving body condition, SMR (absolute and mass-specifically) tended to increase, however, there were no significant differences in SMR between sampling points (initial, mid, final) for the in-air trials (RM ANOVA, all P>0.05). When these four animals are considered separately, there were also no significant differences in SMR between initial and final measurements at 2 and 8 ºC in water. These in-air values were 4.1 to 5.5 times higher than predicted by Kleiber (1975) for similarly sized adult terrestrial mammals. While SMRs measured in air tended to be higher than levels measured on the same animals in water (2, 8 ºC), this trend was not significant (RM ANOVA, all P>0.05).

Opportunistic in-water metabolic measurements on young (8 months of age) sea lion pups yielded average SMRs of 22.7 MJ d^{-1} at 2 ºC, 26.0 MJ d^{-1} at 4 ºC and 21.5 MJ

Table 8. *Mean standard metabolic rate (MJ d^{-1}) for Steller sea lions in stationary and moving water (2, 4, 8 ºC) at each sampling point in the trial*

Age class	Sampling Point	Tw (°C)	SMR Stationary	SMR Moving
Juvenile, 70-100 kg	Initial	2	29.5	51.6
		4	-	-
		8	27.0	43.5
	Mid	2	28.2	45.1
		4	27.1	40.7
		8	27.4	38.0
	Final	2	32.4	49.3
		4	27.1	46.8
		8	26.8	41.8
Juvenile, 100-130 kg	Initial	2	49.1	63.3
		4	44.9	59.1
		8	41.1	49.9
	Mid	2	44.1	58.7
		4	39.8	54.1
		8	33.4	47.6
	Final	2	35.0	52.0
		4	36.4	50.1
		8	32.5	44.1
Sub-adult, >130 kg	Initial	2	46.2	58.7
		4	43.4	55.0
		8	47.5	51.3
	Mid	2	48.2	51.1
		4	43.1	54.0
		8	42.7	51.5
	Final	2	50.9	58.1
		4	42.3	52.1
		8	45.0	46.4

Tw, water temperature; SMR, standard metabolic rate.

d^{-1} at 8 ºC in stationary water. Metabolism was significantly elevated (approximately 15%) at water speeds of 0.5 m s^{-1} (t=4.23, d.f.=11, P=0.0014) at all water temperatures. Individual pups showed greater increases in SMR between water flow speeds at 2 ºC compared with 8 ºC.

Discussion

Water temperature, water flow speed, and changes in body condition influenced metabolic rate in juvenile Steller sea lions. Sea lions showed elevated metabolic rates at colder water temperatures and in flowing water. In stationary water, initial mean SMRs at 2 ºC were 12% higher than at 8 ºC, while final mean SMRs were 10% higher. In flowing water, initial mean SMRs at 2 C were 19% higher than at 8 C, while final mean SMRs were 16% higher. Few studies have examined changes in metabolic rate with varying water temperatures in otariids (Liao, 1990; Donohue et al., 2000; Rutishauser et al., 2004). Liao (1990) measured metabolic rates of sub-adult California sea lions in water (T_w=5 to 35 ºC) and found that their T_{lc} was near 15 ºC. More recently, Mostman and Williams (2005) found a lower T_{lc} of 6 ºC in water for adults and 12 ºC for juvenile animals. Both studies suggest that free-ranging juvenile California sea lions are outside their TNZ while resting in water since these animals normally inhabit waters at or below critical temperatures.

Donohue et al. (2000) looked at how ontogeny influenced the thermal capabilities of pre- and post-molt northern fur seal pups. They found that post-molt pups showed no differences in SMR at water temperatures of 5, 10, and 20 ºC, nor from levels in pre-molt pups. They attributed this response to increased thermoregulatory capacity in the larger pups, suggesting that the T_{lc} for these pups is below 5 ºC. Finally, Rutishauser et al. (2004) measured metabolic rates in pup and juvenile Antarctic fur seals in water and estimated the T_{lc} to be 14 ºC, which is considerably warmer than the waters they inhabit. In the present study, T_{lc} was evident between 4 and 8 ºC in smaller Steller sea lions with reduced body condition, however, once body mass (and in some cases lipid mass) was regained, their was a suggestion that larger animals may still be thermoneutral at 2 ºC. Steller sea lions are the largest of the otariids inhabiting the waters of the North Pacific and thus might be expected to have greater thermal tolerances than smaller species of otariids.

Blubber depths of captive sea lions measured in the present study were thinner than typically measured in free-ranging animals. Steller sea lions are considered to be relatively 'lean' animals with thin blubber (Pitcher et al., 2000). Total body lipid (% of total body mass) levels in Steller sea lions (8 to 21%, recovered) were within the range of levels measured in similar sized free-ranging Steller sea lions (5-32%, L. Rea, personal communication) and in yearling Antarctic fur seals (15%, Rutishauser et al., 2004). In contrast to sea lions, fur seals rely primarily on their dense fur for insulation (Scheffer, 1962), and the addition of subcutaneous adipose tissue further enhances thermoregulation and increased lipid reserves provide a source of energy to pups during times of reduced intake (Donohue et al., 2000; Arnould et al., 2001). While the fur of Steller sea lions confers less of an insulative role than in fur seals, their large body size, compared to other otariids, may help to reduce heat flow due to lower surface area to volume ratios.

While lipid mass does not contribute significantly to total metabolic rate in marine mammals (Rea and Costa, 1992; Aarseth et al., 1999), lipid loss can indirectly affect metabolic rate when it impinges on thermoregulatory capabilities (Rea and Costa, 1992). This is supported by the large elevations in metabolic rate found in those sea lions that experienced large changes in lipid loss at the start of the trials and the subsequent reduction in metabolic rate, which occurred once total mass and lipid mass were regained. Additionally, a T_{lc} was evident between 4 and 8 ºC in both smaller Steller sea lions and those that had undergone large changes in fat mass. However, once body mass or in some cases fat mass was regained, the metabolic increase at 4 ºC was less obvious, and thermoregulatory capacity was restored. This has significant implications for thermal balance of young sea lions in the wild during times of limited or inadequate energy intake. Juvenile sea lions experiencing reductions in energy intake are forced to choose between mobilizing energy reserves for metabolic fuel and defending lipid stores for thermoregulation. Foraging introduces another set of physiological constraints in that in order to find prey animals must leave land to forage in a medium where heat losses are magnified and they must further expend energy while searching for prey. Smaller body size and reduced thermal tolerances make juvenile animals more susceptible to higher metabolic costs in order to survive. It is no surprise then, that under conditions of unpredictable prey availability these metabolic costs are magnified. By understanding the physiological conditions under which juvenile sea lions overcome these challenges allows for better predictions of the factors (biotic and abiotic) affecting juvenile success and survival.

As body condition improved (and original total body mass was regained), total metabolic rate decreased even within the TNZ. Smaller sea lions generally experienced greater relative increases in SMR compared to larger animals, except for young sea lions measured during the summer. When sea lions were in a state of reduced body condition, juveniles increased their heat production 26-40% over that seen when body condition was recovered, whereas sub-adult animals only had to raise metabolism 0-5% when body mass was reduced. Larger body mass confers an advantage in that a large surface area to volume ratio helps reduce heat losses. Over this same time frame, juvenile sea lions have increased their TBL (% of total body mass) by 20%, whereas sub-adult animals only experienced a 5% increase. This is consistent with the notion that changes in total body mass and body composition in younger sea lions have a more detrimental impact on their ability to maintain thermal homeostasis.

Even though sea lions started the experimental trials in a state of reduced body condition, no changes in blubber depth at any of the sites on the body were evident even after sea lion regained, or exceeded, their initial mass. Although individual changes in body composition varied, the general trend was for increases in both lean and lipid masses. The only morphometric parameter to change over the recovery period was axillary girth suggesting that the increase in body mass came from both lean tissue recovery and lipid deposition in visceral and muscle storage sites, not within blubber stores. This suggests that during times of reduced energy intake, sea lions rely on internal lipid stores for fuel while defending the blubber layer for thermoregulation.

Recent studies on young otariids have provided mostly indirect evidence that these animals use lipids as the primary metabolic fuel source in order to spare protein (Rea et al., 2000; Arnould et al., 2001; Donohue et al., 2002), like other adult species that undergo periods of fasting (Castellini and Rea, 1992; Cherel et al., 1994). Directed studies by Beauplet et al. (2003) examining changes in body composition and metabolic fuel use during short-term fasting (2-3 weeks) in Subantarctic fur sea pups confirms that the majority of mass lost (56%) in these animals was comprised of lipid. However, Beauplet et al. (2003) did not examine blubber depth in the study, so there is no way to determine whether preferential use of lipid reserves included the fat from the blubber layer.

Changes in blubber volume were estimated using dual cone approximations of the body of a sea lion. Calculations suggest that most animals were storing a fraction of their lipid gain as blubber (0.28 to 1.66 kg). Despite no significant changes in blubber depth, it is not inconceivable that these small amounts of lipid, spread over the entire body of the sea lion, could result in much more than 1-2 mm difference in blubber thickness, which is within the range of sensitivity of the ultrasounds used to measure blubber depth (±1 mm, G. Worthy, personal communication). However, it should be noted that this type of approximation of blubber mass assumes an overly simplistic view of the uniform blubber depth across the entire body surface of the sea lion. In reality, blubber thickness varies with location across the body of most pinnipeds (e.g., Gales and

Burton, 1987). Additionally, since blubber depth at the thickest location on the body was used in the calculations (axillary), this technique likely overestimated total blubber volume and hence mass. Finally, reducing the sea lion body to a set of cones joined at the base is also a simplification of sea lion body form.

In water, heat is primarily transferred away from the surface of an animal to the surrounding medium through convection. Convective heat loss will change depending on whether the animal is at rest in still water (free convection) or whether the animal is moving (forced convection). In the latter situation, the heat transfer rate across the skin/water boundary will increase with velocity (Hind and Gurney, 1997). The present study is the first one to quantify the increased cost in metabolism in a non-swimming animal due to convective heat loss in flowing water. Sea lions in the present study showed increases in SMR with increasing water speed at each temperature. The magnitude of this response was increased when sea lions were either young (i.e., small) and/or in a state of reduced body condition. The mechanism of heat transfer was likely forced convection, even for animals in still water, as "rest" in these captive Steller sea lions still included some degree of movement in the flume. Interestingly, SMR for some animals at 1.2 m s^{-1} was less than at 1.0 m s^{-1}. At the highest water flow speed, sea lions were observed to engage in greater activity than at lower speeds (L. Hoopes, personal observation). Animals often were observed pushing into the water flow and occasionally swimming for short bursts. This is suggestive of Steller sea lions being able to substitute heat loss in water with increased heat production through increased levels of activity.

The decrease in SMR on average was 6% and for individual animals it was as high as 22%.

Body size was also an important determinant of the metabolic response during recovery from reduced energy intakes. Two animals (F00ED, F00TS) were noteworthy in that they both started the trials having lost similar proportions of body fat (42 and 49% respectively); however, their thermal response to cold water was very different. The smaller sea lion (F00ED) showed some of the largest elevations in SMR in still water (10X Kleiber at 2 ºC), while the larger animal (F00TS) displayed SMRs that were comparable to similarly sized animals. The difference in thermal response between these two animals is likely a function of size, where the larger animal had an easier time defending against heat loss and the smaller animal, by virtue of a larger surface area to volume ratio, could not. Both animals gained proportionally more fat mass than lean mass over the experiment, confirming that decreases in fat mass prior to the trials were impacting thermoregulatory demands. In moving water, this smaller sea lion experienced virtually no increase in SMR at 2 ºC over that measured at same temperature in still water, suggesting this animal was operating near peak metabolic capacity.

Not all animals showed similar recovery responses to the reduced intakes, with the four young sea lions (2 yr old) tested during the summer showing little changes in metabolism across water temperatures and over the course of the experiment as they regained body mass. In contrast, the young sea lion (2.5 yr old) that was tested during the fall showed an elevated metabolic response to temperature, which declined as body mass (and fat mass) was regained. These differing responses suggest that season may be an important variable in determining how sea lions respond to reduced energy intake. Kumagai et al. (2006) found that changes in body mass and body composition in captive Steller sea lions responded differently to reduced energy intake based on season. They found that in-air metabolic rates were highest in the fall and lowest in the summer but no differences in body fat between these two seasons were evident. Molting typically occurs in the month of July for these captive sea lions (D. Rosen, personal communication) and is generally thought to be an energetically expensive activity due to increased metabolism necessary to grow new hair tissue and/or defend against thermoregulatory costs during hair replacement (Worthy et al., 1992; Boily, 1996). Kumagai et al. (2006) suggest that the increases metabolic rates in sea lions in the fall and the lack of change in fat mass may be suggestive of a switch from thermoregulation to somatic growth expenditures. Energy intake reductions during this period of somatic growth, where expenditures were already elevated, could explain the high metabolic costs of thermoregulation in water for the small sea lion F00ED in this study. This could also explain the muted metabolic response of the young sea lions (F03AS, F03IZ, F03MA, and F03WI) in the summer, which also exhibited the slowest daily rates of body mass loss. Food intakes in captive Steller sea lions appear to be lower in the warmer months (Kastelein et al., 1990) and it is possible that natural differences in energy state were influencing metabolism (Kumagai et al., 2006), and hence the thermoregulatory response. This is an area of study that warrants further investigation.

In-air metabolic rates showed the same patterns with changing body condition as seen in the water trials. In-air metabolic rates (mean 13 ºC) were slightly higher, but not significantly so, than in-water rates at 2 and 8 ºC for the same group of juvenile sea lions. While it is generally thought that animals that are able to thermoregulate in water would have trouble coping with the milder properties of air, data collected in the present study do not support this notion. Noren and Mangel (2004) report higher metabolic rates in northern elephant seal pups at air temperatures of 21 ºC compared to animals in 3-8 ºC water, and they suggest that pups may take advantage of warmer air temperatures to reduce total metabolic costs during their first foraging trip. In contrast, in-water metabolic rates were significantly greater than in-water rates (at similar temperatures) for pre-molt Antarctic and northern fur seal pups (Donohue et al., 2000; Arnould et al., 2003).

Steller sea lions may temporarily experience periods of thermal stress during periods of reduced energy intake in the wild. These reduced intakes would primarily impact young animals in water temperatures at the lower limit of their TNZ. As shown in the present study, reduced energy intakes, even lasting as short as 8 days, can severely impact juvenile sea lions whose small body size and the reduced insulatory layer is insufficient to defend against heat losses in the water. Under situations of reduced energy intake (whether from changes in prey quality or quantity) juvenile sea lions cope with the conflicting demands of preserving their insulative layer for thermoregulation and the need to survive by mobilizing fuel (lipid) reserves to survive. Juvenile sea lions must compromise between remaining on land with lower thermal costs, but with no opportunity to acquire energy, or entering the water to forage, with the potential increased cost of energy expenditure for thermoregulation (Rosen et al., 2007). Understanding the thermal constraints to foraging allows for better understanding of the challenges to survival in juvenile Steller sea lions.

CHAPTER III

SPATIAL AND TEMPORAL DEVELOPMENT OF THERMAL WINDOWS IN PUP AND JUVENILE STELLER SEA LIONS

Introduction

The dual aquatic and terrestrial existence of pinnipeds poses thermal challenges to these marine mammals since the heat transfer coefficient in water is significantly higher than that of air at similar temperatures (Nadel, 1984). To cope with the increased thermal conductivity in water, and the potential for increased heat loss, pinnipeds rely on a number of physiological mechanisms, including increasing metabolic rate, allowing a decrease in body temperature, decreasing thermal conductance of the body in contact with water through increased insulation of pelage and/or a subcutaneous blubber layer, increased muscular activity, and/or restriction of blood flow from body core to skin to prevent heat loss (Costa and Williams, 1999). In some cases the response to the thermal challenges of submersion in cold water may be dealt with through heterothermy, whereas other times it is accomplished through increases in metabolic rate (Boyd, 2000). The ability of different species to respond to cold, and the suite of physiological mechanisms that are employed are often poorly understood.

Even though immersion in water is perceived to be the more stressful environment, pinnipeds must also retain the capacity to dissipate excess heat and avoid hyperthermia on land or when active (e.g, Hart and Irving, 1959). Excess body heat in pinnipeds is usually eliminated by utilizing circulatory changes that effectively bypass their thermal insulation. Since blubber is highly vascularized, marine mammals can either constrict or dilate arterioles in the blubber depending on the thermal needs of the animal, easily allowing excess heat to be carried by the vascular system through the blubber and to the body surface (Pabst et al., 1999). Historically, it was presumed that body heat was principally lost through areas of the body that were poorly insulated (e.g., Irving and Hart, 1957; e.g., Hart and Irving, 1959; Matsuura and Whittow, 1974; Gallivan and Ronald, 1979). In pinnipeds, this includes the fore and hind flippers where heat is transferred to the environment through small vessels running close to skin and is aided by the fact that these appendages often have large surface area to volume ratios (Tarasoff and Fisher, 1970; Innes et al., 1990; Pabst et al., 1999).

Studies examining the relative role of heat dissipation via the flippers versus the body trunk have produced mixed results, and have almost always utilized phocid seals. Early physiological studies suggested that the flippers were the main avenue of heat transfer and thermoregulation (Irving and Hart, 1957; Hart and Irving, 1959; Matsuura and Whittow, 1974; Gallivan and Ronald, 1979). Hart and Irving (1959) measured skin temperatures of harbor seals in water and in air and found that in air the temperature of the flippers was much more variable than the temperatures on the trunk and they suggested separate control of heat flow through these appendages. Estimates of heat loss from the flippers of a resting seal in water ranged from 10-30% with an increase up to 80% during exercise (Ryg et al., 1993). Additionally, assuming that skin temperatures were the same on the appendages as on the body trunk, Ryg et al. (1993) found a 20% higher heat loss per unit surface from the flippers.

However, later studies have debated the role of the flippers in heat transfer (Kvadsheim and Folkow, 1997; Kvadsheim et al., 1997; Mauck et al., 2003). Øritsland (1968) recorded surface temperatures in exercising harp seals on land and found areas of heat dissipation both on the flippers and along the body trunk. Warm spots appeared near the base of each flipper and spread out towards the main body of the trunk, and were also observed in irregular patterns along the trunk (Øritsland, 1968). Kvadsheim et al. (1997) found subcutaneous temperatures in the flippers of harp seals exposed to ice water were comparable to subcutaneous temperatures measured along the trunk, and assumed equal rates of heat loss per unit area between the two different body regions. Further study with the same species showed increasing heat loads with increasing water temperatures (1 to 24 ºC), and the fraction of heat lost from the flippers increased, while the fraction of heat lost through the trunk decreased, despite an increase in conductive heat transfer through the blubber layer (Kvadsheim and Folkow, 1997). Recently, Mauck et al. (2003) demonstrated the presence of thermal windows on the trunks of 3 species of phocid seals, although they were neither consistent in time nor space. In the only study incorporating an otariid, Willis et al. (2005) found high loads of heat flux from the shoulder and hip regions of Steller sea lions stationary and swimming in water, suggesting these areas were preferential to dump excess heat. Traditionally, the term 'thermal window' has been used to describe areas of the body that are naked or sparsely haired and poorly insulated (usually the appendages) which would facilitate the transfer of excess heat from the body core to the body surface (Noren et al., 1999; Meagher et al., 2002). However, Mauck et al. (2003) noted that the term 'thermal window' must be used more broadly to include all body surfaces functioning as temporary heat dissipaters during high heat loads.

Infrared thermography has been used in a variety of terrestrial (Phillips and Heath, 1992; Phillips and Heath, 1995; Tattersall and Milsom, 2003) and marine species (Dehnhardt et al., 1998; Meagher et al., 2002; Mauck et al., 2003; McCafferty et al., 2005; Willis et al., 2005) to monitor changes in surface temperatures and thermoregulation. Thermal imaging cameras measure the amount of infrared radiation emitted by the surface and then convert this to a radiative surface temperature using the Stefan-Boltzmann equation for a blackbody emitter:

$$R=\epsilon\sigma T^4$$

where R is the emitted energy (W m^{-2}), ϵ is the emissivity of the surface body, σ is the Stefan-Boltzmann constant (5.67×10^{-8} W m^{-2} K^{-4}), and T is temperature (K) (McCafferty et al., 2005).

Because the balance between heat production and heat loss in cold water can be mediated by the adjustment of blood flow to the skin and extremities, examining surface temperatures along the body after submersion in water can provide information about the mechanism of heat flux in an organism. Heat balance models estimated for marine mammals have assumed the importance of skin temperature for the conduction (both free and forced) of heat away from the body surface to the surrounding water (Lavigne et al., 1990; Boily, 1995; Hind and Gurney, 1997). While skin temperatures have been measured using attached heat sensor devices (e.g., Boyd, 2000; Willis et al., 2005), few studies have successfully measured skin temperatures in otariid seals without having to compromise insulation (e.g., pelage) of the animal (e.g., Liao, 1990). Thermal imaging is a non-invasive technique which provides a complete picture of the heat profile of an entire animal. It provides several advantages over alternate techniques. Unlike direct measures of heat flux using sensors, it avoids *a priori* assumptions of locations where heat loss occurs along the body. While measuring metabolism can provide indications of total energy requirements under different thermal conditions, the physiological mechanisms can not be elucidated.

The goal of this study was to use infrared thermography to (1) assess surface temperatures (as a proxy for skin temperature) in pup and juvenile Steller sea lions after exposure to various water temperatures, and (2) assess patterns of warming and development of thermal windows in pup and juvenile Steller sea lion held at ambient air conditions post-exposure.

Materials and Methods

Animals

Thermograms were recorded from a group of Steller sea lions that had been captured as pups and held at the Vancouver Aquarium Marine Science Centre (Vancouver, BC, Canada). Six female sea lions (4 pups, 8 months of age; 2 juveniles, 3.5 years of age) were included in the study. Prior to the first thermal image being recorded, sea lions were exposed to varying water temperatures (2, 4, 8 ºC), both stationary and flowing, for 100 min as part of larger study to examine the energetic cost of thermoregulation (see Chapter II).

Infrared Thermography

Thermal images were taken using the ThermaCAM P20 thermal imaging camera (FLIR Systems, MA, USA). This system has a thermal sensitivity of 0.08 ºC at 30 ºC, field of view/minimum focal distance of 24º x 18º/0.3 m. Images were produced in real-time and were not affected by animal movement.

In each session, images were taken of sea lions within 1-2 min from the time of emergence from a temperature-controlled swim flume at ambient air temperatures (mean=12.8 ± 2.9 ºC). Initial images were taken when sea lions were wet and held stationary. Images of both the right and left side of the sea lion were taken from animals in their normal 'standing' posture (both fore and hind flippers on the ground) and a third image was taken of the sea lion upright on their hind flippers, in order to get complete coverage of the dorsal surface of the animal. Images of the ventral surface of the sea lions were not taken since this area of the body was in constant contact with the substrate. Subsequent images were taken 15 and 30 min after initial photos in pups, and 30 and 60 min after initial photo in juveniles to monitor changes in appearance of thermal windows. Due to logistical constraints, only one pup was able to be imaged after the 4 ºC water trials. Over 150 images were recorded and stored digitally for later analysis. Sea lions were held in a dry run between photos and their fur was usually dry in these later images. Ambient air temperature and humidity were recorded with a digital thermometer (Model # 61220-670, VWR International, Ontario, Canada) along with distance from the camera to the sea lion (range 2 to 4 m) to correct for absorbed radiation and a drop in transmittance with distance and humidity. The emissivity of the sea lion's fur was set at 0.96, similar to values used for other pinnipeds (Trites, 1990; McCafferty et al., 2005).

All images were analyzed using ThermaCAM Researcher (Version 2.7, FLIR Systems, MA, USA) software and a rainbow color scheme. Images were screened and selected for analysis in which the complete profile of the sea lion was visible. The body of the sea lion was divided into 5 regions for analysis of surface temperature: head, upper body, lower body, fore flipper, and hind flipper. The head area was defined as the tip of the nose to just posterior to the ear flap (including eyes and vibrissae). Upper body included the area just below the ear to axillary girth and excluding the fore flipper. Lower body was defined as the area from the axillary girth to the tail, excluding the hind flipper (Fig. 10). Average surface temperatures were determined within each region and

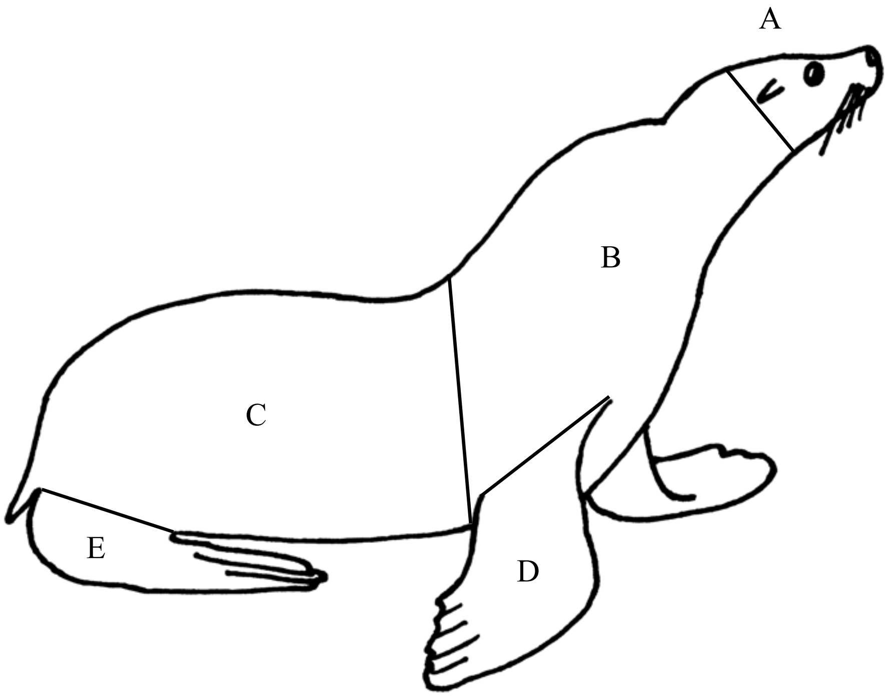

Figure 10. Division of the sea lion body for surface temperature analysis of thermograms: head (A), upper body (B), lower body (C), fore flippers (D), and hind flippers (E).

the location, number, and temperature profile of thermal windows within each region were also recorded.

Statistical Analyses

SigmaPlot (SPSS Science, version 8.0) and SPSS (SPSS Science, version 11.5) software was used for graphical and statistical analyses. Repeated measures analysis of variance (RM ANOVA) was used to account for non-independence of longitudinal data. Friedman repeated measures on ranks was used for repeated-measures distributions with unequal variances. When significant, Tukey-Kramer HSD multiple pairwise comparisons or Tukey-Kramer multiple pairwise comparisons on ranked data were performed. Independent t-tests were employed to examine differences between groups of animals. All data were explored for sphericity, normality and equality of variances where appropriate using Mauchley's, Shaprio-Wilk and Levene's tests, respectively. For non-normal distributions or those with unequal variances, Mann-Whitney rank sum tests were used where indicated. Means are reported with ±1 SEM. Statistical tests were considered significant at $P<0.05$ unless otherwise stated.

Results

Pup and juvenile sea lions displayed similar patterns of warming after emergence from the swim flume (Fig. 11). The head was always the warmest region of the body upon emergence, and the hind flippers the coolest, often only 1 ºC warmer than the colder water temperatures (Table 9). While body region surface temperatures consistently decreased down the length of the animal (from head to tail), only the head was significantly warmer than the remaining regions ($F_{4,129}$=9.20, P<0.001). Although not a significant trend, pups had cooler mean surface temperatures at all body regions upon emergence (2 and 8 ºC water) compared with juveniles. While serial thermal images were taken at different time scales between pups and juveniles, both had photos taken at 30 min post-emergence. Surface temperatures differed between pup and juvenile sea lions in the fore-flippers after submergence in 2 ºC water (t=6.86, d.f.=5, P=0.002), and in the lower body after 8 ºC water (t=3.26, d.f.=5, P=0.031). Sample sizes were not large enough to look at difference between age groups after trials in 4 ºC water.

Mean surface temperatures became significantly warmer with each image taken post-emergence for pups (χ^2(19)=38.0, P<0.001; χ^2(5)=10.0, P=0.007; χ^2(20)=37.54, P<0.001) (Figs 11, 12, 13) and juveniles ($F_{2,16}$=72.89, P<0.001; $F_{2,18}$=158.66, P<0.001; $F_{2,18}$=25.05, P<0.001) (Figs 11, 13, 14) at all water temperatures, with one exception. Mean surface temperatures did not differ between the second (30 min) and final (60 min) set of images in juvenile animals that were exposed to the 8 ºC water. Overall, surface

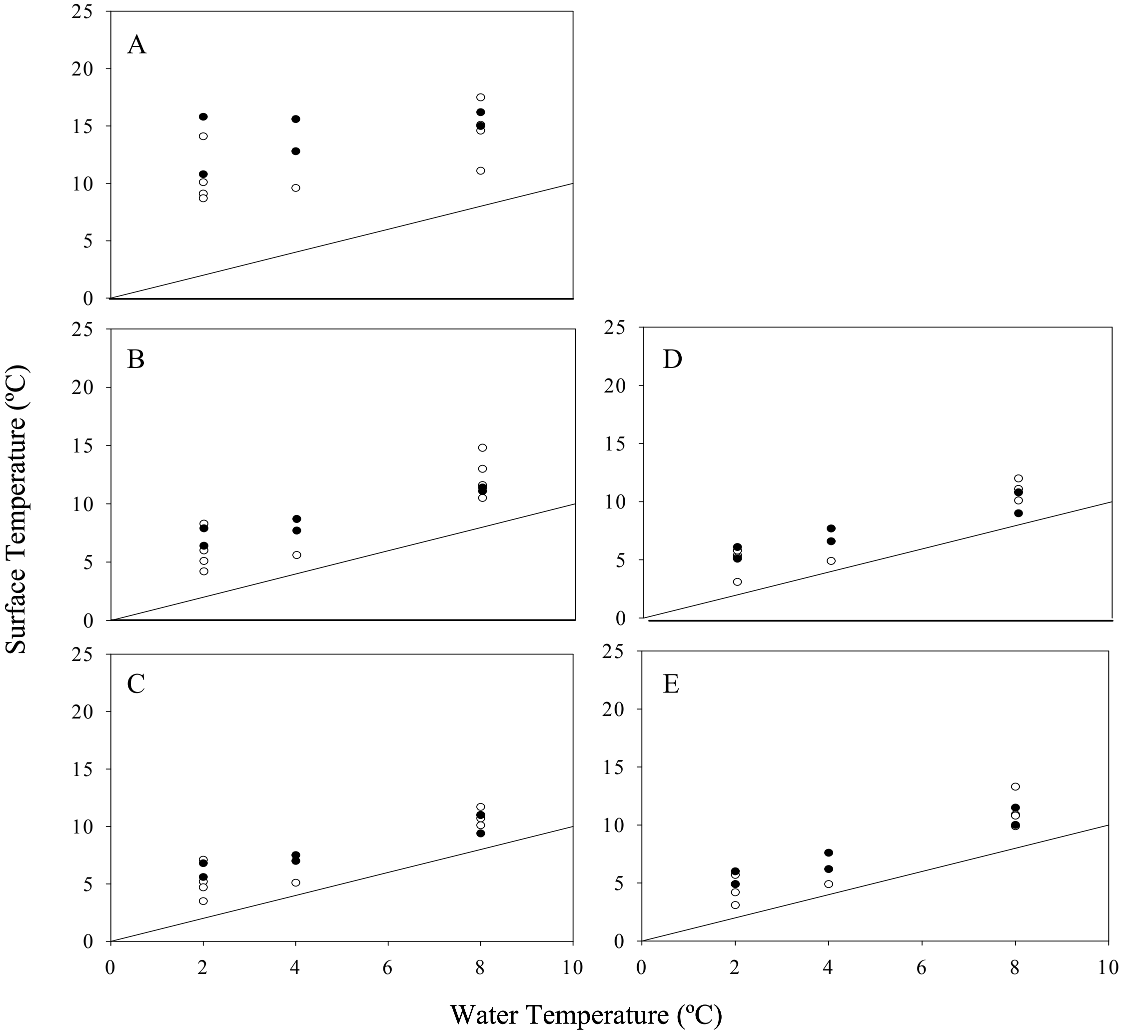

Figure 11. Surface temperatures (ºC) for the head (A), upper body (B), lower body (C), fore flippers (D), and hind flippers (E) of pup (while circle) and juvenile (black circle) Steller sea lions immediately after emergence from water. Solid line represents the line of equality.

Table 9. *Surface temperatures (ºC, mean±SEM) for juvenile (N=4) and pup (N=2) sea lions after emergence from 2, 4, 8 ºC water*

	Region	2 ºC	4 ºC	8 ºC
Juveniles	Head	13.3 ± 2.5	14.2 ± 1.4	15.6 ± 0.6
	Upper	7.2 ± 0.8	8.2 ± 0.5	11.3 ± 0.2
	Lower	6.2 ± 0.6	7.3 ± 0.3	10.2 ± 0.8
	Fore	5.6 ± 0.5	7.2 ± 0.6	9.9 ± 0.9
	Hind	5.5 ± 0.6	6.9 ± 0.7	10.8 ± 0.8
Pups	Head	10.5 ± 1.2	9.6*	14.5 ± 1.3
	Upper	5.9 ± 0.9	5.6*	12.5 ± 0.9
	Lower	5.1 ± 0.7	5.1*	10.9 ± 0.3
	Fore	4.9 ± 0.6	4.9*	11.0 ± 0.4
	Hind	4.5 ± 0.6	4.9*	11.2 ± 0.7

*N =1.

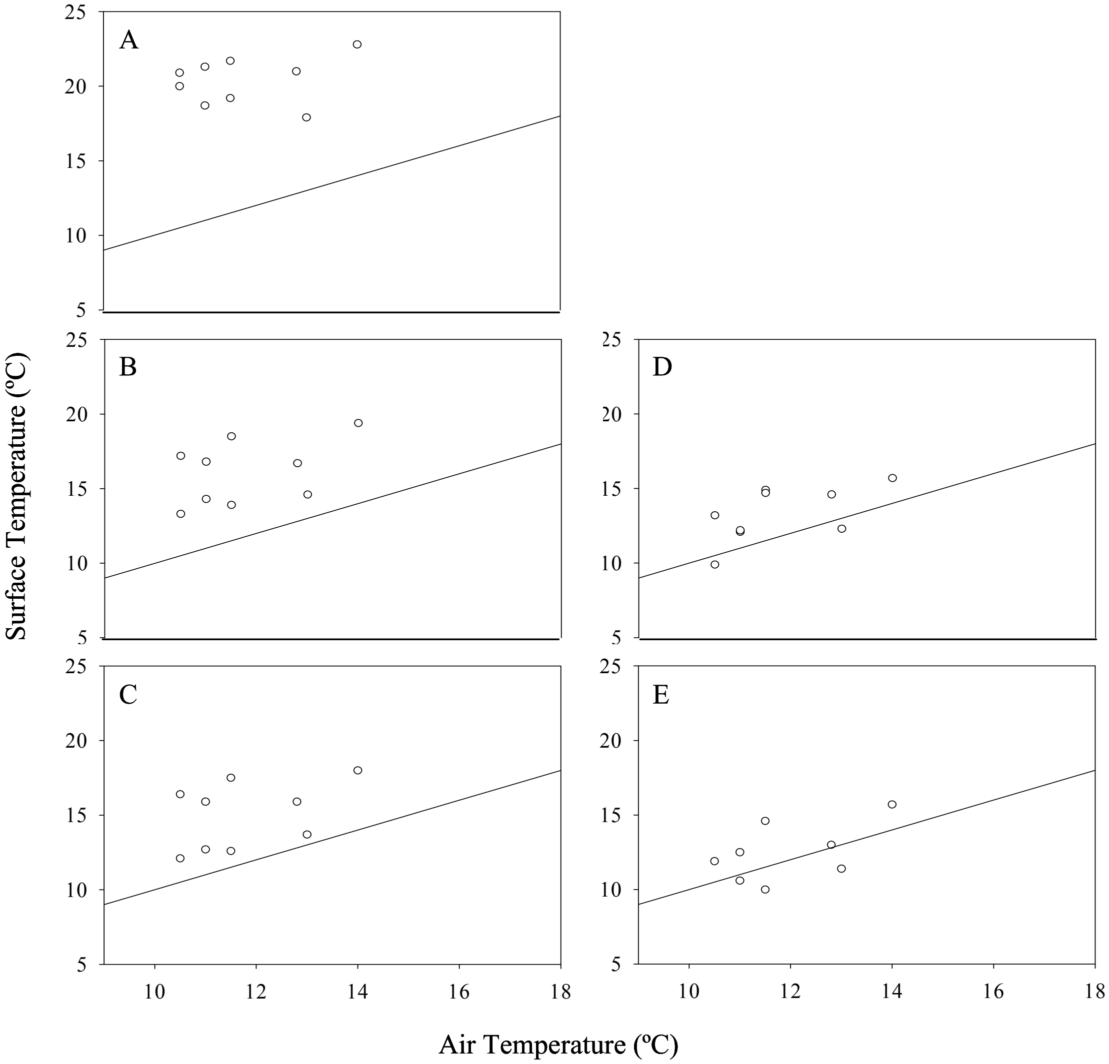

Figure 12. Surface temperatures (ºC) for the head (A), upper body (B), lower body (C), fore flippers (D), and hind flippers (E) of pup Steller sea lions in air 15-minutes post-emergence. Solid line represents the line of equality.

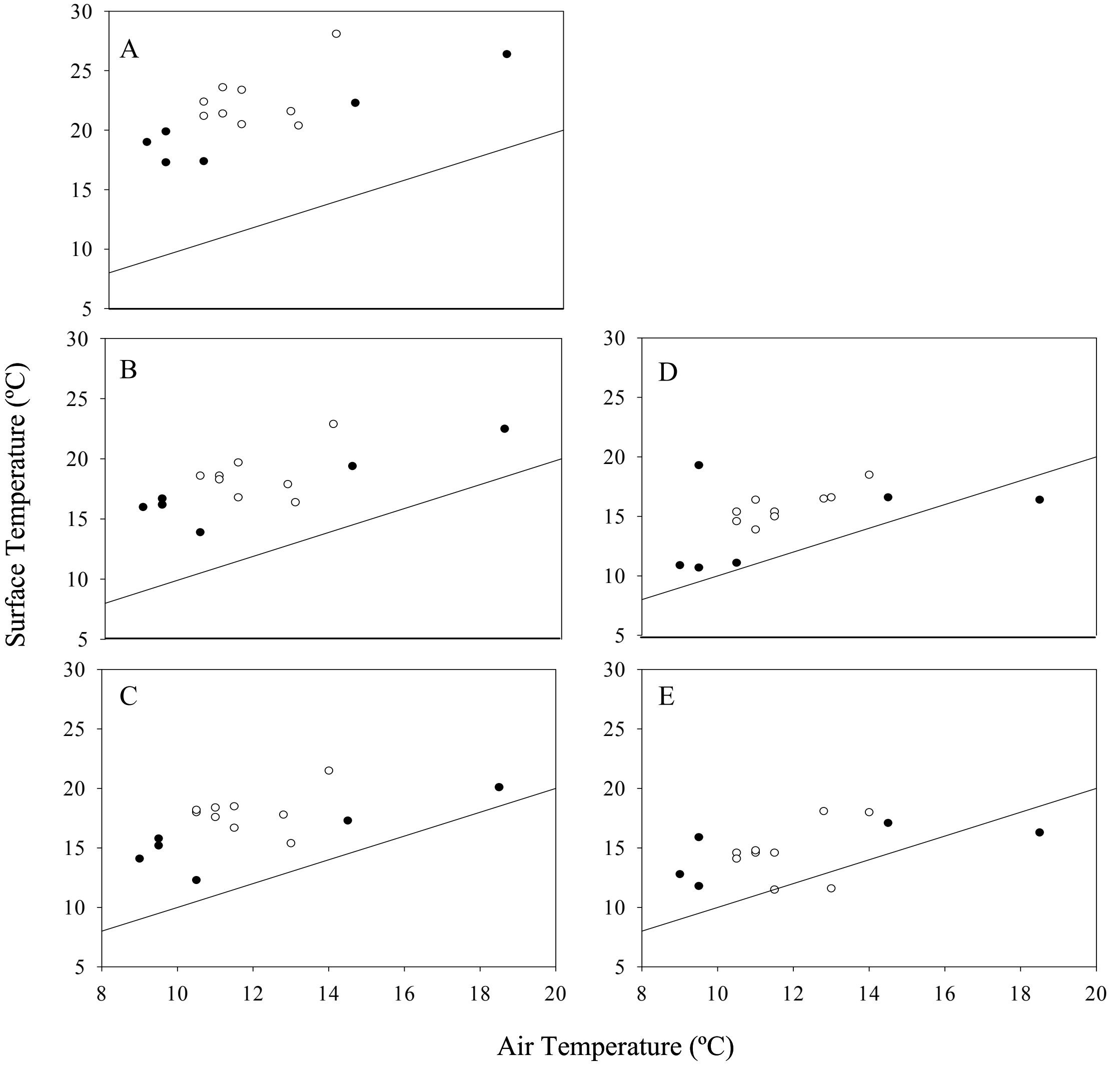

Figure 13. Surface temperatures (ºC) for the head (A), upper body (B), lower body (C), fore flippers (D), and hind flippers (E) of pup (white circles) and juvenile (black circles) Steller sea lions in air 30-minutes post-emergence. Solid line represents the line of equality.

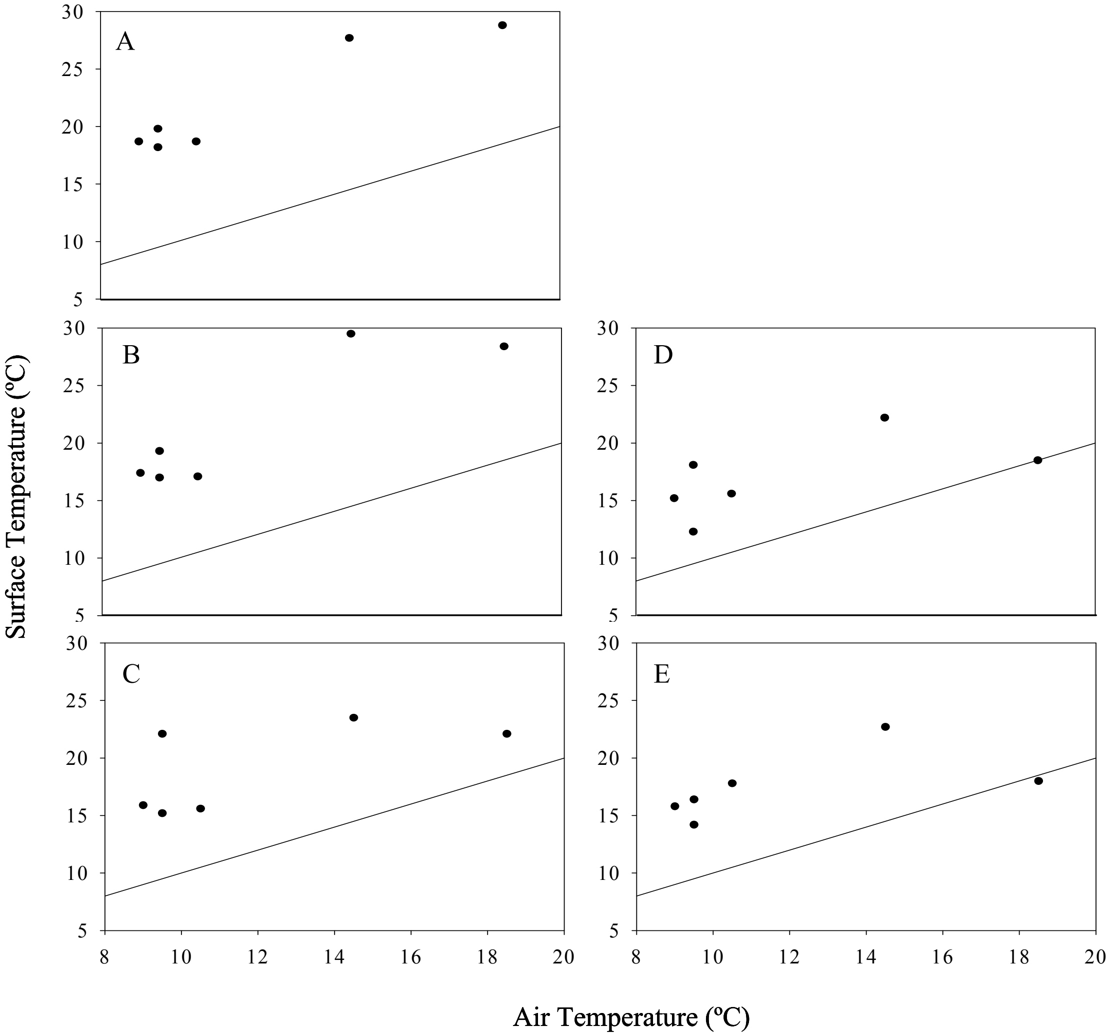

Figure 14. Surface temperatures (ºC) for the head (A), upper body (B), lower body (C), fore flippers (D), and hind flippers (E) of juvenile Steller sea lions in air 60-minutes post-emergence. Solid line represents the line of equality.

temperatures recorded in air were variable within an age class and between age classes (Figs. 11, 12, 13, 14).

Assuming a constant and linear increase in surface temperature warming in sea lions over time, the rate of warming of each body region was calculated as the increase in temperature divided by the amount of time between measurements (ºC min^{-1}). Mean rates of warming did not differ between pups and juveniles based on flume water temperatures nor body region, with the exception of the fore flippers that had warmed up 2 times faster in pups (0.36 ºC min^{-1}) compared to juveniles (0.18 ºC min^{-1}) after trials at 2 ºC. Given the lack of significant differences between the rate of warming in pups and juveniles, warming rates were pooled for all animals to look at differences between body compartments. The fore flippers were excluded from these comparisons using the pooled data since differences were observed in this region.

The mean rate of surface warming did not differ across the four body compartments (head, upper body, lower body, hind flippers) for a given water temperature. However, when individual body regions were compared across water temperatures, differences were evident in the rate of warming after trials in 8 ºC water. Mean rates of warming were generally slower for animals emerging from 8 ºC water in each region of the body, although this trend was only significant in the upper body ($F_{2,12}$=4.538, P=0.034, 4 v. 8: P=0.033) and hind flipper regions ($F_{2,11}$=9.419 P=0.004, 2 vs. 8: P=0.034, 4 vs. 8: P=0.005). All other comparisons of the rate of warming between body regions did not differ based on water temperature.

Thermal windows were classified in sea lions as areas of the body surface showing higher temperatures than the rest of the body and that are separated from the surrounding areas by an edge that transitions to lower temperatures (Mauck et al., 2003); e.g. Fig. 15). Thermal windows typically appeared in both pups and juveniles in the upper and lower regions of the body. In the upper region of the body windows appeared predominately in the area of insertion of the fore flipper to the body. Thermal windows in the lower region of the body typically appeared around the hips and even on portions of the hind flippers (see Fig. 15).

Windows initially appeared in the thermograms as small, circular areas with moderate temperature differences to surrounding areas. Over time these areas increased in temperature and adjoining windows often became merged together to form larger patches. These warm patches could become quite large, and in several instances, occupied nearly half the visible body surface (e.g., Fig. 16). Individual thermal windows could easily be followed over time, as their centers generally remained in the same location, and surface temperatures compared (Fig. 17), however, the time scale in which images were taken for this study was not fine enough to detect the spatial pattern of window development along the sea lion trunk nor long enough to determine whether windows had fully developed.

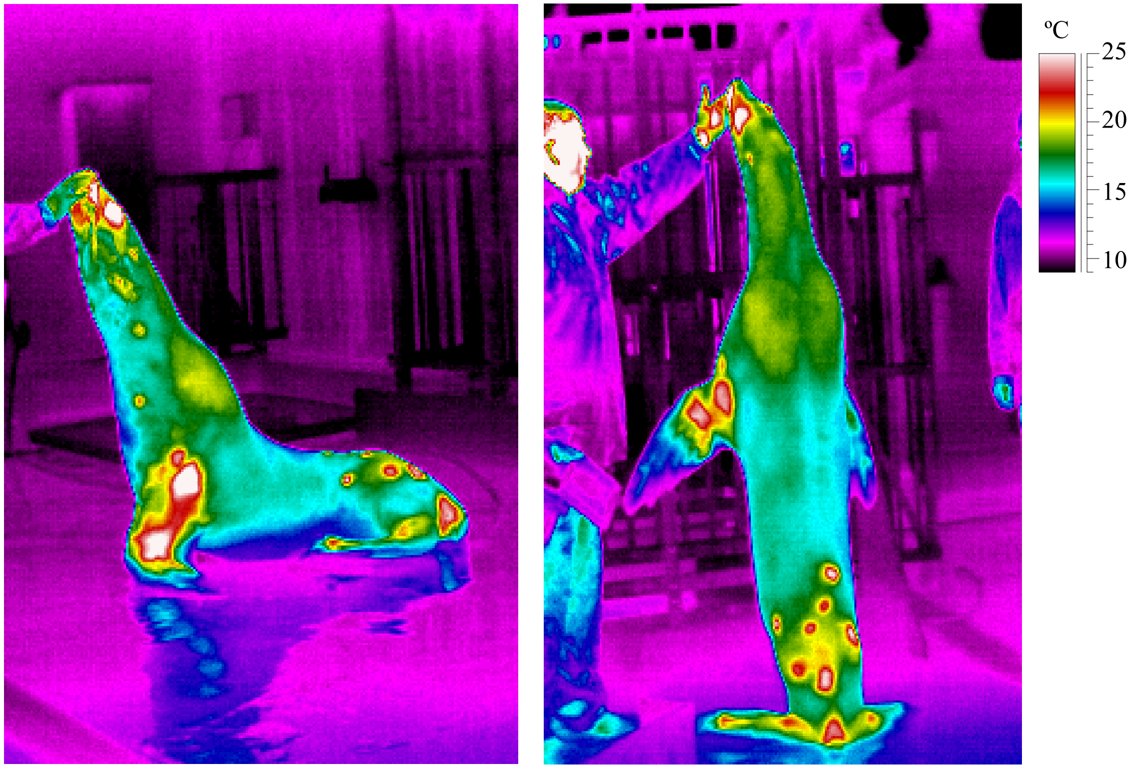

Figure 15. Thermograms of a juvenile sea lion (F00NU) 60 min. after emergence from 2 ºC water at an ambient air temperature of 10.5 ºC. Note the presence of several thermal windows in the upper and lower body regions and the large patch of merged windows on the fore flipper.

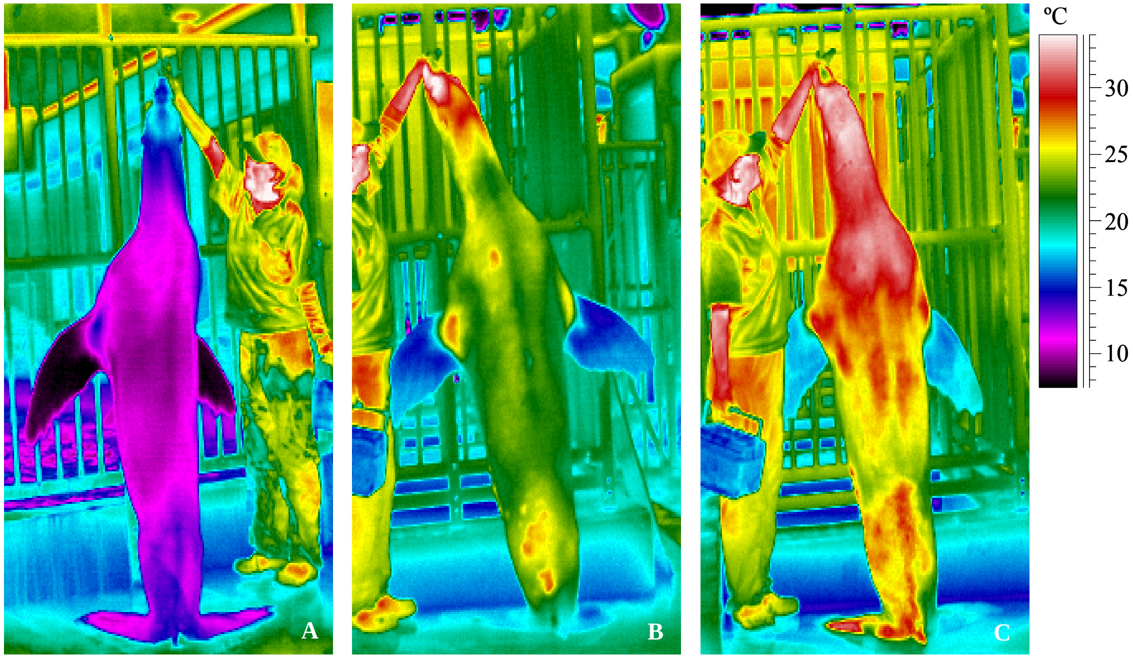

Figure 16. Thermograms of a juvenile sea lion (F00YA) immediately after emergence from 4 ºC water (A), and 30 min. (B) and 60 min. (C) post-emergence at an ambient air temperature of 18.5 ºC.

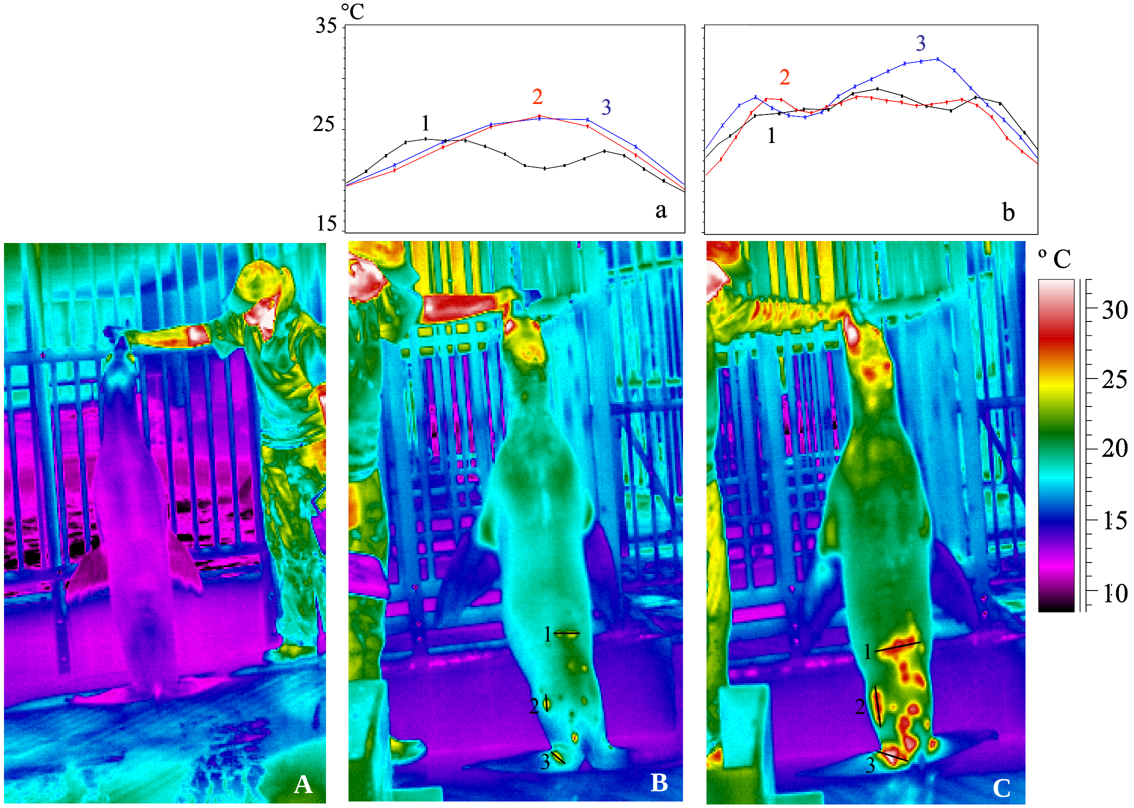

Figure 17. Thermograms of a sea lion pup (F03WI) immediately after emergence from 8 ºC water (A), and 15 min. (B) and 30 min. (C) post-emergence at an ambient air temperature of 11 ºC. Surface temperature profiles at three thermal windows (1, 2, and 3) were followed between the 15 (a) and 30-min (b) images.

Discussion

As expected, the head was always the warmest region of the sea lions body. Given the minimal insulation and the need to maintain the brain at core body temperatures, it is not surprising that this region has a significant energetic cost associated with it. Pinnipeds and likely other aquatic mammals preferentially direct warm blood to the regions surrounding the eyes and vibrissae in order to maintain sensory function while in the water (Dehnhardt et al., 1998; Mauck et al., 2000). It was evident in the images that these regions were much warmer than the surrounding surface area of the head and were a major reason for the higher average surface temperature for this body region. Additionally, sea lions tended to avoid submergence of their head while in the swim-flume, especially at colder water temperatures, which may have contributed to warmer surface temperatures immediately after emergence from the water. When images of the head region were reexamined excluding the eyes and vibrissae, surface temperatures were 1-2 ºC cooler.

The fore flippers were consistently warmer than the hind flippers after emergence from the water and throughout the serial images. The fore flippers of sea lions are large and make up roughly 16% of the total surface area of the body (Feldkamp, 1987; Stelle et al., 2000). Based on surface area alone the fore flippers would experience greater heat loss than the smaller hind flippers. Additionally, the fore flippers are the primary propulsive appendage in otariids during swimming (Feldkamp, 1987; Stelle et al., 2000), and although the animals were not actively swimming in the flume, at the higher water flow speeds an increase in activity was noticeable. Given their proximity to the primary propulsive musculature, the fore flippers would be expected to experience increased heat losses to cold water due to greater heat production during activity from the large swimming muscles. Additionally, the temperature differential (ΔT) between the flippers and the water upon emergence was smaller for the extremities than for areas along the body trunk. This suggests that in cold water, sea lions maintain tight peripheral control of blood flow through utilization of arteriovenous anastomoses (AVA) and/or countercurrent vascular heat exchange systems in the poorly insulated flippers to conserve heat as compared to insulated portions of the body. This was evident by the low surface temperatures of the hind flippers in this study. This was also the case for a variety of other (usually phocid) pinnipeds (Irving and Hart, 1957; Watts et al., 1993; Kvadsheim and Folkow, 1997; Kvadsheim et al., 1997). Alternatively, as temperature increases, flippers can become significant dissipaters of heat by bypassing the heat exchanger (e.g., Tarasoff and Fisher, 1970).

Thermal windows were consistently present in the region of the body where the fore flipper attaches to the base of the body, and grew to be quite large (extending both onto the flipper and up the body) and warm over time. Both Øritsland (1968) and Willis et al. (2005) also noted high surface temperatures in this area. This area must be preferential for dumping heat generated during locomotion given the fact that the fore flipper is the sea lions primary propulsive mechanism and its proximity the pectoral swimming muscles.

Large animals have a smaller surface area to volume ratio than smaller animals and therefore relatively less surface area is available for heat exchange (Innes et al., 1990). McNab (1983) suggested that given the relationship between metabolic rate and body mass, temperature regulation (e.g., heat loss) should also scale with body mass in vertebrates. Phillips and Heath (1995) tested this idea by measuring surface temperatures on a variety of terrestrial animals ranging in size from a mouse to an elephant and verified that the ability to control surface temperature becomes more important as the surface area to volume ratio decreases. Under these principles, it would be expected that the larger sea lions in this study would warm more slowly than smaller pups and that their larger size should have buffered them against heat loss in the colder water temperatures compared with the younger animals. This appears to be the case as pups had cooler surface temperatures upon emergence from the water than juveniles, especially in the fore flippers, and the fore flippers warmed at a slower rate in the larger sea lions.

Rates of warming for all sea lions were slower across the body surface after emergence from the warmest water temperature (8 ºC). This is not unexpected given the smaller difference in skin temperature and ambient air temperature after emergence from the water. It was also more likely that individual sea lions were within their thermal neutral zone at this water temperature (see Chapter II) and would have less need to acquire heat as would animals emerging from the colder water temperatures. Juvenile sea lions exposed to 8 ºC-water had reached thermal equilibrium in air within 30 minutes and no changes surface warming were evident beyond this time point.

Willis et al. (2005) used infrared thermography to identify ‘hot’ and ‘cold’ spots along the trunk of adult Steller sea lions for the purposes of attaching heat flux sensors.

They noted that surface temperatures of the shoulders and hips were warmer than other areas along the trunk and heat flux from these areas were consistently greater than from mid-trunk and axillary regions. Serial thermal photos in this study confirm the development of thermal windows in the areas around the shoulder (upper body) and hip (lower body) areas. Thermal window development in the hip area was generally more pronounced and was evident in pups within 15 minutes of emergence from the water. Additionally, warm patches were evident at the base of the fore flipper in the larger sea lions immediately after emergence from the water, and continued to increase in size and temperature over time. Willis et al. (2005) also noted the base of the fore flipper as a 'hot' spot; however, no data on heat flux was collected from this location or any of the flippers due to the difficulty in sensor attachment.

Understanding the mechanisms of heat loss in water requires understanding how sea lions modify blood perfusion to the skin and extremities. It has been presumed that sea lions would minimize heat flux in cold water by minimizing skin temperature. Thus as water temperature gets colder, the difference (ΔT) between water and skin temperature should increase up until a point where skin temperature cannot be decreased any further. This is supported by the surface temperature data in this study for animals immediately after emergence from the water where surface temperatures of the wet pelage are assumed to be comparable to skin temperatures. Other studies have noted that temperature at the skin/blubber interface can be several degrees warmer than ambient temperature (Hart and Irving, 1959; McGinnis et al., 1972; Hampton and Whittow, 1976; Worthy, 1985; Folkow and Blix, 1987; Folkow and Blix, 1989; Kvadsheim et al., 1997). However, ΔT in free-swimming and diving Antarctic fur seals were much more variable in cold water (mean 1.8-2.9 ºC) and the strategy of minimizing skin temperature to control heat loss was not observed in these animals (Boyd, 2000). It is not surprising that skin temperature was highly variable in Boyd’s (2000) study given the range of diving and swimming activities undertaken by free-ranging animals and given the complex interaction of exercise and cold water where individuals are generating excess heat from muscular work while trying to minimize heat loss. The present study has attempted to minimize the activity component of heat production since water was flowing past the sea lion in the swim flume (although see Discussion in Chapter II) and the assumed net transfer of heat was loss, through convection. Surface temperatures were recorded immediately upon emergence from the water at these high flow speeds and were assumed to be the same as skin temperature.

Although the present study was not as extensive as the one undertaken in three species of phocid seals by Mauck et al. (2003), thermal windows in Steller sea lions also developed primarily along the body trunk of the animal. Thermal windows were observed in all sea lions during all sessions. While the present study was unable to identify the initial location of the first thermal window and subsequent order in which they developed, it was clear that there were variations in window size, shape, and number between individual sea lions. Mauck et al. (2003) speculated on the physiological mechanism of heat transfer as evaporation of condensed water vapor contained in the pelage. They noticed ‘steaming’ of animals on colder days and the development of windows in areas of the pelage that were still wet. Mauck et al. (2003)

suggested that the thermal window was driving the drying process in a particular region through ‘forced evaporation’ of water from the pelage. They noted this would be a thermoregulatory advantage to the animal as it serves to restrict heat loss to a small area and could be more efficiently controlled by the animal over time given the limited storage capacity of water in the pelage per unit body surface. By this mechanism, after water has completely evaporated from a thermal window, blood flow could be directed to another region to ‘force’ evaporation (create another window) or could be restricted if no further heat loss is required (Mauck et al., 2003). Sea lions in the current study were not observed between serial photos and it was unclear whether any ‘steaming’ occurred. Given the mild air temperatures during this time of year in Vancouver, the possibility seems unlikely.

This was the first study to examine surface temperatures in pup and juvenile Steller sea lions after exposure to cold water temperatures, and to look at serial thermal window development during warming at ambient air temperatures. Serial photos in this study were taken at a fairly broad scale (15-30 min intervals) and during a relatively warm month. Continued examination of the development of thermal windows over a finer scale, across seasons, and with changing body condition would provide a clearer picture as to the physiological mechanisms that control thermoregulation in juvenile sea lions.

CHAPTER IV

EFFECTS OF AGE, REGION, AND TEMPERATURE ON IN-AIR RESTING METABOLIC RATES OF FREE-RANGING STELLER SEA LIONS

Introduction

Juvenile pinnipeds experience energetic challenges unique to their life history stage that can impact individual survival and even species population dynamics (York, 1994; Craig and Ragen, 1999). Compared to adults, juveniles must deal with a different set of energetic constraints associated with growth, thermoregulation, and foraging. Elevated metabolic rates in young pinnipeds are thought to be associated with growth and at least partially reflect the heat produced in association with biochemical synthesis of new tissue. The relatively small size of young pinnipeds can potentially constrain thermoregulatory abilities given that smaller animals are prone to greater heat losses due to their high surface area to volume ratio (Irving and Hart, 1957). While this can be a concern for species on land at higher latitudes, pinnipeds must eventually enter the water to forage where the potential for heat loss is considerably higher upon submergence. Juvenile pinnipeds naïveté with regard to foraging strategies and behavior during the transition from weaning to independence is a critical factor determining survival in these animals (Baker and Donohue, 2000). Juvenile seals are faced with the constraints of reduced diving capabilities (e.g., smaller total oxygen stores, high metabolic rates) compared with larger more experienced divers, potentially making some prey resources difficult or impossible to access (Burns, 1999; Hindell et al., 1999; Irvine et al., 2000;

Fowler et al., 2006; Richmond et al., 2006). To better understand energetic constraints to survival in juveniles, knowledge of the animals' basic physiology is required. Understanding maintenance requirements in young animals can provide the key for developing energy budgets, bioenergetics models, and identifying thermal and diving constraints on immature life history stages. This information becomes even more critical when resource availability, such as access to prey, becomes unpredictable or scarce (e.g., Hindell, 1991).

Under conditions of reduced food availability, animals can either increase foraging effort or limit energy expenditures, if they are to maintain energy balance. Increased foraging effort is a short-term strategy with the tradeoff being increased energy expenditure in the hopes of finding prey resources to offset the expense on the front end. Limiting energy expenditures is a longer term strategy used by animals during predictable or prolonged shortages in energy intake (Rosen and Trites, 2002a). The criteria in which an animal chooses one strategy over the other is not well understood.

Animals can limit energy expenditures through decreased activity, increased sleep, and/or a lowering of core body temperature and/or metabolism. One of the most common physiological responses to undernutrition or fasting is a lowering of basal metabolic rate, known as metabolic depression (Guppy and Withers, 1999). Adaptations to periods of food restriction requires not only adjustments in metabolism, but also in fat deposition, in hormonal regulation, and in mobilization of fuel reserves in order to extend the time an organism can survive (e.g., Robin et al., 1988). Metabolic depression has been observed in nearly all major animal taxa (Guppy and Withers, 1999) including several species of marine mammals (Heath et al., 1977; Ashwell-Erickson and Elsner, 1981; Gallivan and Best, 1986; Nordøy et al., 1990; Rea and Costa, 1992; Boily and Lavigne, 1995; Markussen, 1995; Rosen and Renouf, 1998; Rosen and Trites, 1999; Rosen and Trites, 2002a).

Metabolic depression occurs during natural periods of fasting (usually associated with weaning and molting) in phocid pinnipeds (Worthy and Lavigne, 1987; Nordøy et al., 1990; Rea and Costa, 1992; Boily and Lavigne, 1995; Markussen, 1995). Juvenile otariids, on the other hand, generally do not undergo extensive periods of fasting associated with changes in life history. Rather, these species tend to experience periods of reduced intake associated with unpredictability in prey availability over spatial and temporal scales. Diet restriction studies with captive Steller sea lions showed reductions in metabolic rate (31%) when animals were fasted or on a low-energy diet, however, these reductions were not sufficient to prevent body mass loss (Rosen and Trites, 2002a; Rea et al., 2007).

Steller sea lion populations have shown precipitous declines starting in the late 1970's throughout their western Alaskan range (Aleutian Islands and Gulf of Alaska), while numbers in Southeastern Alaska, British Columbia and Oregon have remained stable or slightly increased. Current population trends (1989-2006) in the west reflect continued declines in central and western Aleutian Islands, with counts of non-pups holding stable in the eastern Aleutian Islands and western and eastern Gulf of Alaska (National Marine Mammal Laboratory, unpublished data; www.afsc.noaa.gov/NMML). One hypothesis proposed to explain the rapid decline of western Alaskan Steller sea lions is that these populations experienced either a reduction in overall prey abundance or change in relative abundance of the type and quality of prey available (Trites and Donnelly, 2003). Reductions in prey abundance would be particularly hard on juvenile animals, thus potentially reducing recruitment of this life history stage into the breeding population (e.g., York, 1994).

If Steller sea lions are continuing to decline in western Alaska due to reductions in prey abundance (i.e., nutritional stress hypothesis) then we would expect to see evidence of this reflected in maintenance requirements between sea lions from the differing regions. The goal of the present study was to better understand energetic maintenance requirements in individuals at a variety of ages and interpret these data in reference to ambient temperature and the magnitude of insulative fat stores. The principle objective was to determine if there was evidence of metabolic depression in animals from the declining western population.

Materials and Methods

Study Area and Subjects

Free-ranging Steller sea lions (*N*=91) ranging in age from 2 to 44 months were captured throughout their Alaskan range between 2003 and 2005. Individual animals originated from three distinct geographical regions and populations: (1) Southeast Alaska (SEA) ranging from the southern border of Alaska north to Cape Suckling, (2) Prince William Sound (PWS), west of Cape Suckling, and (3) the Aleutian Islands chain (AI) (Fig. 18). Within each region, sea lions were captured in the waters surrounding known haul out or rookery locations using SCUBA divers and an underwater capture technique developed by the Alaska Department of Fish and Game (ADFG) (see Raum-Suryan et al., 2004). Occasionally sea lions were captured on land with hoop nets when weather or current conditions prevented in-water capture. All research conformed to the guidelines of the ADFG Animal Care and Use Committee (# 03-0002) and was conducted under MMPA permit #358-1564.

Animal Processing

Approximately 1-2 h post-capture, sea lions were weighed (Ocean King D-6, TCI Scales, Inc., Mukilteo, WA, ±0.5 kg) in their capture boxes and then immobilized under gas anesthesia based on methods detailed by Heath et al. (1997). Age and sex were determined. With the exception of young pups, whose age was estimated by average pupping date (June 15, Pitcher et al., 2001), age determination for older sea lions was estimated using date, body size, and degree of tooth eruption or canine length (King et

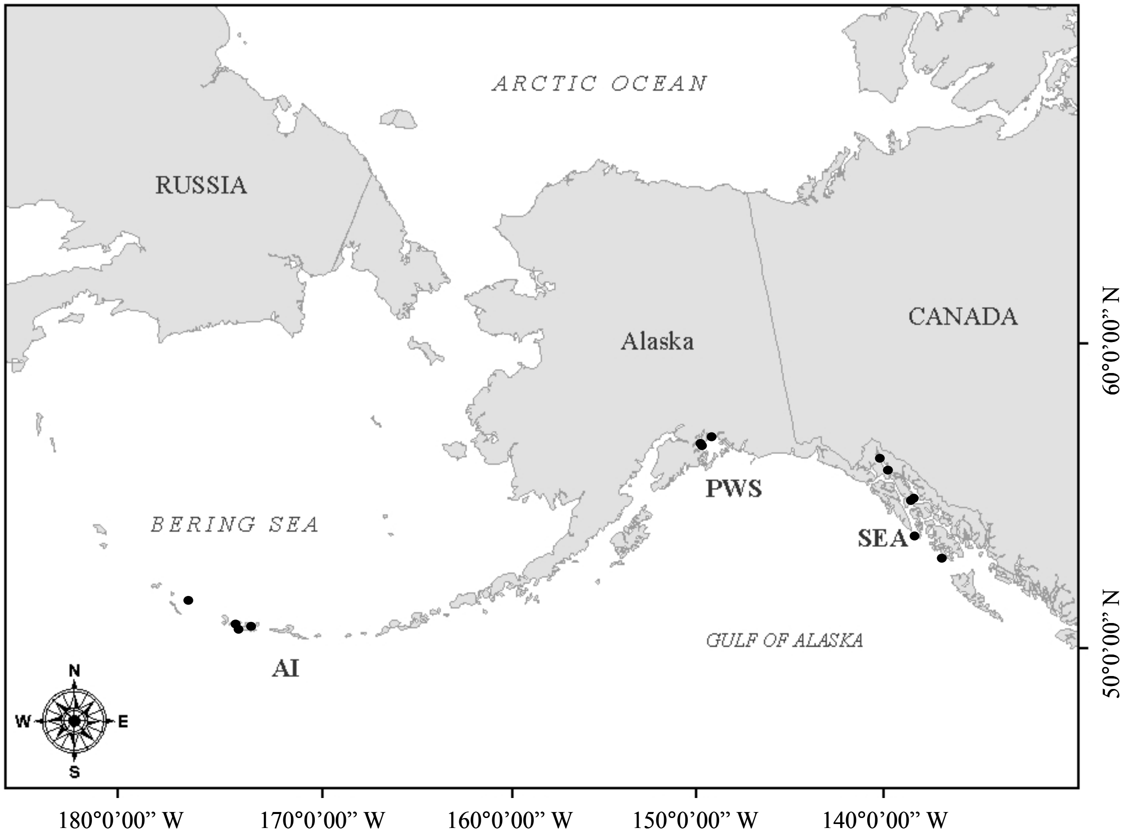

Figure 18. Map of Alaska showing capture locations (black circles) within each of the three sampling regions: Southeast Alaska (SEA), Prince William Sound (PWS), and the Aleutian Islands (AI).

al., 2003). Animals were intubated to collect gastric contents in order to determine whether animals had recently been nursing or feeding. Standard length and girths at five locations along the body (neck, chest, axilla, mid trunk, and hips) were also recorded.

Body Condition

Body condition was assessed in sea lions by measuring the thickness of the subcutaneous blubber layer (*N*=87) and by measuring body composition (*N*=90) calculated from total body water (TBW). Blubber thickness was measured with the SonoSite 180PLUS, ultrasound (SonoSite Inc., WA, USA) at a single site, dorsally, on the right hip.

Total body water was determined using the deuterium isotope (^2H_2O) dilution method. Blood samples to assess background isotope levels were collected either from the interdigital rear flipper vein or the caudal gluteal vein prior to the intermuscular administration of deuterium oxide (dose 0.5 g kg^{-1}). Additional blood samples were collected during a subsequent anesthesia procedure at a minimum of 2 and 2.5 h after deuterium dosing to ensure equilibrium with body water (L. Rea, personal communication). Sera and reference samples were stored on dry ice in the field, and at -80 ºC upon return to the lab. Samples were analyzed for TBW by ADFG using infra-red (FTIR) spectrophotometry. Calculated TBW was converted to total body lipid (TBL) using predictive equations derived for pinnipeds (Bowen and Iverson, 1998) and correcting for the approximate 4% overestimation of TBW (Reilly and Fedak, 1990).

Lean body mass (LBM) was calculated as the difference between total body mass (TBM) and TBL and then expressed as percentage of total mass (%TBL, %LBM).

Resting Metabolism

Resting metabolic rate (RMR) was measured under ambient air temperature conditions using open-flow respirometry. Sea lions held in ventilated metal cages where placed on the wooden base of the metabolic chamber, over which, a plexiglass lid (231×79×56 cm) was fitted into metal tracking. An airtight seal between the lid and base was ensured by filling the track initially with seawater, and later with foam-rubber. Fans were mounted in each of the four corners of the lid to ensure continuous air mixing. Temperature inside the chamber, monitored by a thermocouple inserted into the chamber through air intake tubing and attached to a weather station (Weather Monitor II, Davis Instruments, CA, USA), was recorded every 5 min. The chamber was outdoors, located on the deck of the vessel, and thus air temperature in the chamber was influenced by ambient conditions. Chamber temperature was usually 2 to 8 ºC warmer than ambient conditions. Under sunny summer conditions, tarps were erected over the chamber to help keep chamber temperatures from getting too warm. On these days, sea lion behavior was closely monitored along with chamber temperature, and trials were immediately ended when chamber temperatures approached 24 ºC or if sea lions exhibited signs of heat stress (e.g. open mouth breathing).

Air was drawn through the chamber at a constant rate (70-200 l min^{-1}, based on animal size) and was regulated by a flow controller (500H Flow Kit , Sable Systems

International, NV, USA). Oxygen and carbon dioxide concentrations within a desiccated sample of expired air were measured by an oxide cell analyzer (FC-1B, Sable Systems International, NV, USA) and an infrared gas analyzer (CA-2A, Sable Systems International, NV, USA), respectively. The amount of oxygen consumed during the trial was calculated from the difference in oxygen concentration between air entering and leaving the chamber, with flow corrected to STPD. Carbon dioxide was not removed from the sampled air stream and oxygen concentrations were determined by Datacan V software (Sable Systems International, NV, USA) using equation 3b from Withers (1977):

$$\dot{V}O_2 = f \times 60 \times (FIO_2 - FEO_2) - \dot{V}CO_2 \times FIO_2/(1 - FIO_2)$$

where $\dot{V}O_2$ is the rate of oxygen consumption (ml h^{-1}), f is the rate of airflow through the chamber (ml min^{-1}), FIO_2 is the fractional concentration of oxygen gas entering the respirometer, FEO_2 is the fractional concentration of oxygen gas leaving the respirometer, and $\dot{V}CO_2$ is the rate of carbon dioxide production (ml h^{-1}). The entire system was calibrated using gases of known concentration both before and after each trip (Fedak et al., 1981). Oxygen consumption (and carbon dioxide production) data was recorded for a minimum of 1 h, and up to 2 h. Resting metabolic rates were calculated from the lowest rate of oxygen consumption during a minimum 15 min period where the animal was inactive and chamber temperatures held stable. Oxygen consumption rates were converted to energy utilization assuming that 1 l O_2 = 20.0 kJ.

Resting metabolism was measured on animals post-anesthesia, during deuterium equilibration with body water. To determine the potential effects of anesthesia on metabolism, oxygen consumption was measured before and after anesthesia in a subset of animals (*N*=19), varying in age from 5 to 20 months. All sea lions were awake when placed in the chamber. Some animals initially struggled or paced in their cage when placed in the chamber, but most animals were immediately calm or attempted to sleep. Sea lion activity was monitored and recorded every 5 min, or whenever activity levels changed, and tapping on the chamber prevented animals from sleeping. For grouping purposes, sea lions < 12 months in age were considered “young of the year” (YOY), while animals ≥ 12 months of age were considered “juveniles”.

Statistical Analysis

SPSS (version 11.0) and JMP (version 6.0) statistical software were used for all analyses. The relative contribution of factors such as age, total body mass, lean mass, lipid mass, and ambient temperature to RMR was determined by multiple linear regression. Linear regression was calculated by least squares and stepwise methods, and significance of the relationships was determined by *F*-tests. Paired *t*-tests were used to compare metabolic rates pre- and post-anesthesia. Sexes were compared using two-sample *t*-tests. One-way ANOVA was employed to compare group means across age categories and Tukey-Kramer HSD test was used for post hoc pairwise comparisons. ANCOVA was employed to identify differences in metabolic rate between locations while accounting for the influence of total mass, LBM, air temperature, and/or age on metabolism. Interaction terms using the general linear model were used to compare slopes of relationships. All means are presented with ± SEM. Results were considered significant at $P<0.05$, unless otherwise noted.

Results

Morphometrics

Male sea lions were generally heavier than females for each age class in which comparisons could be made (Table 10). Both male and female juvenile sea lions were significantly heavier than YOY ($t=4.08$, d.f.=60, $P<0.001$; $t=4.19$, d.f.=26, $P<0.001$ respectively). Although there were differences in age between regions, sea lions in PWS were larger than animals in SEA (Fig. 19). For their age, AI YOY were larger than similarly aged animals from the other capture locations and were of similar total mass to juvenile animals (Fig. 19).

Dorsal standard length and all girth measurements (neck, chest, axillary, mid trunk, hips) were significantly longer/larger in male sea lions compared to female sea lions (all $P<0.05$). Mean length and girths generally differed between sexes within each age class, with the exception of 17 and 26 mo. animals where males and females were similar in all morphometric measurements (Tables 11, 12, 13, 14 ,15, 16). Non-linear increases in length and girth were evident with increasing sea lion size/age (Fig. 20). All morphometric parameters showed similar patterns, however, only data for dorsal length, axillary girth, and hip girth are presented here (Figs. 21, 22, 23). Morphometric comparisons between similarly aged animals in PWS and SEA showed no significant differences in length or girth ($P>0.05$) between location with the exception of PWS sea lions (both 8 and 20 mo. animals) having significantly larger ($P<0.05$) neck girths than SEA sea lions.

Body Composition

Total body water (TBW) ranged from 44.0 to 69.0% across all aged sea lions. No significant differences in %TBW were evident between male and female sea lions (all $P>0.05$). Mean %TBW levels varied with sea lion size, especially in YOY, where smaller animals had a significantly higher water pool than larger YOY (Fig. 24). Little difference in %TBW was observed among juvenile sea lions (Fig. 24).

Total body lipid (TBL) ranged from 2.8 to 35.3% and varied longitudinally with ontogeny but also individually within an age class (Table 17). There were no significant differences ($P>0.05$) in mean %TBL between male and female sea lions at all age categories where sufficient sample sizes allowed comparisons (Table 17). Sea lions from PWS maintained a relatively constant proportion of %TBL as they increased in total mass since mean %TBL did not significantly differ with age ($F_{4,29}=1.55$, $P=0.218$, Fig. 25). Conversely, sea lions captured in SEA showed significant differences in mean %TBL among the age classes in this location ($F_{4,43}=6.94$, $P=0.0003$, Fig. 26). Specifically, among the yearlings, 2 mo. old animals had significantly lower ($P<0.05$) mean %TBL than 8 mo. old sea lions (Fig. 26). No differences in mean %TBL were evident among juvenile sea lions in SEA (Fig. 26). Sea lion YOY from AI had the greatest mean %TBL at 27.4% (Fig. 27). Pups captured in PWS had similar ($P=0.55$) mean %TBL values to sea lions in SEA of the same age (8 mo), while juvenile sea lions

Table 10. *Mean body mass values (kg, ±SEM) for female and male Steller sea lions captured in the Aleutian Islands, Prince William Sound, and Southeast Alaska*

Location	Age (mo)	Females		Males		*P*
AL	10.5	106.8 ± 7.0	(6)	145.7 ± 8.6	(10)	**0.007**
PWS	5	61.7 ± 5.7	(4)	82.7 ± 3.2	(11)	**0.006**
	8	99.0 ± 3.0	(2)	86.0	(1)	
	17	124.1 ± 2.4	(3)	137.5 ± 6.5	(5)	0.180
	20			150.5 ± 13.4	(3)	
	44			232.5	(1)	
SEA	2	34.6 ± 1.5	(4)	42.2 ± 0.7	(5)	**0.002**
	8	70.6 ± 5.4	(6)	82.8 ± 3.7	(12)	0.077
	14	104.0	(1)	100.6 ± 5.5	(8)	
	20			127.4 ± 6.4	(5)	
	26	143.3 ± 16.8	(2)	146.0 ± 3.0	(2)	0.887

Sample sizes are in parentheses; significant comparisons at $P < 0.05$ are in bold.
AI, Aleutian Islands; PWS, Prince William Sound; SEA, southeast Alaska.

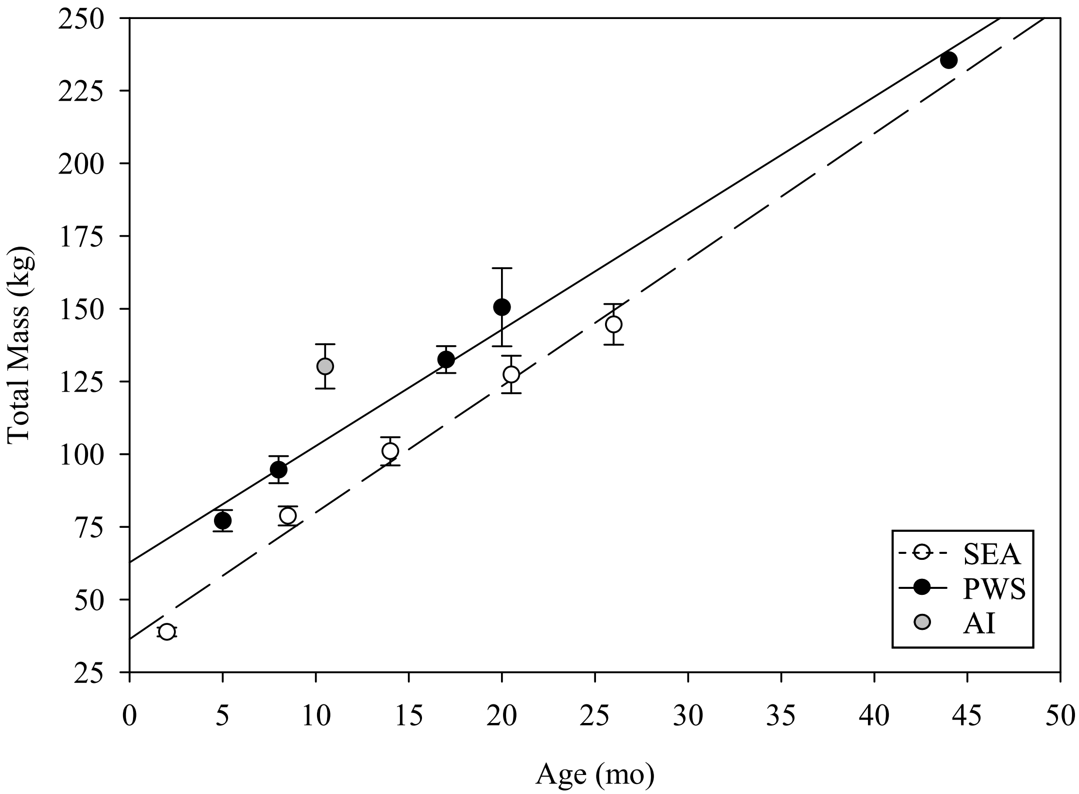

Figure 19. Mean total body mass (kg) ± SEM per age category for sea lions captured in Southeast Alaska (SEA), Prince William Sound (PWS), and the Aleutian Islands (AI). The regression equation for SEA animals is y=4.39x+38.46 (r^2=0.85, P<0.001; dashed line) and the regression equation for PWS animals is y=4.28x+57.76 (r^2=0.88, P<0.001; solid line).

Table 11. *Mean dorsal standard length (cm, ±SEM) for female and male Steller sea lions captured in the Aleutian Islands, Prince William Sound, and Southeast Alaska*

Location	Age (mo)	Females		Males		*P*
AL	10.5	166.0 ± 2.7	(6)	182.6 ± 2.5	(10)	**0.001**
PWS	5	141.8 ± 2.3	(4)	153.9 ± 2.0	(11)	**0.005**
	8	159.3 ± 0.3	(2)	150.5	(1)	
	17	185.7 ± 1.8	(3)	189.4 ± 2.6	(5)	0.349
	20			191.5 ± 5.4	(3)	
	44			220.0	(1)	
SEA	2	114.0 ± 2.3	(4)	123.4 ± 0.9	(5)	**0.018**
	8	145.8 ± 3.8	(6)	152.7 ± 2.6	(12)	0.160
	14	174.0	(1)	169.3 ± 3.9	(8)	
	20			183.4 ± 4.6	(5)	
	26	189.9 ± 12.8	(2)	190.8 ± 9.8	(2)	0.956

Sample sizes are in parentheses; significant comparisons at $P < 0.05$ are in bold; comparisons were made where adequate sample sizes allowed.

AI, Aleutian Islands; PWS, Prince William Sound; SEA, southeast Alaska.

Table 12. *Mean neck girth (cm, ±SEM) for female and male Steller sea lions captured in the Aleutian Islands, Prince William Sound, and Southeast Alaska*

Location	Age (mo)	Females		Males		*P*
AL	10.5	72.2 ± 2.2	(6)	79.9 ± 1.9	(10)	**0.021**
PWS	5	57.5 ± 2.6	(4)	64.1 ± 1.1	(11)	**0.014**
	8	72.5 ± 5.5	(2)	68.0	(1)	
	17	70.3 ± 0.9	(3)	76.0 ± 2.0	(5)	0.084
	20			82.0 ± 2.3	(3)	
	44			87.0	(1)	
SEA	2	50.8 ± 1.9	(4)	54.6 ± 0.7	(5)	0.132
	8	59.0 ± 2.1	(6)	65.7 ± 1.2	(12)	**0.026**
	14	73.0	(1)	70.6 ± 1.8	(8)	
	20			73.8 ± 1.7	(5)	
	26	74.5 ± 3.5	(2)	79.5 ± 3.5	(2)	0.419

Sample sizes are in parentheses; significant comparisons at $P < 0.05$ are in bold; comparisons were made where adequate sample sizes allowed.

AI, Aleutian Islands; PWS, Prince William Sound; SEA, southeast Alaska.

Table 13. *Mean chest girth (cm, ±SEM) for female and male Steller sea lions captured in the Aleutian Islands, Prince William Sound, and Southeast Alaska*

Location	Age (mo)	Females		Males		*P*
AL	10.5	111.2 ± 3.5	(6)	121.5 ± 2.3	(10)	**0.021**
PWS	5	98.8 ± 3.2	(4)	107.6 ± 2.3	(11)	0.061
	8	111.0 ± 8.0	(2)	104.0	(1)	
	17	126.7 ± 1.7	(3)	121.6 ± 2.6	(5)	0.222
	20			129.7 ± 6.0	(3)	
	44			154.0	(1)	
SEA	2	75.5 ± 1.3	(4)	83.4 ± 1.1	(5)	**0.020**
	8	94.5 ± 2.9	(6)	106.9 ± 2.4	(12)	**0.007**
	14	115.0	(1)	111.4 ± 3.7	(8)	
	20			121.0 ± 3.9	(5)	
	26	121.5 ± 2.5	(2)	124.0 ± 1.0	(2)	0.492

Sample sizes are in parentheses; significant comparisons at $P < 0.05$ are in bold; comparisons were made where adequate sample sizes allowed.

AI, Aleutian Islands; PWS, Prince William Sound; SEA, southeast Alaska.

Table 14. *Mean axillary girth (cm, ±SEM) for female and male sea lions captured in the Aleutian Islands, Prince William Sound, and Southeast Alaska*

Location	Age (mo)	Females		Males		*P*
AL	10.5	119.8 ± 3.8	(6)	132.7 ± 3.2	(10)	**0.026**
PWS	5	93.8 ± 4.1	(4)	105.0 ± 1.8	(11)	**0.013**
	8	115.3 ± 2.3	(2)	109.5	(1)	
	17	129.5 ± 2.3	(3)	125.3 ± 2.5	(5)	0.310
	20			127.2 ± 3.9	(3)	
	44			146.0	(1)	
SEA	2	78.5 ± 1.5	(4)	84.3 ± 1.0	(5)	**0.003**
	8	101.8 ± 3.5	(6)	109.3 ± 1.8	(12)	0.093
	14	114.0	(1)	110.4 ± 2.8	(8)	
	20			128.0 ± 3.8	(5)	
	26	123.1 ± 3.0	(2)	123.3 ± 1.3	(2)	0.956

Sample sizes are in parentheses; significant comparisons at $P < 0.05$ are in bold; comparisons were made where adequate sample sizes allowed.

AI, Aleutian Islands; PWS, Prince William Sound; SEA, southeast Alaska.

Table 15. *Mean mid-trunk girth (cm, ±SEM) in female and male Steller sea lions captured in the Aleutian Islands, Prince William Sound, and Southeast Alaska*

Location	Age (mo)	Females		Males		*P*
AL	10.5	113.8 ± 4.4	(6)	128.7 ± 3.7	(10)	**0.023**
PWS	5	89.5 ± 4.3	(4)	98.7 ± 2.4	(11)	0.070
	8	106.5 ± 2.5	(2)	107.0	(1)	
	17	111.3 ± 2.4	(3)	119.0 ± 3.0	(5)	0.133
	20			119.0 ± 5.5	(3)	
	44			143.0	(1)	
SEA	2	75.0 ± 1.2	(4)	81.8 ± 0.9	(5)	**0.005**
	8	98.3 ± 2.4	(6)	105.0 ± 2.2	(12)	0.059
	14	106.0	(1)	107.1 ± 2.1	(8)	
	20			118.8 ± 3.9	(5)	
	26	117.0 ± 5.0	(2)	120.0 ± 3.0	(2)	0.668

Sample sizes are in parentheses; significant comparisons at $P < 0.05$ are in bold; comparisons were made where adequate sample sizes allowed.

AI, Aleutian Islands; PWS, Prince William Sound; SEA, southeast Alaska.

Table 16. *Mean hip girth (cm, ±SEM) for female and male Steller sea lions captured in the Aleutian Islands, Prince William Sound, and Southeast Alaska*

Location	Age (mo)	Females		Males		*P*
AL	10.5	82.5 ± 2.3	(6)	97.3 ± 2.9	(10)	**0.003**
PWS	5	62.3 ± 2.9	(4)	74.9 ± 1.3	(11)	**0.004**
	8	80.0 ± 4.0	(2)	76.0	(1)	
	17	84.3 ± 2.8	(3)	88.8 ± 2.1	(5)	0.245
	20			89.0 ± 1.2	(3)	
	44			107.0	(1)	
SEA	2	56.8 ± 1.4	(4)	59.6 ± 0.9	(5)	0.141
	8	72.2 ± 1.5	(6)	76.2 ± 1.5	(12)	0.081
	14	79.0	(1)	79.0 ± 1.1	(8)	
	20			87.8 ± 4.4	(5)	
	26	85.0 ± 4.0	(2)	84.0 ± 2.0	(2)	0.850

Sample sizes are in parentheses; significant comparisons at $P < 0.05$ are in bold; comparisons were made where adequate sample sizes allowed.

AI, Aleutian Islands; PWS, Prince William Sound; SEA, southeast Alaska.

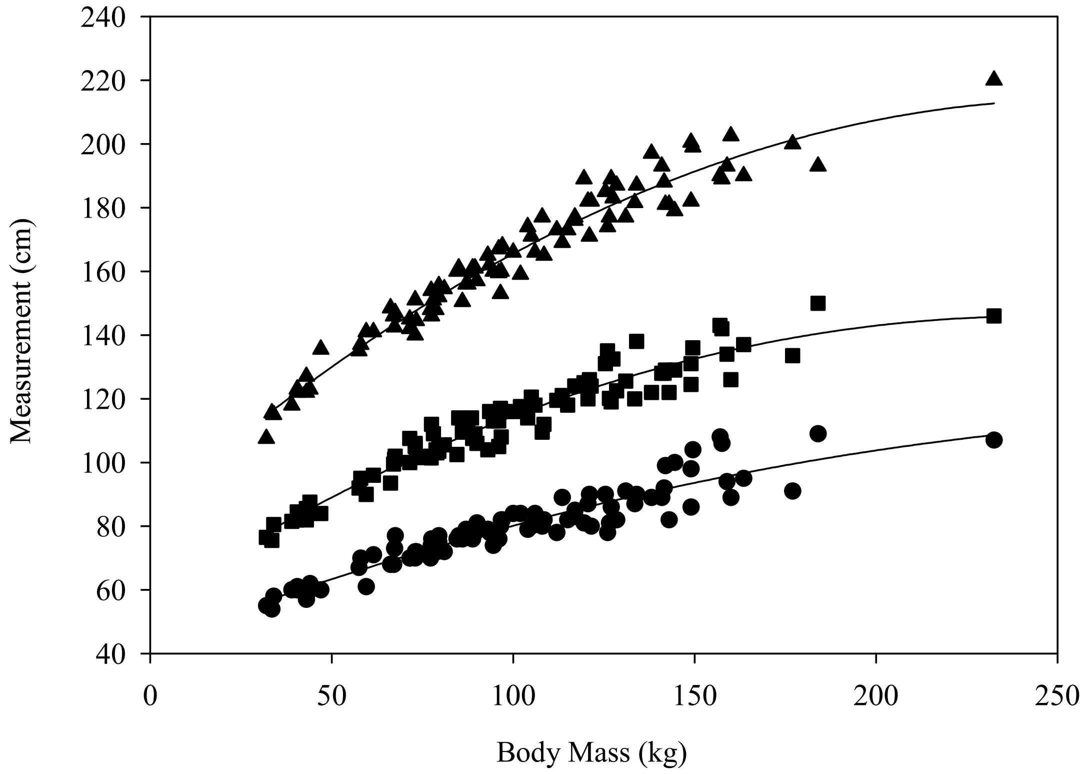

Figure 20. Non-linear relationship between total body mass (kg) and dorsal standard length (r^2=0.95, $F_{2,88}$=904.1, P<0.0001, triangles), axillary girth (r^2=0.92, $F_{2,88}$=578.1, P<0.0001, squares), and hip girth (r^2=0.88, $F_{2,88}$=311.2, P<0.0001, circles). Relationships between body mass and chest (r^2=0.85, $F_{2,88}$=251.7, P<0.0001), mid-trunk (r^2=0.91, $F_{2,88}$=422.9, P<0.0001) and neck (r^2=0.88, $F_{2,88}$=331.5, P<0.0001) girths were similar, however, these data are not shown.

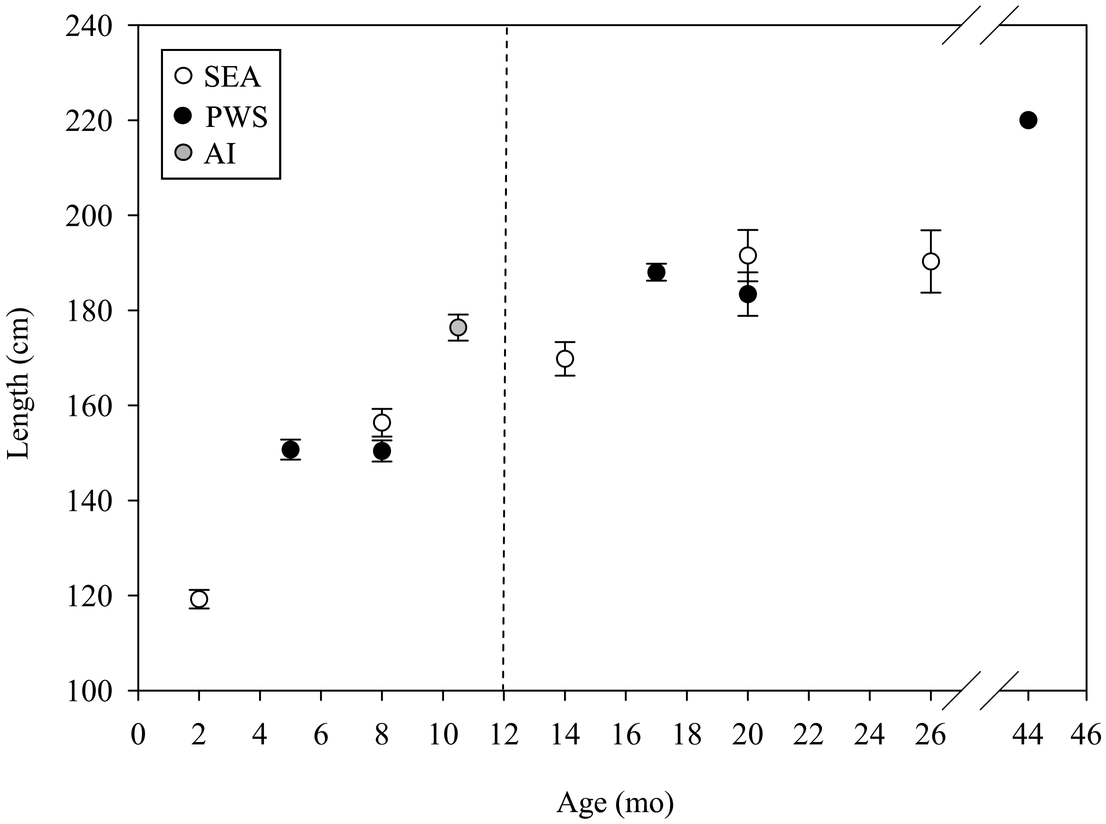

Figure 21. Mean dorsal standard length (cm) ± SEM as a function of age (mo) for sea lions captured in Southeast Alaska (SEA), Prince William Sound (PWS), and the Aleutian Islands (AI).

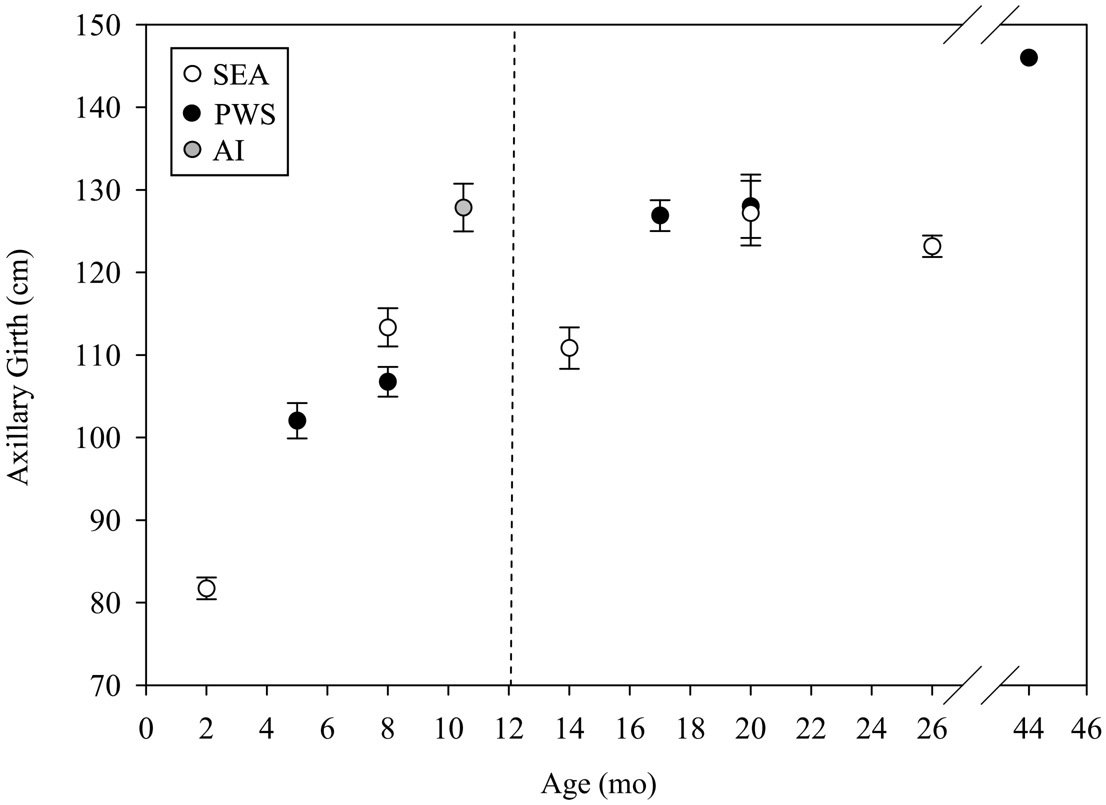

Figure 22. Mean axillary girth (cm) ± SEM as a function of age (mo) for sea lions captured in Southeast Alaska (SEA), Prince William Sound (PWS), and the Aleutian Islands (AI).

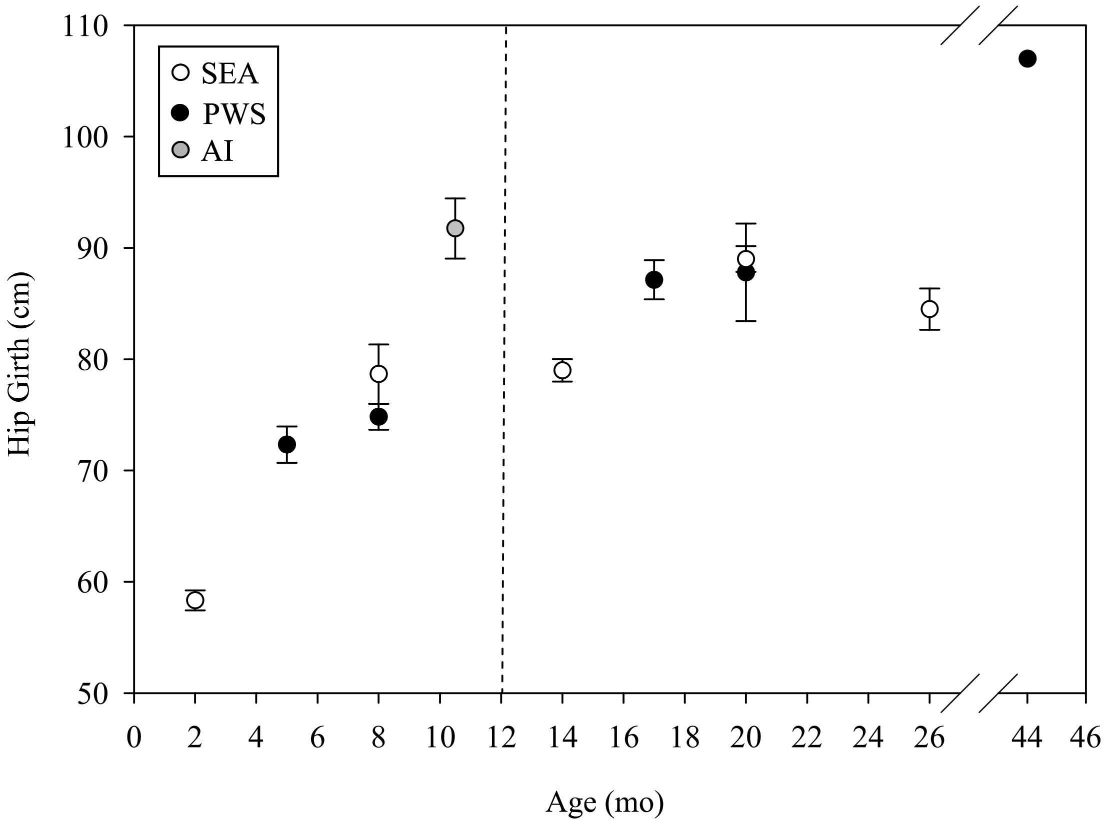

Figure 23. Mean hip girth (cm) ± SEM as a function of age (mo) for sea lions captured in Southeast Alaska (SEA), Prince William Sound (PWS), and the Aleutian Islands (AI).

in PWS had lower, but non-significant ($P=0.06$), mean %TBL values than similar aged animals (20 mo) in SEA (Fig. 28).

Lean body mass (LBM) ranged from 64.7 to 95.1% in all free-ranging sea lions. Percent LBM was similar ($P>0.05$) between male and female sea lions at each age category. Similar to %TBL, %LBM did not differ significantly by age in PWS ($F_{4,29}=1.55$, $P=0.218$). However, %LBM did differ between sea lion age groups in SEA ($F_{4,43}=11.14$, $P<0.001$). Specifically, 2 mo. old pups had significantly higher %LBM than 8 mo. YOY and 20 mo. old juveniles ($P<0.05$), but no differences in lean mass compared to 14 and 26 mo. old juveniles ($P>0.05$, Fig. 25). In comparisons of similarly aged animals %LBM was similar between regions for 8 mo. old animals ($t=0.367$, $P=0.718$) and slightly higher, but not significantly so ($t=-2.35$, $P=0.06$), for 20 mo. sea lions in PWS compared to SEA.

Blubber depth ranged from 1.0 to 2.7 cm across all animals. Among the YOY, 2 mo. old animals had the smallest mean blubber depths (1.1±0.2 cm), which were significantly thinner than blubber depths from older pups (Fig. 29). Sea lion YOY captured in AI had the thickest mean blubber depths measured (2.2±0.1 cm, Fig. 29). While blubber depths were slightly higher in SEA 8 mo. YOY compared to animals of the same age in PWS, these values were not significantly different ($P>0.05$, Fig. 29). Among juveniles, no significant differences in blubber depth were evident across the age classes nor the differing capture regions (Fig. 29).

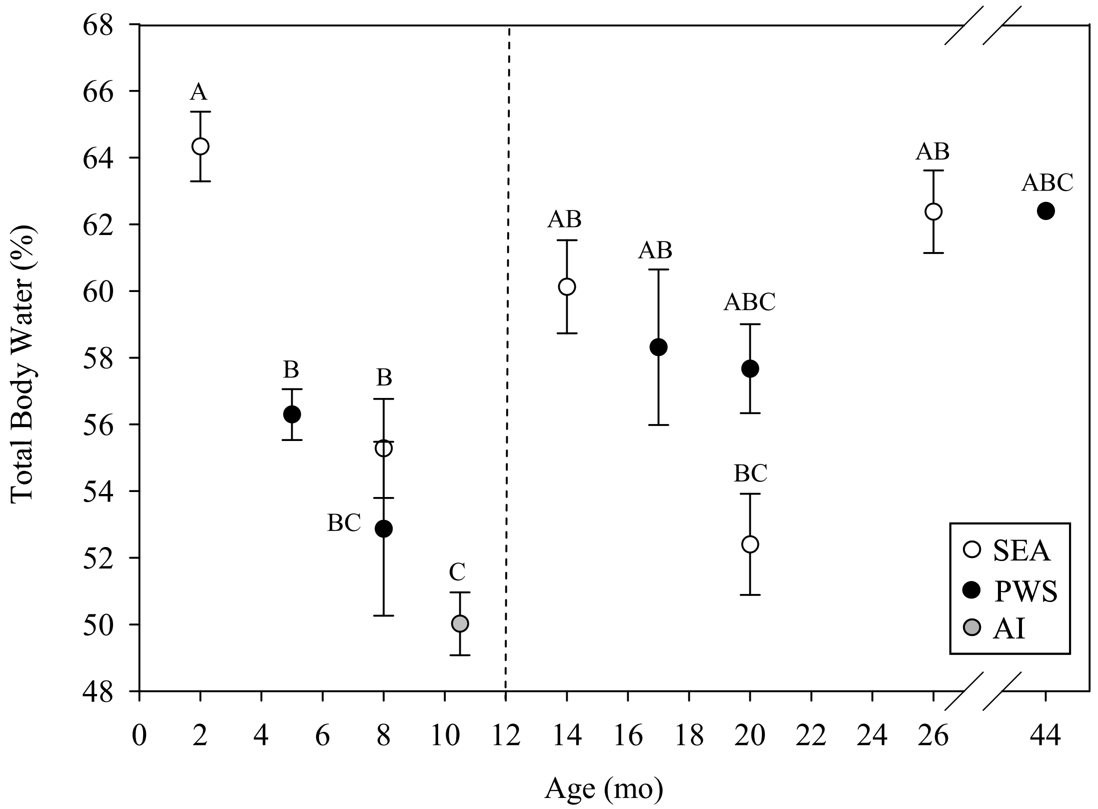

Figure 24. Mean percent total body water (%TBW) ± SEM for sea lions captured in Southeast Alaska (SEA), Prince William Sound (PWS), and the Aleutian Islands (AI). Levels of %TBW with different letters were considered significantly different, while levels with the same letter were not significantly different.

Table 17. *Mean total body lipid levels (%, ±SEM) for female and male Steller sea lions captured in the Aleutian Islands, Prince William Sound, and Southeast Alaska*

Location	Age (mo)	Females		Males		*P*
AL	10.5	27.0 ± 2.4	(6)	27.8 ± 1.4	(10)	0.760
PWS	5	21.6 ± 1.9	(4)	18.6 ± 1.1	(11)	0.196
	8	21.6 ± 4.5	(2)	28.2	(1)	
	17	20.9 ± 4.9	(3)	13.9 ± 3.8	(5)	0.302
	20			17.4 ± 1.7	(3)	
	44			11.2	(1)	
SEA	2	10.2 ± 2.1	(4)	8.4 ± 1.9	(5)	0.549
	8	23.9 ± 3.3	(6)	19.1 ± 2.4	(12)	0.254
	14	22.7	(1)	13.1 ± 1.6	(8)	
	20			24.3 ± 2.0	(5)	
	26	11.1 ± 2.8	(2)	11.3 ± 2.8	(2)	0.965

Sample sizes are in parentheses; significant comparisons at $P < 0.05$ are in bold; comparisons were made where adequate sample sizes allowed.

AI, Aleutian Islands; PWS, Prince William Sound; SEA, southeast Alaska.

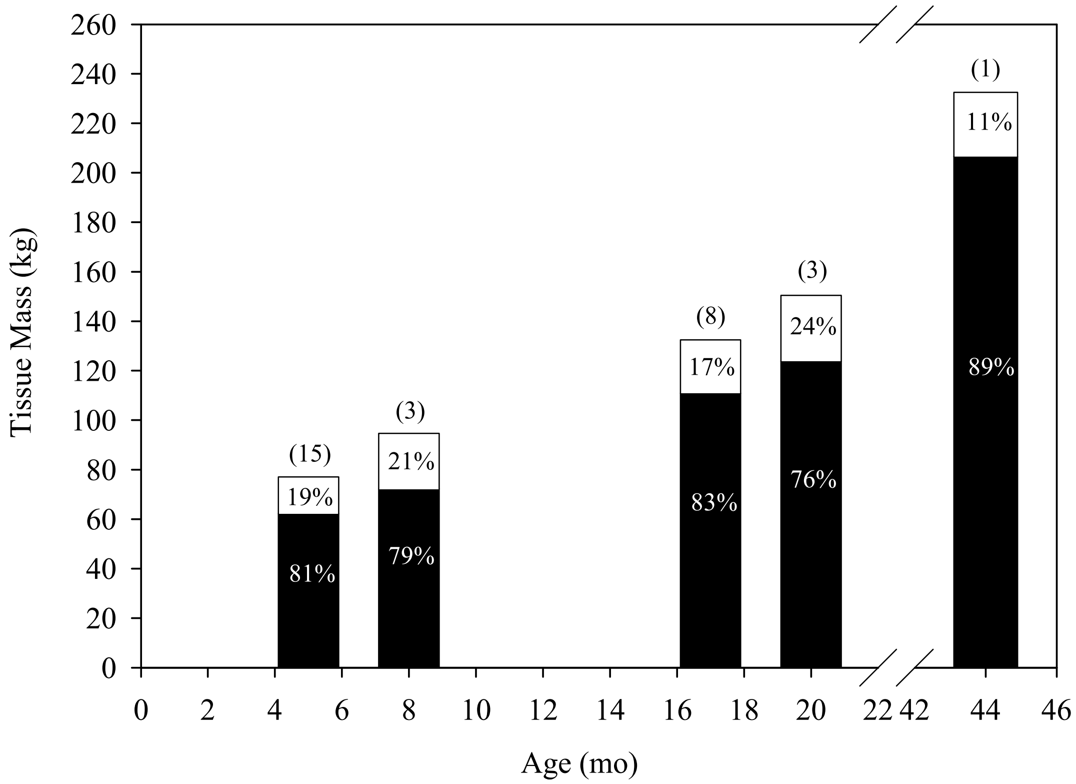

Figure 25. Mean total body mass and proportion of lean and lipid tissue for sea lions captured in Prince William Sound (PWS) as determined by total body water. Numbers in parentheses represent sample size. There were no significant differences in %TBL with age (*P*>0.05).

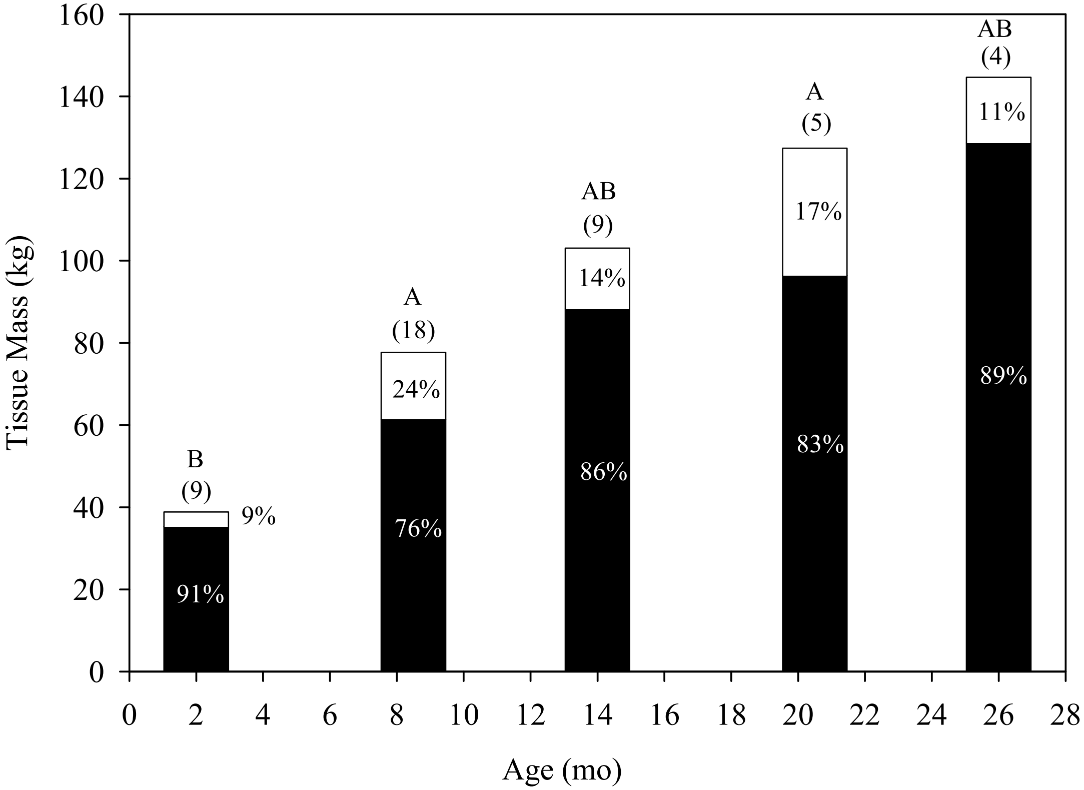

Figure 26. Mean total body mass and proportion of lean and lipid tissue for sea lions captured in Southeast Alaska (SEA) as determined by total body water. Numbers in parentheses represent sample size. Significant difference in %TBL with age are indicated by lipid levels with differing letters. Levels that share the same letter are not considered significantly different at P>0.05.

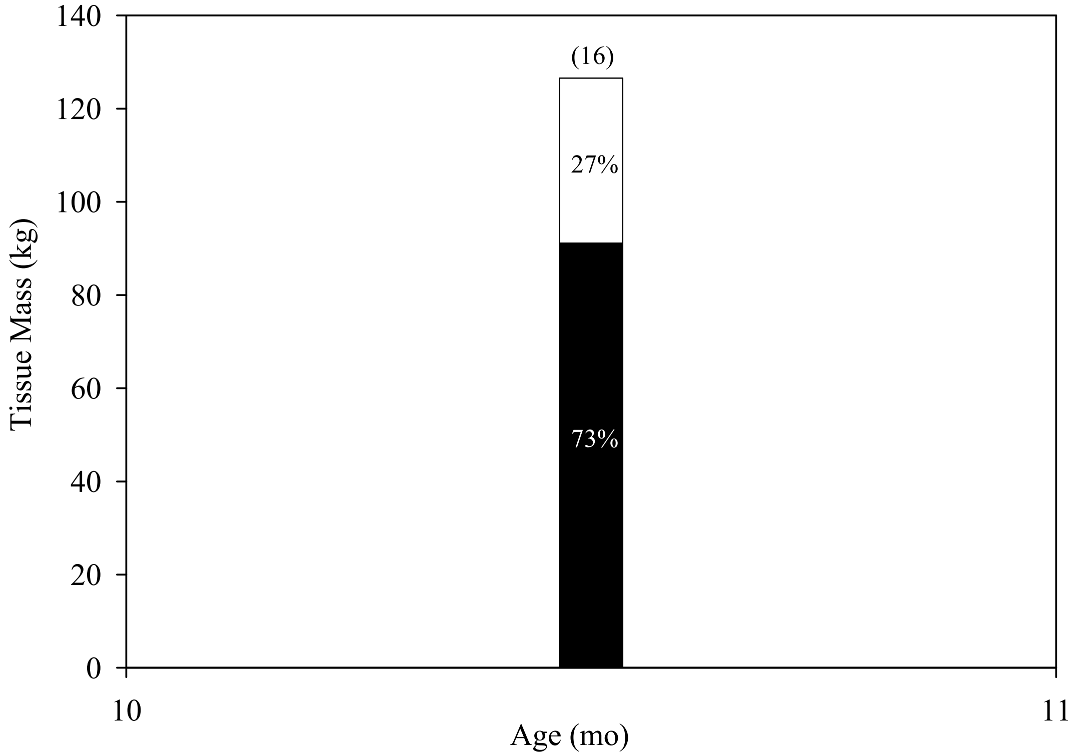

Figure 27. Mean total body mass and proportion of lean and lipid tissue for sea lions captured in Aleutian Islands (AI) as determined by total body water. Numbers in parentheses represent sample size.

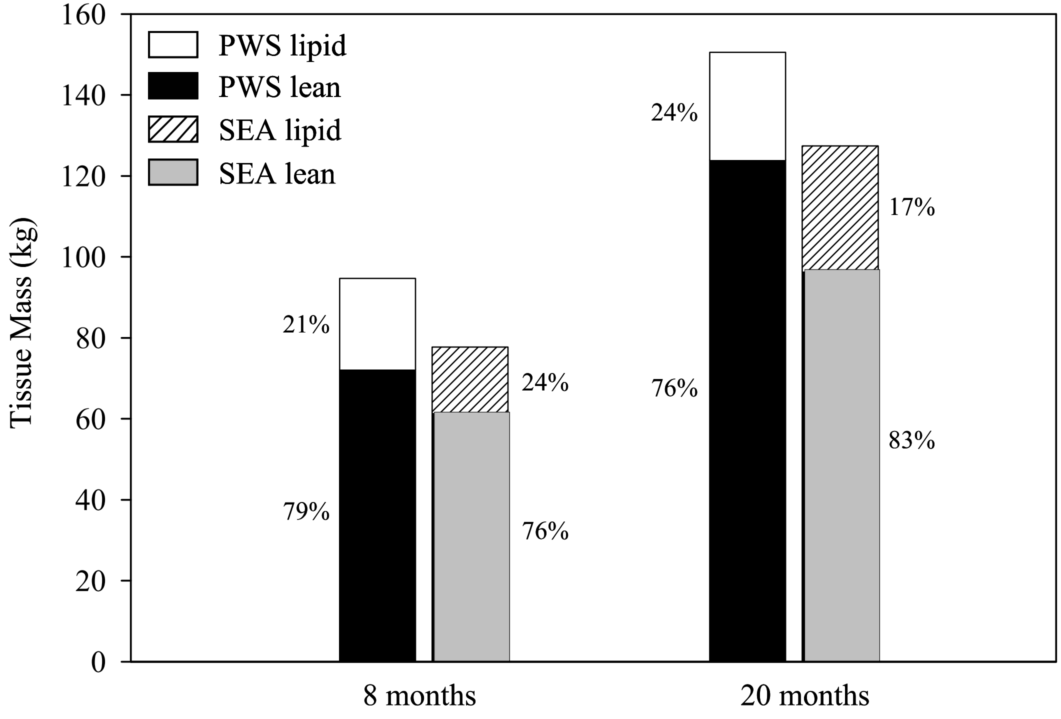

Figure 28. Mean total body mass and proportion of lean and lipid tissue for 8 and 20 month old sea lions captured in Prince William Sound (PWS), and Southeast Alaska (SEA) as determined by total body water.

Metabolic Rate

Resting metabolic rate (MJ d^{-1}) measured on a subset of sea lions (N=19) showed no significant differences (t=1.29, d.f.=18, P=0.214) between pre- and post-anesthesia measurements. Thus, anesthesia was not considered to be a factor influencing metabolic rate. Milk was discovered in the stomach of a small number of animals (N=14) ranging in age from 2-17 months, with all but one animal being younger than 12 mo. of age. No significant differences in RMR (all P>0.05) were detected between animals with and without milk according to age class and location, therefore, the potential increase in metabolism resulting from the biochemical work associated with digestion was not evident. Therefore, all sea lions, regardless of the presence of milk, were included in the remainder of analyses.

Metabolic rate was determined for free-ranging sea lions captured over varying ambient air temperatures (range -6.5 to 19.2 ºC). Multiple linear regressions incorporating combinations of total body mass, age, gender, ambient air temperature, location, %TBL, %LBM, and blubber depth for all sea lions were run to determine the most parsimonious model with respect to RMR. Three variables (total body mass, age, ambient temperature) explained most of the variation in metabolic rate (r^2=0.75, $F_{10,89}$=24.18, P<0.001). While ambient temperature had a non-significant (P=0.087) effect on metabolic rate, both age and total body mass were significant (both P<0.001). These results were confirmed by sequential tests (Type I sums of squares).

Mean RMR in air ranged from 6.7 to 36.2 MJ d^{-1} and varied with sea lion age/size. Younger sea lions generally had lower absolute metabolic rates than older

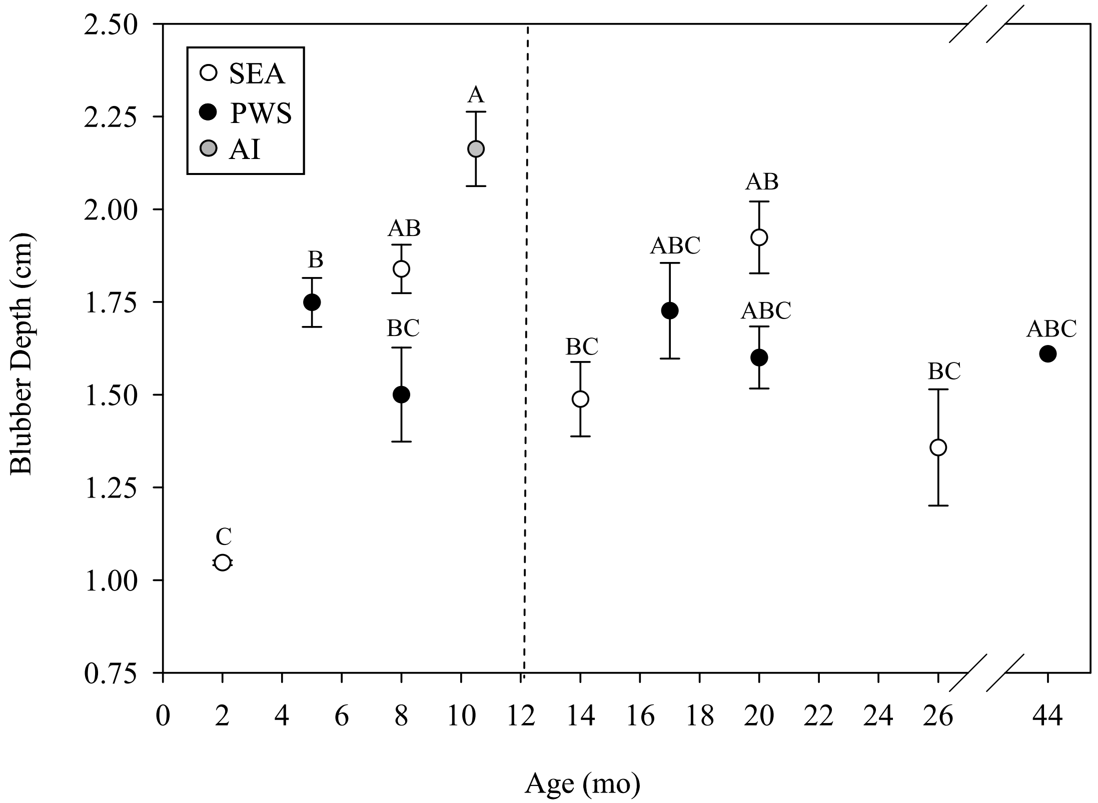

Figure 29. Mean blubber depth (cm) ± SEM as a function of age (mo) for sea lions captured in Southeast Alaska (SEA), Prince William Sound (PWS), and the Aleutian Islands (AI). Blubber depth levels with similar letters showed no significant differences, while levels with differing letters were considered significantly different at $P<0.05$.

juveniles (Fig. 30). Among the YOY, 2 mo. old animals (SEA) had the lowest mean RMR at 11.00±1.38 MJ d^{-1} (*N*=9) and 10.5 mo. old animals (AI) had the highest mean RMR at 21.19±1.07 MJ d^{-1} (*N*=15). However, mean RMR in the AI YOY was not significantly different ($P>0.05$) from mean RMR values in 5 (20.97±1.07 MJ d^{-1}, *N*=15), 8 (SEA: 16.40±0.98 MJ d^{-1}, *N*=18; PWS: 14.95±2.39 MJ d^{-1}, *N*=3), 14 (20.65±1.38 MJ d^{-1}, *N*=9), 20 (SEA: 20.15±1.85 MJ d^{-1}, *N*=5; PWS: 22.96±2.39 MJ d^{-1}, *N*=3), and 44 (29.23±4.14 MJ d^{-1}, *N*=1) mo. old animals (Fig. 30). Among juvenile sea lions, 26 mo. old animals had highest mean RMR (32.35±2.07 MJ d^{-1}, *N*=4).

In general, metabolic rates increased linearly with total body mass, lean mass and lipid mass. Resting metabolic rate was slightly more strongly correlated to LBM ($r^2=0.55$, $P<0.001$) than to total body mass ($r^2=0.52$, $P<0.001$), and weakly correlated to TBL ($r^2=0.13$, $P=0.0004$). Conversely, metabolic rates for all sea lions were not correlated with %TBL ($P=0.72$) nor %LBM ($P=0.95$). A backward stepwise regression incorporating all mass compartments (total body mass, LBM, and TBL) revealed that RMR could be predicted solely by LBM ($r^2=0.55$, $P<0.001$).

ANCOVA results suggested no significant differences in the metabolic rates of sea lions from different capture locations when ambient temperature and LBM were included as covariates in the model ($r^2=0.59$, $F_{2,85}=1.29$, $P=0.280$).

Young of the year from AI (10.5 mo., 130.2 kg, *N*=15) were compared to 17 and 20 mo. juvenile sea lions of similar size from PWS and SEA. Since there were no significant differences in total body mass between the 17 mo. PWS, 20 mo. PWS and 20

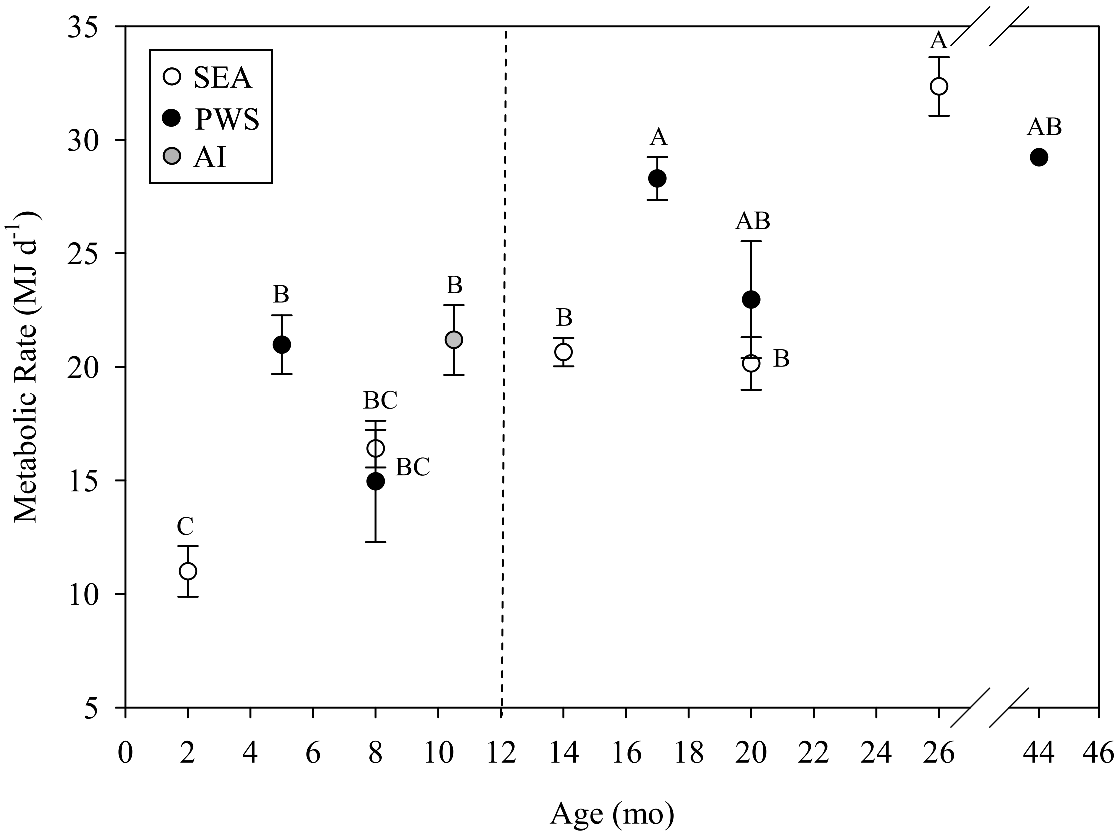

Figure 30. Mean metabolic rate (MJ d^{-1}) ± SEM as a function of age (mo) for sea lions captured in Southeast Alaska (SEA), Prince William Sound (PWS), and the Aleutian Islands (AI). Metabolic rate levels with similar letters showed no significant differences, while levels with differing letters were considered significantly different at $P<0.05$.

mo. SEA animals ($F_{2,15}$=2.19, P=0.152), juvenile sea lions were pooled (134.3 kg, N=16) so that they could be compared to the AI YOY. While there were no significant differences in total body mass between AI YOY and similarly sized juveniles (t=0.493, P=0.626), AI sea lions displayed significantly higher blubber depths (t=-3.16, P=0.004) and %TBL values (t=-3.70, P<0.001) than juvenile sea lions of the same size. ANCOVA comparisons of RMR showed no differences between AI YOY and (pooled) juveniles ($F_{1,27}$=0.087, P=0.770) with ambient temperature and LBM as covariates in the model.

Resting metabolic rate as a function of total body mass was plotted for sea lions from each capture location (Figs. 31, 32, 33). Data from SEA showed the strongest correlation (r^2=0.75, P<0.0001, Fig. 31) while data from PWS had the weakest (r^2=0.29, P=0.0022, Fig. 32).

Comparison of RMR Among Various Otariid Species

Metabolic rates measured on individual sea lions in the present study are equivalent to 1.1-4.1 (overall mean 2.2±0.1) times the predicted basal metabolic rate of similarly sized adult terrestrial mammals (Kleiber, 1975) (Fig. 34). The mean value of 2.2 times predicted RMR for all animals was similar to predicted levels when sea lions were considered by age (Table 18). Log-log regression produced the following predictive equation: RMR=1.33 $M^{0.62}$ (r^2=0.58, N=91, P<0.001), where RMR is expressed as MJ d^{-1} and M represents total body mass in kg. In-air RMRs from this

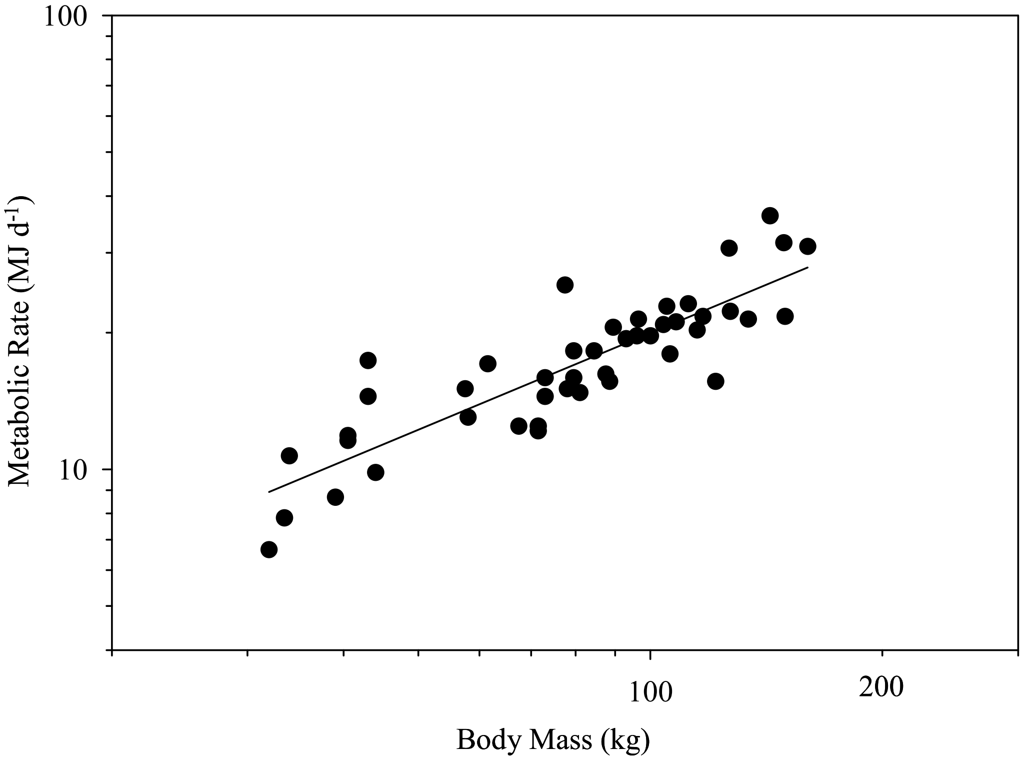

Figure 31. Resting metabolic rate (MJ d^{-1}) as a function of body mass (kg) for sea lions captured in Southeast Alaska (SEA). The regression equation is y=0.55x0.71 (r^2=0.75, P<0.0001).

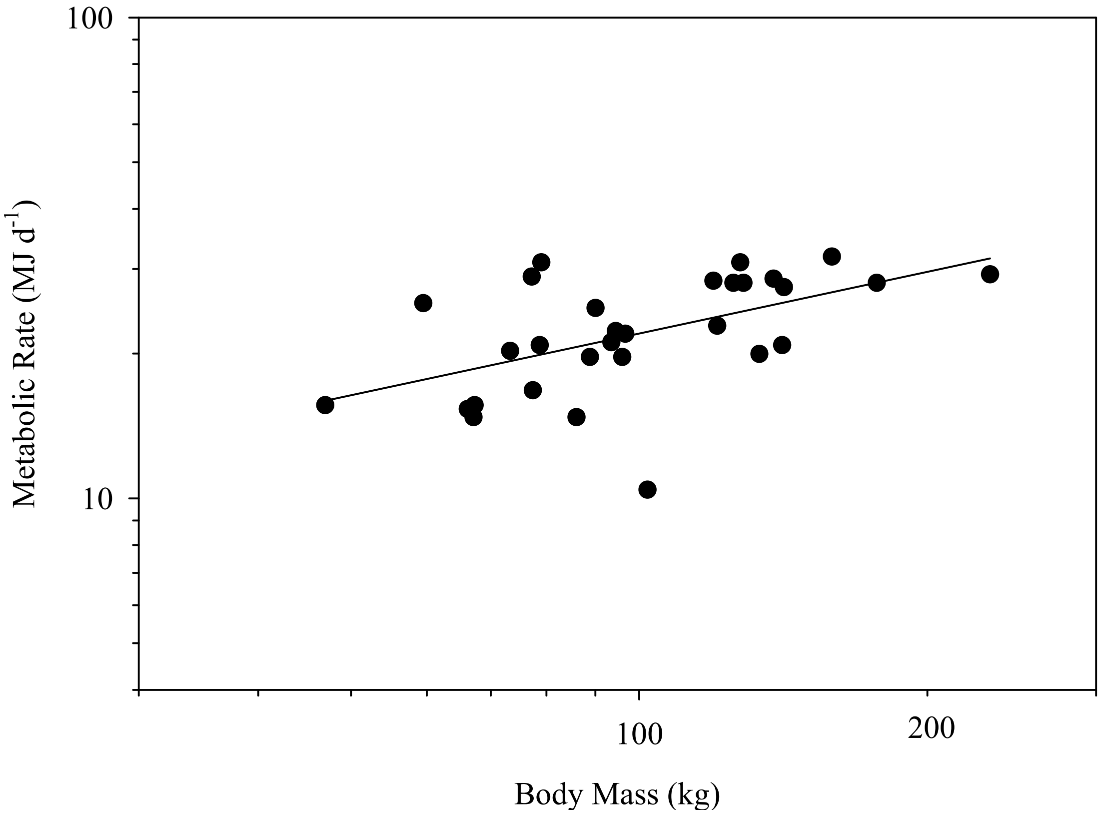

Figure 32. Resting metabolic rate (MJ d^{-1}) as a function of body mass (kg) for sea lions captured in Prince William Sound (PWS). The regression equation is y=13.51x0.43 (r^2=0.29, P=0.0022).

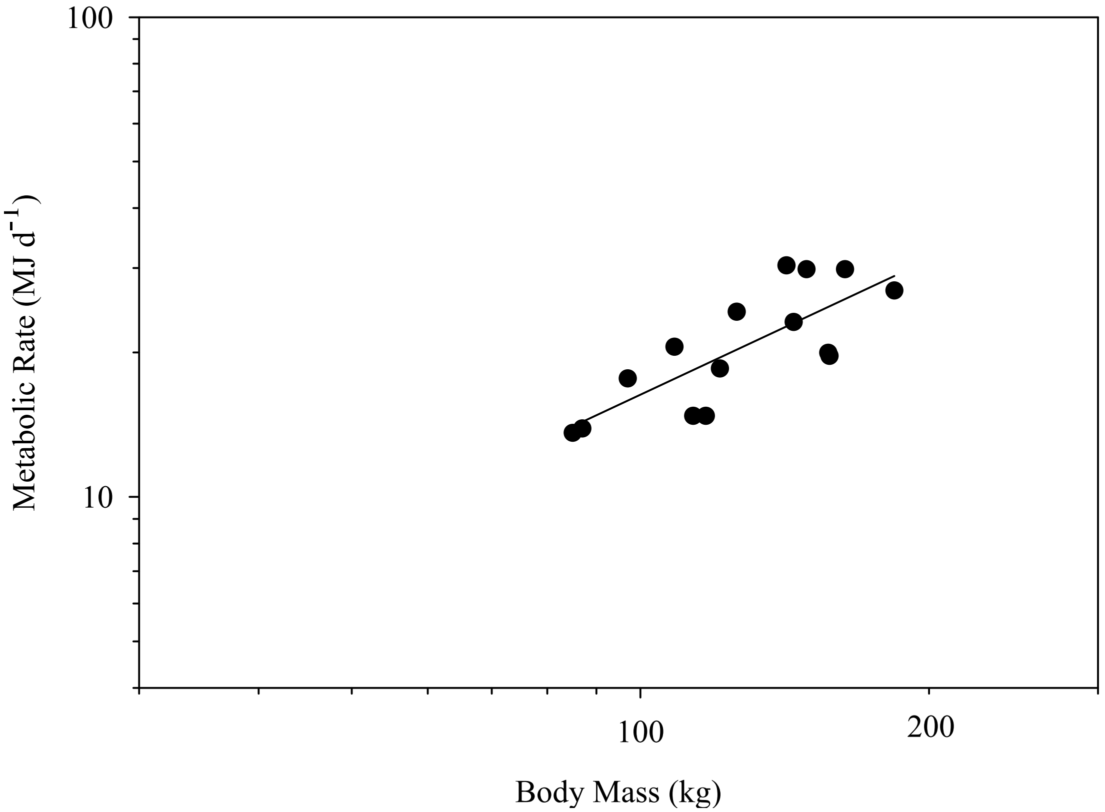

Figure 33. Resting metabolic rate (MJ d^{-1}) as a function of body mass (kg) for sea lions captured in the Aleutian Islands (AI). The regression equation is $y=0.03x0.93$ ($r^2=0.60$, $P=0.0007$).

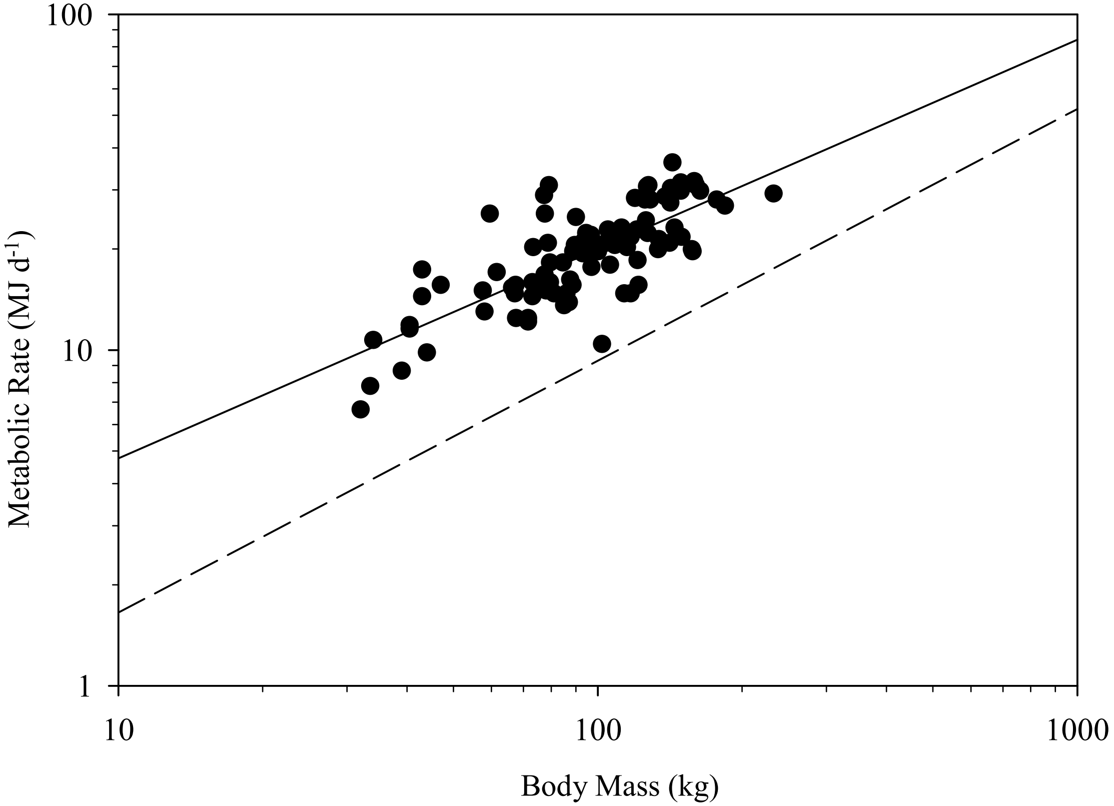

Figure 34. Log-log plot of metabolic rate (MJ d^{-1}) expressed as a function of total body mass (kg). This relationship is best described by the equation: RMR=1.33 *M*0.62 (r^2=0.58, *N*=91, *P*<0.001; solid line). RMR in young Steller sea lions is about 2.2 times the predicted levels for adult terrestrial mammals of similar size (Kleiber, 1975; dashed line).

Table 18. *A comparison between mean standard metabolic rate measured for Steller sea lions of varying age and predicted basal metabolic rate*

Age (mo)	*N*	SMR (MJ d^{-1})	BMR (MJ d^{-1})	Factor by which SMR exceeds BMR
2	9	11.0 ± 1.12	4.6	2.4
5	15	21.0 ± 1.29	7.6	2.8
8	21	16.2 ± 0.78	7.9	2.1
10.5	15	21.0 ± 1.45	11.3	1.9
14	9	20.6 ± 0.63	9.3	2.2
17	8	28.3 ± 0.95	11.5	2.5
20	8	21.2 ± 1.21	11.7	1.8
26	4	32.3 ± 1.29	12.2	2.7
44	1	29.2	17.5	1.7

Predicted basal metabolic rates (BMR) were estimated according to Kleiber, 1975.

Values for standard metabolic rate (SMR) are means ± SEM, N=number of subjects.

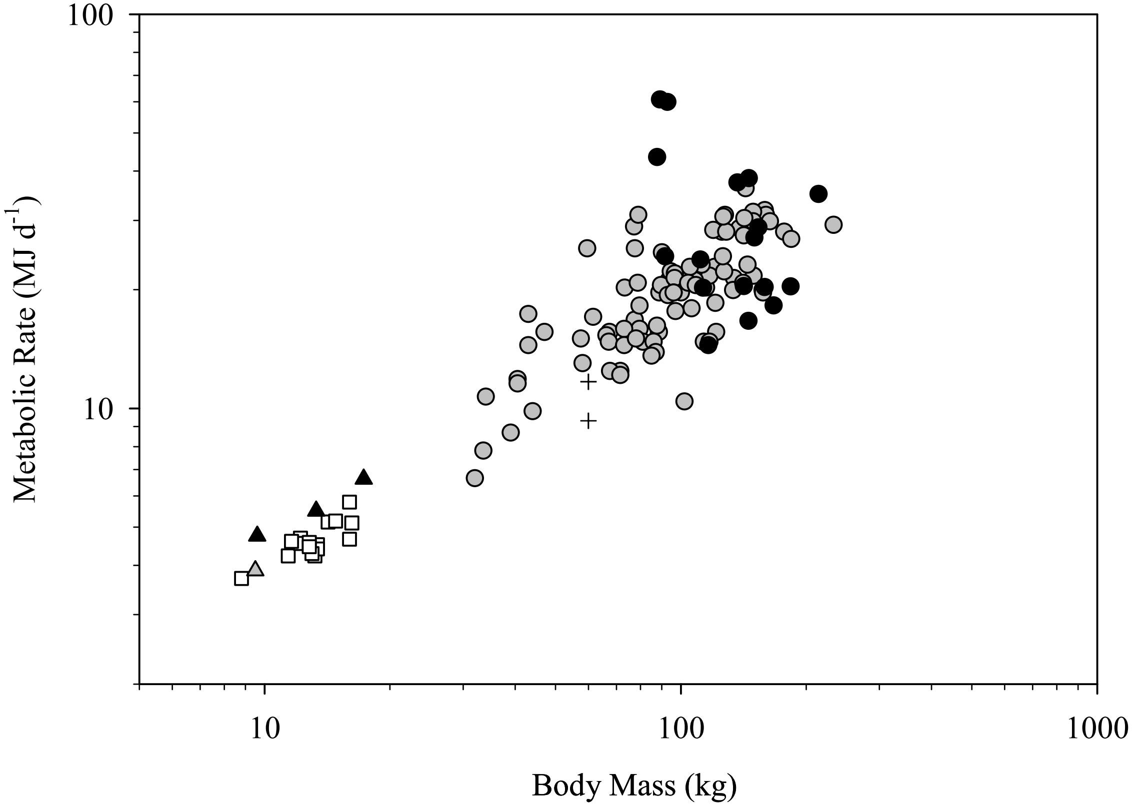

Figure 35. Log-log plot of resting metabolic rate (measured in air) as a function of body mass for a variety of otariid species: free ranging (this study; grey circles) and captive (Rosen and Trites, 1997; Rosen and Trites, 1999; Rosen and Trites, 2002; Rosen and Trites, 2003; L. Hoopes, unpublished data; black circles) Steller sea lions, California sea lions (Matsuura and Whittow, 1973; crosses), northern fur seal pups (Donohue et al., 2000; squares), Antarctic fur seal pups (Arnould et al., 2003; Rutishauser et al., 2004; black triangles), and subantarctic fur seal pups (Arnould et al., 2003; grey triangles).

study and from the literature on other otariids were complied and plotted as a function of total body mass (Fig. 35). Metabolic data taken from the literature (Matsuura and Whittow, 1973; Rosen and Trites, 1997; Rosen and Trites, 1999; Donohue et al., 2000; Rosen and Trites, 2002a; Arnould et al., 2003; Rosen and Trites, 2003; Rutishauser et al., 2004) were converted to like units (MJ d^{-1}, assuming 1 l O_2 = 20.1 kJ) and both individual and mean values were included.

For comparison to other studies, RMR was expressed mass-specifically (MJ d^{-1} kg^{-1}). Mass-specific RMR declined with increasing sea lion age (size), with younger sea lions (2, 5 mo.) having significantly higher RMRs than older animals (Fig. 36). Log-log regression produced the following relationship: $RMR=1.41\ M^{-0.38}$ ($r^2=0.34$, $F_{1,89}=46.54$, $P<0.0001$). ANCOVA results failed to detect difference in mass-specific RMR with regard to location when ambient temperature, total body mass and LBM were covariates in the model ($F_{2,84}=1.63$, $P=0.201$).

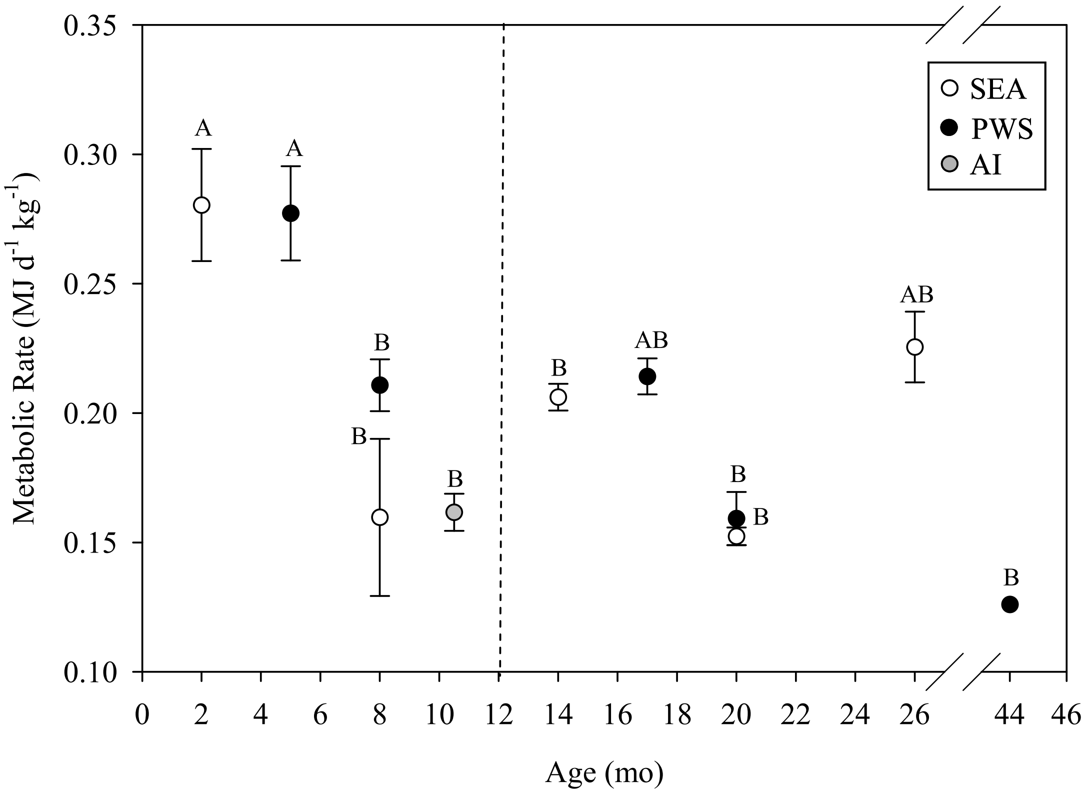

Figure 36. Mean mass-specific metabolic rate (MJ d^{-1} kg^{-1}) ± SEM as a function of age (mo) for sea lions captured in Southeast Alaska (SEA), Prince William Sound (PWS), and the Aleutian Islands (AI). Metabolic rate levels with similar letters showed no significant differences, while levels with differing letters were considered significantly different at $P<0.05$.

Discussion

Male YOY sea lions were 15 to 27% heavier than female sea lions (depending on age). This difference in size was less pronounced in juvenile male sea lions, which were only 2 to 10% heavier than juvenile females. Overall, male sea lions were greater in length (7.5%) and neck (8.8%), chest (8.3%), axillary (6.9%), mid-trunk (8.6%), and hip (9.1%) girth than female sea lions. Sexual dimorphism is a characteristic of all otariids and its influence is evident early in development where, maternal investment during gestation, average birth mass, mass at weaning, and growth rates of male pups are generally greater than those of females (Kovacs and Lavigne, 1992; Trites, 1992). This disparity in size is evident in neonate Steller sea lion pups (Brandon et al., 2005), and several other otariids young, including, Antarctic fur seals (Goldsworthy, 1995; Guinet et al., 1999), California sea lions (Ono and Boness, 1996), northern fur seals (Boltnev et al., 1998; Boltnev and York, 2001), South African/Australian fur seals (Arnould and Hindell, 2002; Gamel et al., 2005), South American sea lions (Lima and Páez, 1995), and southern sea lions (Cappozzo et al., 1991). The more similar body masses of male and female juvenile sea lions compared to YOY may be indicative of weaning status, where juveniles may no longer rely on milk but instead on foraging and diving ability amongst a patchy prey resource. In other words, females may have a chance to "narrow the gap" in body mass in an environment of equally unpredictable prey availability.

Size differences were also evident between capture regions. Sea lions from SEA were smaller in total body mass at every age compared with sea lions from PWS and AI. This pattern of increasing body mass from east to west is indicative of larger scale trends across a greater variety of age categories for Steller sea lions in Alaska (Merrick et al., 1995; Fadely et al., 2004). Additionally, AI animals were heavier, longer, and fatter (body mass, axillary girth, blubber depth, %TBL) than YOY from other regions. While a direct comparison of size based on age between all three regions was not feasible, other studies have noted that both neonate and juvenile sea lions from the AI were larger and of a higher body fat content than similarly aged animals in other regions of Alaska (Merrick et al., 1995; Fadely et al., 2004; Brandon et al., 2005).

Although sample sizes were small, no differences in %TBW or %TBL were evident between male and female Steller sea lions in this study. Brandon et al. (2005) similarly reported no difference in %TBW and %TBL between male and female Steller sea lion pups (1-5 days of age). Average %TBL in 2 mo. old sea lions was low (9% of total body mass), only 3% higher than levels measured at birth (Brandon et al., 2005), reflecting the fact that Steller sea lion pups are born with small energy reserves and exposure to short-term fasting during periods of maternal foraging results in slower gains in lipid mass compared to phocid seals.

Lean mass in Steller sea lions appears to be the primary contributor to total metabolic rate, with fat mass contributing little to this value. The strong correlation between both total body mass (r^2=0.55) and lean body mass (r^2=0.52) with metabolic rate and the lack of a relationship between fat mass (r^2=0.13) and metabolic rate support this conclusion. Other studies with pinnipeds have determined that changes in metabolism in juvenile animals were strongly correlated with increases in lean mass instead of fat mass (Rea and Costa, 1992; Aarseth et al., 1999; Donohue et al., 2000). It has been suggested that lipid mass could indirectly influence metabolism if decreases in blubber stores impact the thermoregulatory capacity of the animal (Rea and Costa, 1992). Lean mass has also been correlated to metabolic rate in humans (Cunningham, 1980; Roza and Shizgal, 1984; Elliot et al., 1989; Forbes and Brown, 1989) and lean body mass metabolic rates were similar in obese and non-obese individuals, suggesting that additional fat mass did not increase metabolic rate (Felig et al., 1983; Ravussin et al., 1986; Segal et al., 1989).

Average %TBL increased with age for YOY sea lions from SEA and PWS, although the latter trend was non-significant. Juvenile sea lions however, displayed fluctuating, but non-significant %TBL levels with age. As a percentage of body mass, TBL ranged from 2.8 to 35.3% in YOY and from 6.9 to 32.7% in juveniles and was highly variable within and between age categories and locations. Young Antarctic fur seals decrease their lipid stores (and increase lean tissue) from 31% of total body mass as 2-3 mo. old pups to 15% of total mass as weaned yearlings (Rutishauser et al., 2004). A similar decrease in %TBL would be expected at independence in Steller sea lions; however, a clear decrease in %TBL (increase in %LBM) was not evident in the data from this study likely due to variation in weaning time in this small sample size. Steller sea lions typically wean at one year of age, but juveniles have been observed suckling up to three years (Pitcher and Calkins, 1981; Trites and Porter, 2002). Similarly, it would be expected that sea lions would be leaner post-weaning due to the switch in energy intake (lipid to protein) and the increased energetic expenditure necessary to forage independently. In the present study, one juvenile sea lion (17 mo. old) had milk in their stomach. This animal's %TBL values were significantly higher ($P<0.05$, 23.1% TBL) than mean levels measured in the other juveniles (16.6% TBL). While differences in %LBM by age were evident in SEA animals, it was not feasible to determine weaning status in this small dataset.

Steller sea lions are considered to be relatively 'lean' animals, with thin blubber layers (Pitcher et al., 2000). Blubber depths measured in this study were comparable to depths measured in captive Steller sea lion juveniles (Chapter II), but were considerably thinner than measured in phocid seals (e.g., Gales and Burton, 1987; Slip et al., 1992; Rosen and Renouf, 1997). Additionally, for their large size, 2 mo. old Steller sea lions had much lower mean %TBL (9%) compared to smaller species such as northern fur seals (15%, 1 mo. of age, Donohue et al., 2000), Antarctic and subantarctic fur seals (22-31%, 2-3 mo. of age, Arnould et al., 2003; Rutishauser et al., 2004) and California sea lions (1.5-19.3%, 1-3 mo. of age, Oftedal et al., 1987). This difference is largely a reflection of life history strategy since northern fur seals and Antarctic fur seals typically wean quicker (4 months, Arnould et al., 2003) than Steller sea lions.

While differences in blubber depth were not evident between sea lions from the stable and declining populations, animals from SEA were considerably smaller and had slightly less lean tissue mass than sea lions from other regions. While size differences could be attributed to genetic differences in eastern and western stocks (Bickham et al., 1996), it is more likely that increasing numbers of sea lions in SEA (Calkins et al. 1999) are imposing density-dependent constraints on growth and ultimately body size of juvenile sea lions. As animals approach their carrying capacity in a given environment there is greater competition for food, resulting in smaller body size. Pups in SEA have been shown to have slower growth rates and be smaller in body mass than animals in western regions (Merrick et al., 1995; Rea et al., 1998). Population density pressures are evident in other age classes of sea lions. Female Steller sea lions in SEA undertake longer foraging trips and travel further to find resources (Milette and Trites, 2003). As a result, pups in SEA have a longer period of dependence on their mothers, as a higher proportion of young are nursed into their second year compared to animals in western areas. Finally, higher parasite loads (e.g., hookworms) in SEA pups is further evidence of overcrowding (Beckmen et al., 2005; Hughes et al., 2006). Conversely, decreased competition for food due to lower population density could result in increased body size. Increases in body size in northern fur seals have been observed during periods of population decline (Trites and Bigg, 1992). This could explain the larger size attained by AI YOY in this study given that rookeries throughout the Aleutian Island chain are sparsely populated compared to areas in SEA.

Given that AI YOY appear to be larger in both total mass and lipid mass than their cohorts from other regions, and even some juvenile animals, it was no surprise that their RMR was similar to other juvenile animals. However, given the larger body size of these YOY compared to similar aged animals in PWS and SEA, it would be expected that the AI animals would have lower mass specific RMRs compared to their cohorts.

The finding that Steller sea lion juveniles have RMRs on average two times, but up to four times, the level predicted by Kleiber (1975) for adult mammals of similar body size is consistent with the elevated metabolic rates of juveniles. Studies with other young otariids show similar elevations in metabolic rate (Thompson et al., 1987; Donohue et al., 2000; Rutishauser et al., 2004). However, sea lions in this study were also influenced by temperature and this could further inflate already elevated RMR levels. To account for body size, energy measurements are often compared on the basis of metabolic size. RMR of sea lions in this study scaled to $M^{0.61}$, giving a scaling coefficient similar to the value of 0.66 reported in intraspecific studies of metabolic rate (Heusner, 1982).

Under short-term conditions of deceased energy intake (nutritional stress) sea lions would be expected to increase foraging efforts since further expenditure of energy is likely off-set by a reasonable expectation of success during the forage. However, when faced with predictable (e.g., seasonal availability) or large-scale shortages (e.g., El Niño) of energy intake, physiological adaptations that would limit energy expenditures include a reduction in activity, thermoregulation, and metabolism, also known as metabolic depression. These strategies serve to increase survival time by limiting the loss of body mass. Animals undergoing periods of reduced energy intake would also be expected to be in ‘poorer’ condition than animals with continuous access to food. These animals should have thinner blubber and lipid stores due to catabolism of these tissues for fuel. If sea lions from the western declining population are experiencing either short-term or chronic periods of reduced food intake, we should see evidence of this in dive profiles, measures of body composition, and studies of RMR.

Evidence of population differences in diving performance between eastern and western stocks of juvenile Steller sea lions is not clear cut. Pitcher et al. (2005) found maximum dive depths, dive durations, and dive rates in animals from the eastern stock, while sea lions from the western population spent greater amounts of time at sea. They concluded that these differences may relate to differences in bathymetry and prey distribution in the two areas, and cannot be interpreted as greater foraging effort. Loughlin et al. (2003) reported deeper dives in yearlings Steller sea lions from Washington (eastern stock) compared with animals from the Gulf of Alaska and the Aleutian Islands (western stock) but also pointed at differences in prey habitat as a likely explanation. Finally, trip duration was longer for juvenile Steller sea lions in the western population compared to the eastern population, suggesting either earlier weaning or greater difficulty in locating sufficient prey in the west (Raum-Suryan et al., 2004)

Evidence of metabolic depression has been documented during periods of experimental and natural fasting in both marine (Heath et al., 1977; Ashwell-Erickson and Elsner, 1981; Gallivan and Best, 1986; Markussen et al., 1992; Rea and Costa, 1992; Nordøy et al., 1993; Boily and Lavigne, 1995; Rosen and Trites, 1999) and terrestrial (Grande et al., 1958; Harlow, 1981; Heldmaier et al., 2004; Reidy and Weber, 2004) mammals. Rosen and Trites (1999) showed that when captive juvenile Steller sea lions were switched from a high to low energy diet for 14 days a resulting depression (16-26%) in RMR was observed. However, shorter periods of reduced energy intake (8-9 d) in the same animals did not necessarily elicit decreases in metabolism despite evidence of mass loss and varied seasonally (Kumagai et al., 2006), suggesting the relationship between metabolism and energy intake is more complex. Few studies have sought to measure metabolic depression as a response to prey limitations in free-ranging marine mammals and results from this study do not provide evidence of metabolic depression between sea lions from differing populations. This suggests that either during the periods of measurement sea lions were getting adequate energy intake or that nutritional stress in not the cause of continuing declines in the western stock of Steller sea lions.

In a related study, Myers et al. (2006) found decreasing concentrations of thyroid hormones (total T_3, T_4 and free T_3, T_4) with Steller sea lion maturity. These results support RMR measurements in the current study which show elevations in metabolism in younger sea lions. One of the roles of thyroid hormones is to regulate energy metabolism and levels correspond directly with energy intake. Myers et al. (2006) also noted differences in thyroid concentrations by region. They found that pups from SEA had the lowest concentration of thyroid hormones compared to western regions of Alaska and Russia and suggested that elevated levels in the west were suggestive of increased metabolism in order to maintain thermal homeostasis. However, in the present study, no differences in metabolic rate were evident between sea lions from the three capture regions. This disparity is likely due to differences in age and ambient temperature (Myers et al., 2006) which certainly influenced RMR in the present study.

Extremes in air temperatures had an obvious influence on metabolism in young sea lions. Resting metabolic rate of 5 and 17 mo. old sea lions from PWS were measured at an average air temperature of -1.8 ºC, while RMRs of 2, 14, and 26 mo. old sea lions from SEA were measured in much warmer air temperatures (mean 15.4 ºC, range 12 to 19 ºC). Resting metabolic rate plotted as function of air temperature suggests that elevated RMRs at the temperature extremes may suggest that young sea lions were outside their thermoneutral zone in air (Fig. 37). Little is known of the thermal limits in air of many otariids species, including Steller sea lions. Thompson et al. (1987) measured RMR in 3-5 week old California sea lion pups at ambient air temperature ranging from 16 to 26 ºC and found some individuals near hypothermic at ambient air temperatures below 19 ºC. Alternatively, it appears that yearling California sea lions are unable to maintain thermal equilibrium at warmer air temperatures between 28 and 36 ºC (Whittow et al., 1972; South et al., 1976). While it is intuitive that the TNZ would expand as animals mature due to a decrease in the surface area to volume ratio that comes with larger size and freedom from the need to allocate energy into growth, few studies have examined how TNZ changes with ontogeny. Hansen and Lavigne (1997a) measured an 11 ºC decrease in the lower critical temperature and a 3.5 ºC increase in the upper critical temperature of 3 yr old harbor seals compared to the same animals as yearlings.

Because temperature was such an important component to predicting RMR, this relationship was explored further to see if inferences of thermal limits could be made for free-ranging Steller sea lions. Plotting RMR as a function of the ambient temperatures under which the measurements were made (regardless of sea lion age) reveals that the relationship is best fit by a polynomial curve (r^2=0.22, $F_{2,89}$=12.92, P<0.0001, Fig. 37). Using linear and nonlinear regression techniques described by Nickerson et al. (1989) and Nickerson (1991), a two-phase model was fit to the data to estimate a lower critical temperature (T_{lc}), below which sea lions would be expected to increase RMR to compensate for increased heat losses on land. A three-phase model was not possible to

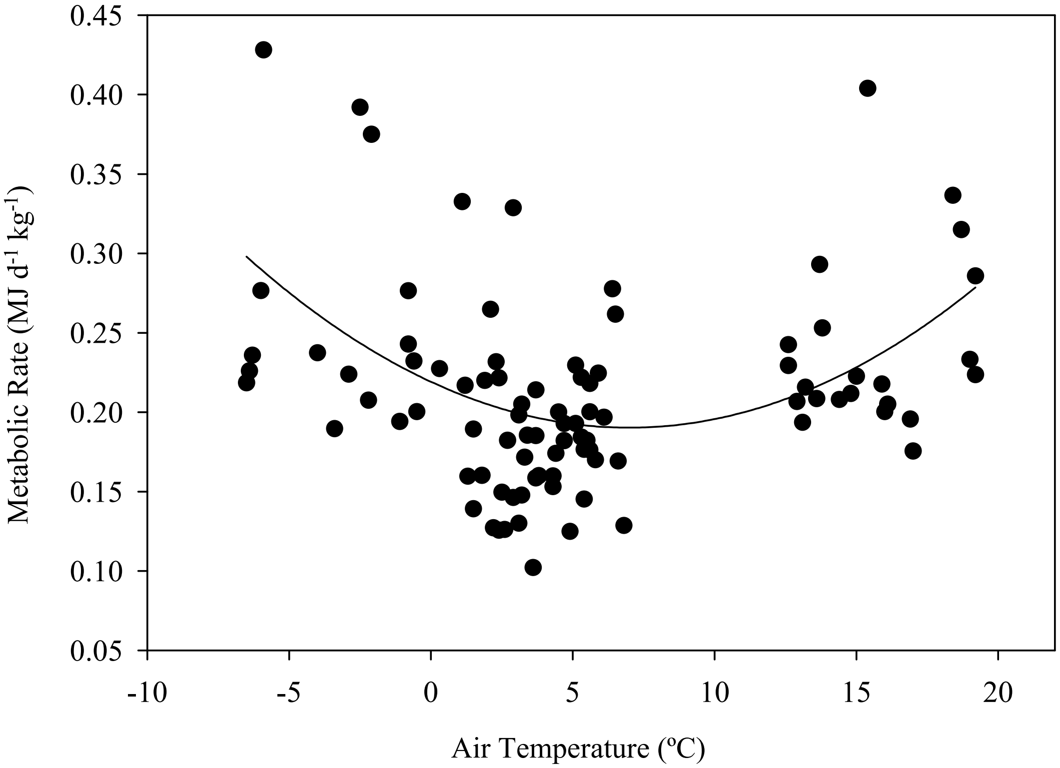

Figure 37. Metabolic rate (expressed mass specifically, MJ d^{-1} kg^{-1}) as a function of ambient air temperature (ºC) for all sea lions captured in the study. Relationship is best expressed as a polynomial curve (r^2=0.23, $F_{2,87}$=12.92, P<0.0001). Note the gap in measurements between 7 and 12 ºC.

fit (providing also an upper critical temperature) due to the lack of RMR measurements at air temperatures between 6 and 12 ºC.

The best fit model included mass specific RMRs (MJ d^{-1} kg^{-1}) and ambient air temperatures less than 10 ºC. The fitted model produced an inflection point of 4.1 ºC, with data points at colder temperatures having a significant negative slope ($P<0.0001$), while data points at warmer temperatures had a slightly positive, but non-significant (from zero) slope ($P=0.356$) (Fig. 38). When the data set was split according to age class, the inflection point for yearlings was similar at 4.3 ºC. The smaller juvenile data set failed to converge upon an inflection point (air temperature) due to the absence of RMR measurements at a sufficient range of temperatures.

This T_{lc} of approximately 4 ºC in air suggests that yearling and possibly juvenile sea lions may be living outside their TNZ during colder months. Average winter air temperatures in the Gulf of Alaska can range as cold as 0 to -15 ºC (Nov-Feb, National Ocean and Atmospheric Association, NOAA). Young sea lions exposed to these air temperatures would have to increase heat production in order to maintain thermal homeostasis. This becomes especially significant during times of reduced food intake, where sea lions would have to rely on lipid reserves for fuel, further compromising thermoregulatory abilities. Models predicting the environmental impacts on thermal tolerances in Steller sea lions suggests that solar radiation plays a large part in sea lions maintaining thermoneutrality (Roscow, 2001). Under cloudy conditions or at night modeled heat losses suggested that Steller sea lions experienced a thermoregulatory cost, and small animals with thin blubber depths incurred the greatest costs. This is supported

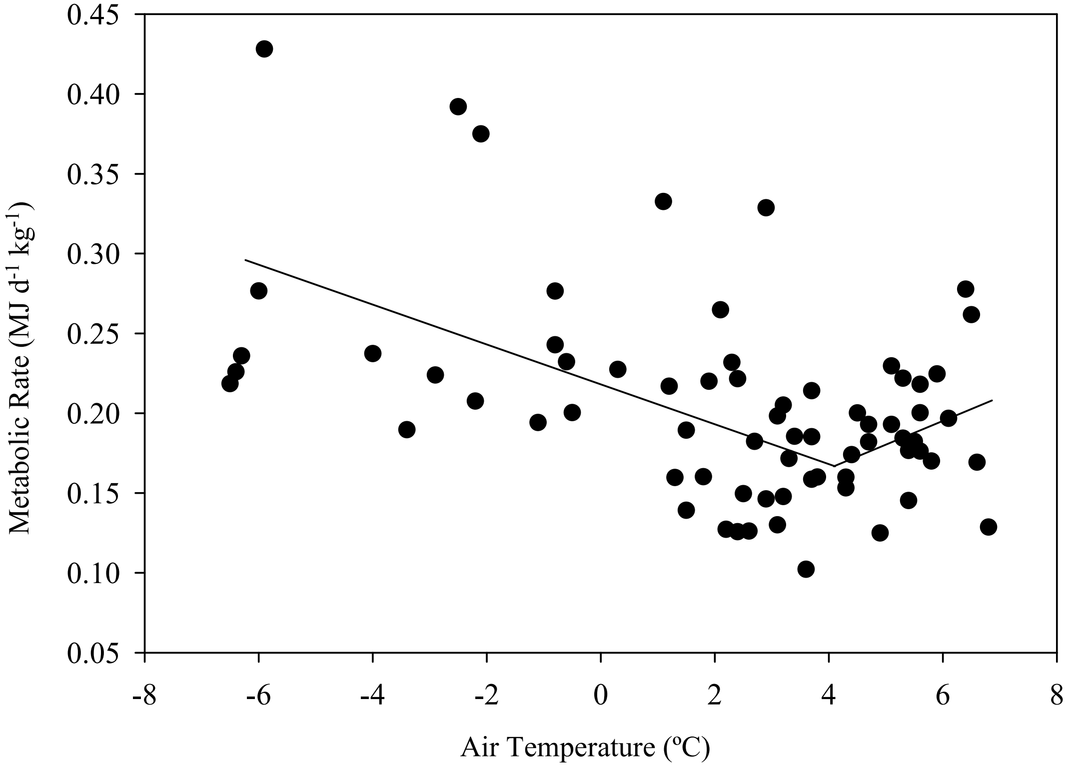

Figure 38. Metabolic rate (expressed mass specifically, MJ d^{-1} kg^{-1}) as a function of air temperatures (ºC) below 10 ºC for all sea lions captured in the study. Results of two-phase nonlinear regression techniques (Nickerson et al. 1989, Nickerson 1991) suggest a lower critical temperature (T_{lc}) of 4.1 ºC.

by limited field data suggesting that at air temperatures of 10-15 ºC Steller sea lions tend to stay dry and cover their flippers to expose minimal surface area to the air (Gentry 1973).

While evidence of metabolic depression was not evident in sea lions from the western population, it is unclear whether or not the metabolic response might be greater at times of year when sea lions are more likely to experience decreased energy intakes (e.g., winter) or in conjunction with life history events that are energetically expensive (e.g. molting). Although sampling trips were conducted at various times of year, small sample sizes and non-similar age classes between locations prevented comparisons of RMR by season. While food restriction experiments with arctic foxes revealed no effect of season on the degree of metabolic depression (Fuglei and Oritsland 1999), short-term reductions in energy intake over the course of a year revealed seasonally dependent changes in metabolism, body composition and body mass in captive Steller sea lions (Kumagai et al., 2006). Sea lions responded differently to reduced intakes depending on season and differences in body mass loss based on diet type (high lipid or low lipid) suggested that diet composition may pose an additional impact during certain times of the year (Kumagai et al., 2006). Steller sea lions undergo their annual molt in the fall (Aug/Sept), when it is predicted that metabolism would be elevated due to new hair growth and/or thermoregulatory costs. Metabolic studies in otariids have shown that pups have elevated metabolic rates during molt (Donohue et al., 2000; Beauplet et al., 2003), but no studies have been done with juveniles or adults. Sampling of sea lions during the molt was avoided for this study to avoid potential confounding effects on resting metabolism, however, future studies looking at seasonal changes in metabolism would be valuable to making better predictions about energetic requirements.

This is the first study to measure RMR in free-ranging juvenile Steller sea lions and subsequently identify a T_{lc} for animals in air. Understanding basic physiological parameters such as metabolism have important implications to understanding diving limitations and capabilities, maintenance requirements for energy budgets, thermoregulatory constraints, and bioenergetics modeling of complicated physiological systems.

CHAPTER V

EVALUATION OF A THERMAL MODEL FOR JUVENILE STELLER SEA LIONS

Introduction

Mathematical models can often provide insight into complex biological and/or physiological systems that may be impossible to measure and integrate in a natural setting. Thermoregulation is an example of such a complex physiological control system, that despite the complexity, can be described by simple thermodynamic equations linked together in a quantitative form (e.g., Birkebak et al., 1966; e.g., Luecke et al., 1975). The guiding principle in modeling thermal capabilities in endotherms attempts to balance heat losses and heat gains based on exchanges of energy between and animal and its environment. Under steady state conditions heat storage of an animal is zero and body temperature remains constant. The standard heat balance equation balances metabolic heat production, evaporative heat losses, and heat exchange through radiative, convective, and conductive means (Schmidt-Nielsen, 1983). The extreme conditions that an animal can withstand are determined by balancing this equation with body temperature, metabolic rate, and evaporative water loss within sustainable physiological limits. Thermal modeling in this fashion allows for predictions of heat balance and energy production under differing environmental conditions in species where it can be difficult to make experimental measurements (Luecke et al., 1975).

The dual aquatic and terrestrial existence of pinnipeds exposes these animals to potentially severe thermal challenges from their environment, especially those species living at high latitudes. Pinnipeds must venture into the water to forage in a medium which promotes heat loss during swimming and diving (Nadel, 1984), yet still retain the capacity to dissipate excess heat and avoid hyperthermia on land or when active (e.g., Hart and Irving, 1959). Maintaining thermal balance is an essential feature in the life of pinnipeds and a wide variety of adaptations have evolved to control heat loss to the environment (Irving, 1969). Pinnipeds employ both morphological (fur, highly vascularized subcutaneous blubber layer, heat exchangers) and behavioral (e.g., huddling) mechanisms to mediate heat exchange with their environment. Despite a large body of literature examining thermoregulation in pinnipeds (e.g., Tarasoff and Fisher, 1970; Odell, 1974; Heath et al., 1977; Costa and Kooyman, 1982; Pierotti and Pierotti, 1983; Thompson et al., 1987; Whittow, 1987; Watts et al., 1993; Andrews et al., 1994; Hansen et al., 1995; Boily and Lavigne, 1996; Hind and Gurney, 1997; Boyd, 2000; Donohue et al., 2000; Noren, 2002; Mauck et al., 2003; Willis et al., 2005), it is not clear, in spite of these adaptations, whether pinnipeds need to increase their metabolic rates to maintain body temperature under certain environmental conditions.

Steller sea lions are the largest of the otariid (eared) seals and are distributed throughout the North Pacific basin from California north through the Gulf of Alaska, the Aleutian Islands and the Bering Sea, and extending south into the waters surrounding Japan. Like other otariids, Steller sea lions have relatively thin blubber layers compared to similarly sized phocid seals (e.g., Bryden and Molyneux, 1978, L. Hoopes, unpublished data). Juvenile sea lions, by virtue of their smaller size and immature physiology, likely experience greater challenges to thermoregulation than adults.

Reductions in body condition (blubber thickness) arising from nutritional deficiencies would certainly impact the thermoregulatory abilities of young Steller sea lions, ultimately compromising survival. While evidence of the impact of nutritional stress in Steller sea lions (e.g., change in survival rates, body size and/or condition) has been sought to explain the continued declines in western Alaskan populations, supporting evidence is inconclusive and sometimes contradictory (Castellini, 1993; Merrick et al., 1997; Rea et al., 1998; Milette and Trites, 2003; Rea et al., 2003). Exploring the thermal constraints placed on juvenile Steller sea lions by their changing environment through the application of an energetics model may provide insight to the physiological challenges these young animals face, and ultimately to understanding the potential for population decline.

Thermoregulation is often neglected in bioenergetics models for pinnipeds by assuming animals are always operating within their thermal neutral zone (TNZ) (Ashwell-Erickson and Elsner, 1981; Olesiuk, 1993; Mohn and Bowen, 1996) or by lumping thermal costs into activity costs (Winship et al., 2002). Roscow (2001) created a series of individual-based predictive models for assessing thermal constraints of Steller sea lions across both terrestrial and aquatic habitats. The model inputs include parameters such as body mass, blubber depth, temperature (air or water), velocity (air or water), and examines the interactions between the heat generated from locomotion and digestion with the heat needed for thermoregulation. The model predicted that Steller sea lions require additional energy to maintain thermal balance in water, unless they are moving and generating additional heat from locomotion. Steller sea lions were within their TNZ when resting in air during clear sunny days as a result of direct solar radiation. However, at night, under cloudy conditions, or at extremely cold air temperatures sea lions needed to generate extra heat for thermoregulation. These additional expenditures could be partially or completely offset if sea lions could use the heat generated from digestion. For all conditions tested, smaller sea lions of those with poor body condition (1 cm blubber thickness) incurred the greatest thermal costs (Roscow 2001).

While the model is the first of its kind and is useful for exploring the physical and environmental conditions under which sea lions experience thermal imbalance, the predictive power of the model is limited by the reliability of data used to construct it. At the time it was created, little information on basic physiological parameters (e.g., resting metabolic rate, skin temperature) was available for Steller sea lions and data for California sea lions or other phocids were used instead. Since the model was completed, several studies on Steller sea lions (e.g., Rosen and Trites, 2002b; Willis et al., 2005, current study) have been conducted which have the potential to improve the predictive power of the model. Thus, the objectives of this exercise were to (1) compare predicted metabolic expenditures to actual measured expenditures presented in Chapters II and IV to identify how well the model predicts the thermal reality of juvenile Steller sea lions in water and air, (2) modify model parameters based on whether the model underestimates or overestimates heat losses, and finally (3) re-run the model and identify parameters which limit the predictive power of the model.

Materials and Methods

Comparisons to Experimental Data

Parameter inputs were modified for Roscow's (2001) thermal bioenergetics model so that predicted thermoregulatory costs could be compared to available experimental data from captive and free-ranging juvenile Steller sea lions. For in-water comparisons, data from captive Steller sea lions that had recovered their body mass were used (see Chapter II). For in-air measurements, data for free-ranging Steller sea lions within their TNZ (4 to 10 ºC, see Chapter IV) were utilized. In order to better predict the thermal constraints on young Steller sea lions, mass estimates for the model were set to 50, 75, 100, 125, 150 and 175 kg, comparable to the range of masses for sea lions in the experimental datasets. Water temperatures were modeled at 2, 4, and 8 ºC, while air temperatures were set to -5, 0, 5, and 10 ºC. In order to assess the portion of convective (lumped with conduction) heat lost for sea lions at rest but in moving water, water velocity inputs were restricted to 0.1, 0.5, 1.0, and 1.2 m s^{-1}. Experimental data for free-ranging Steller sea lions in air were collected from animals in a metabolic chamber which protected them from wind. Every effort was made to keep the chamber shaded to minimize the impacts of solar radiation on the animal. Based on these measurement conditions, the in-air model simulating night time conditions (wind velocity set to 0 m s^{-1}) was chosen for comparison to actual data since solar radiation is minimal at this time.

Body condition in the original model was assessed using maximum blubber depths of 1, 3, and 5 cm, representing animals in poor, average, and good body condition, respectively. Captive and free-ranging juvenile Steller sea lions rarely displayed blubber depths greater than 2.0 cm (see Chapters II, IV). Therefore, maximum juvenile blubber depths were modeled at 1, 2, and 3 cm, better representing the range of potential blubber depths for juvenile Steller sea lions in the size categories that were modeled.

Skin temperature (T_s) in the original model (Roscow, 2001) was varied between 0 and 3 ºC above ambient temperatures (T_a) to simulate the varying response in peripheral blood perfusion to varying environmental conditions. It was unclear how T_s varied in response to T_a in the original design of the model. Surface temperatures recorded from Steller sea lions immediately after emergence from the water were often 2-4 ºC warmer than ambient (depending on location, see Chapter III). Therefore, skin temperatures was arbitrarily set to be 3 ºC above ambient in the model. Model output was converted to MJ d^{-1} for comparisons to experimental data collected on captive Steller sea lions over a limited range of water temperatures (see Chapter II) and on free-ranging Steller sea lions in air (see Chapter IV). Experimental data are presented as linear regression estimates (as determined by least squares) ± 95% confidence intervals.

Results

Comparison of Predicted Versus Actual Expenditures

Comparison of predicted metabolic expenditures to actual expenditures of juvenile sea lions in stationary water (2, 4, 8 ºC) suggests that model predictions for sea lions with 1-2 cm of blubber were within range of actual measurements (Figs. 39, 40, 41). In moving water (1.2 m s^{-1}) the model appeared to overestimate heat losses at all water temperatures (Figs. 42, 43, 44). This overestimation ranged from 4-120% depending on sea lion size and temperature. Overall, the model tended to overestimate thermal losses for larger animals and at the warmer water temperatures (Figs. 42, 43, 44).

Within their terrestrial TNZ, predicted metabolic expenditures for sea lions with 2-3 cm of blubber were within the range of actual measurements, while values predicted for sea lions modeled with thinner blubber overestimated actual expenditures (Fig. 45). Below the TNZ (< 4 ºC), predicted estimates of expenditure were similar to actual measured values at 0 and -5 ºC (Fig. 46 and 47, respectively).

Changes to the Model

While the model did a good job of predicting metabolic expenditures in stationary water and in air, the model overestimated heat losses for sea lions in moving water. Parameters in the model that could be contributing to the overestimation of heat loss in modeled sea lions relates to heat transfer from the core of the sea lion to its surface. Heat flow is dependent on blood perfusion due to vasoconstriction and

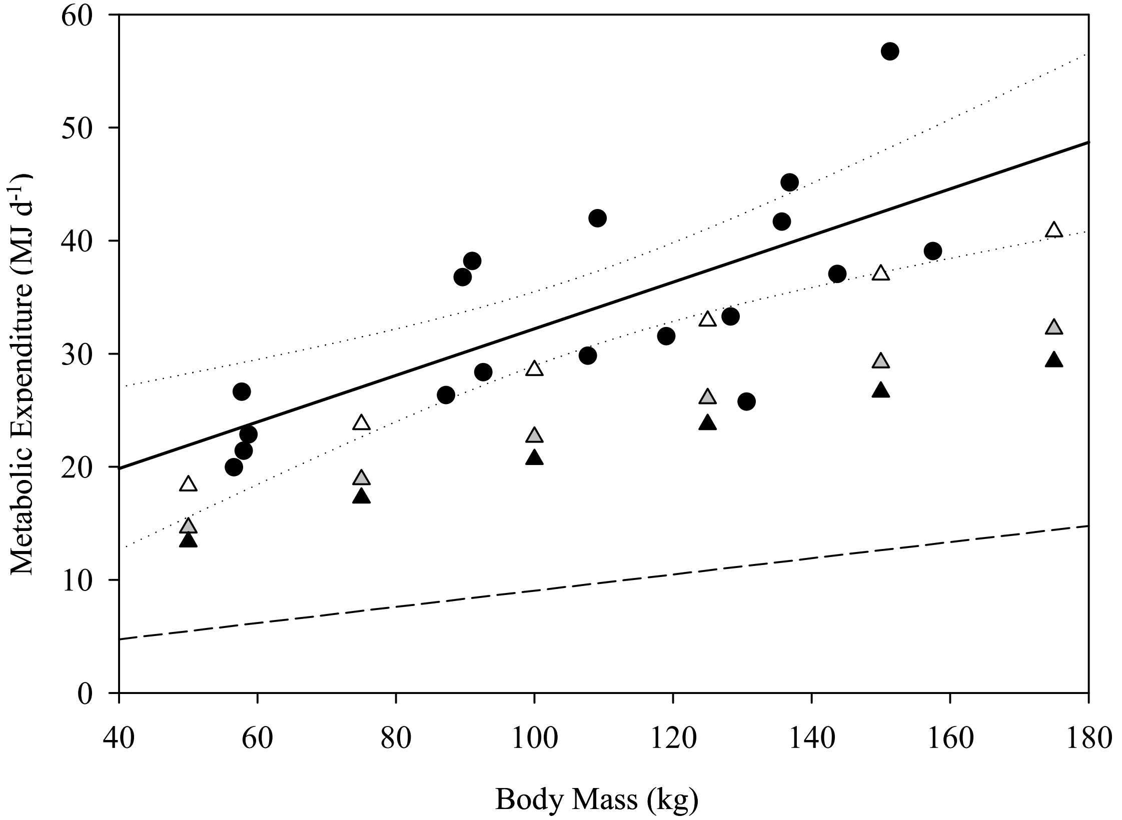

Figure 39. Comparison of the metabolic expenditures (MJ d^{-1}) for captive Steller sea lions (black circles, solid line) in 2 ºC water and predicted results for similar sized sea lions modeled with 1 (white triangles), 2 (grey triangles), and 3 (black triangles) cm of blubber in stationary water. Dashed line represents BMR as predicted by Kleiber (1975) for similarly sized adult terrestrial mammals. Dotted lines represent the ±95% confidence intervals around the regression line.

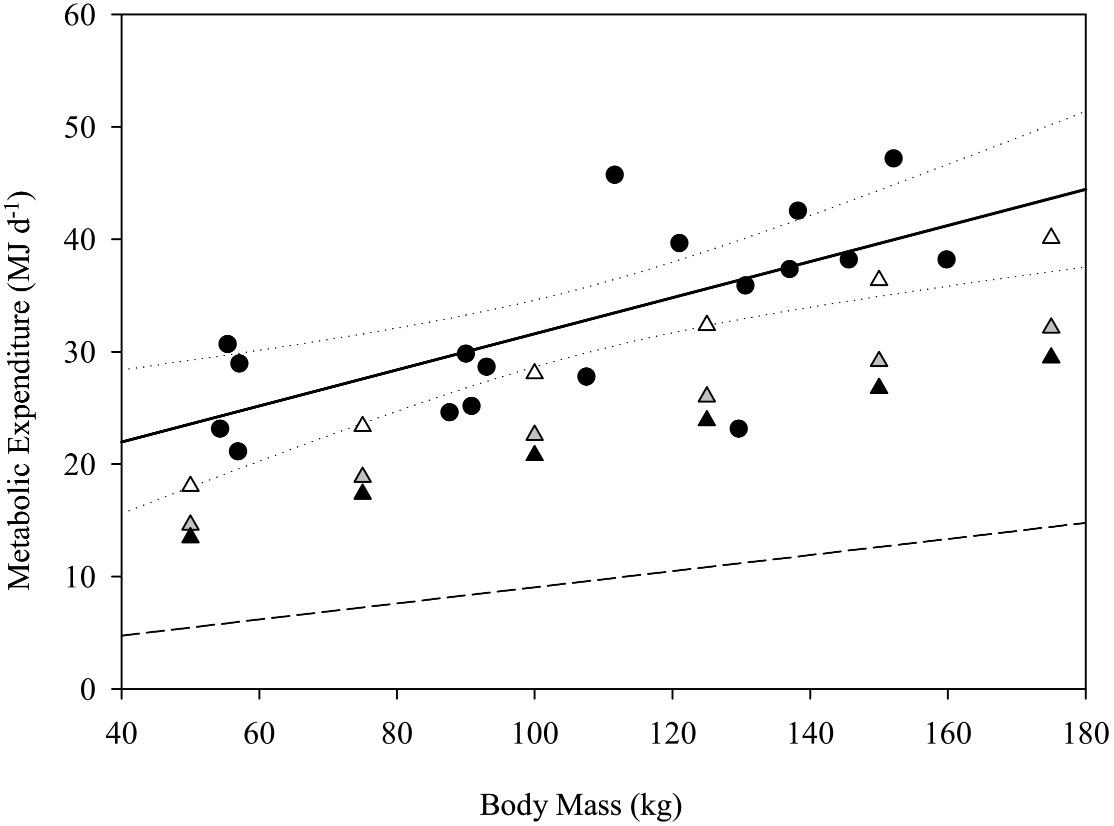

Figure 40. Comparison of the metabolic expenditures (MJ d^{-1}) for captive Steller sea lions (black circles, solid line) in 4 ºC water and predicted results for similar sized sea lions modeled with 1 (white triangles), 2 (grey triangles), and 3 (black triangles) cm of blubber in stationary water. Dashed line represents BMR as predicted by Kleiber (1975) for similarly sized adult terrestrial mammals. Dotted lines represent the ±95% confidence intervals around the regression line.

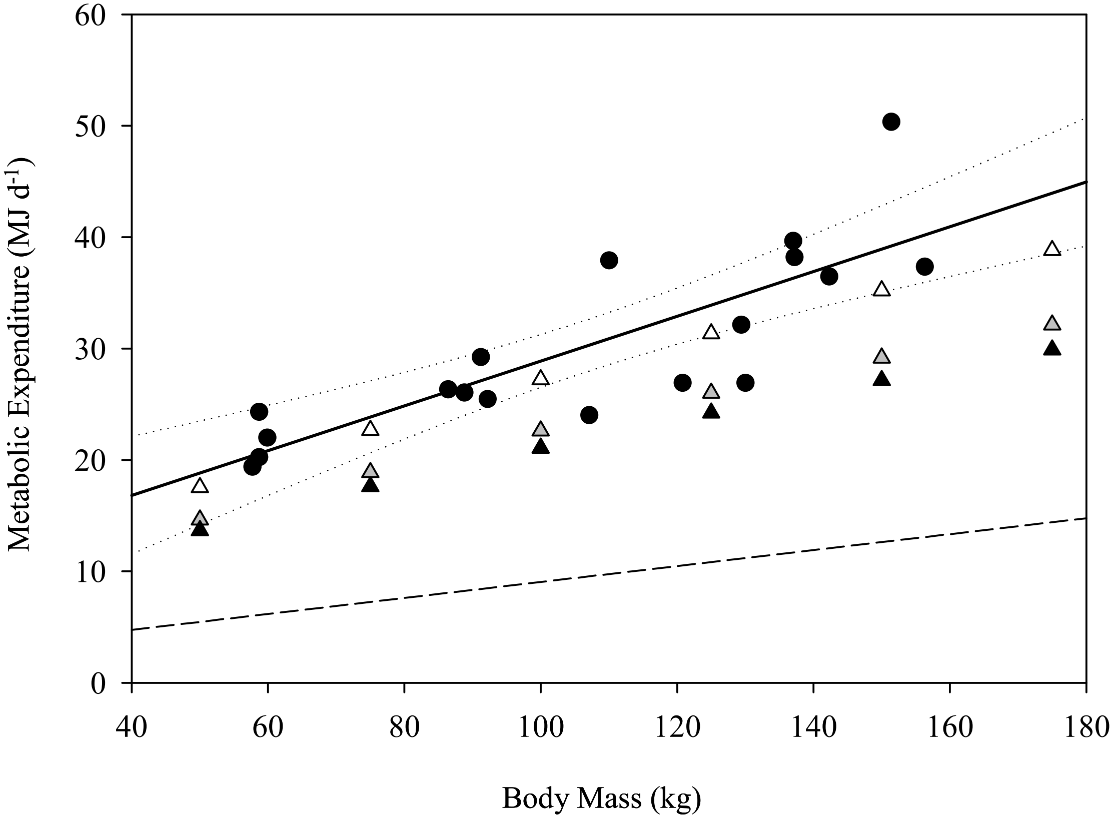

Figure 41. Comparison of the metabolic expenditures (MJ d^{-1}) for captive Steller sea lions (black circles, solid line) in 8 ºC water and predicted results for similar sized sea lions modeled with 1 (white triangles), 2 (grey triangles), and 3 (black triangles) cm of blubber in stationary water. Dashed line represents BMR as predicted by Kleiber (1975) for similarly sized adult terrestrial mammals. Dotted lines represent the ±95% confidence intervals around the regression line.

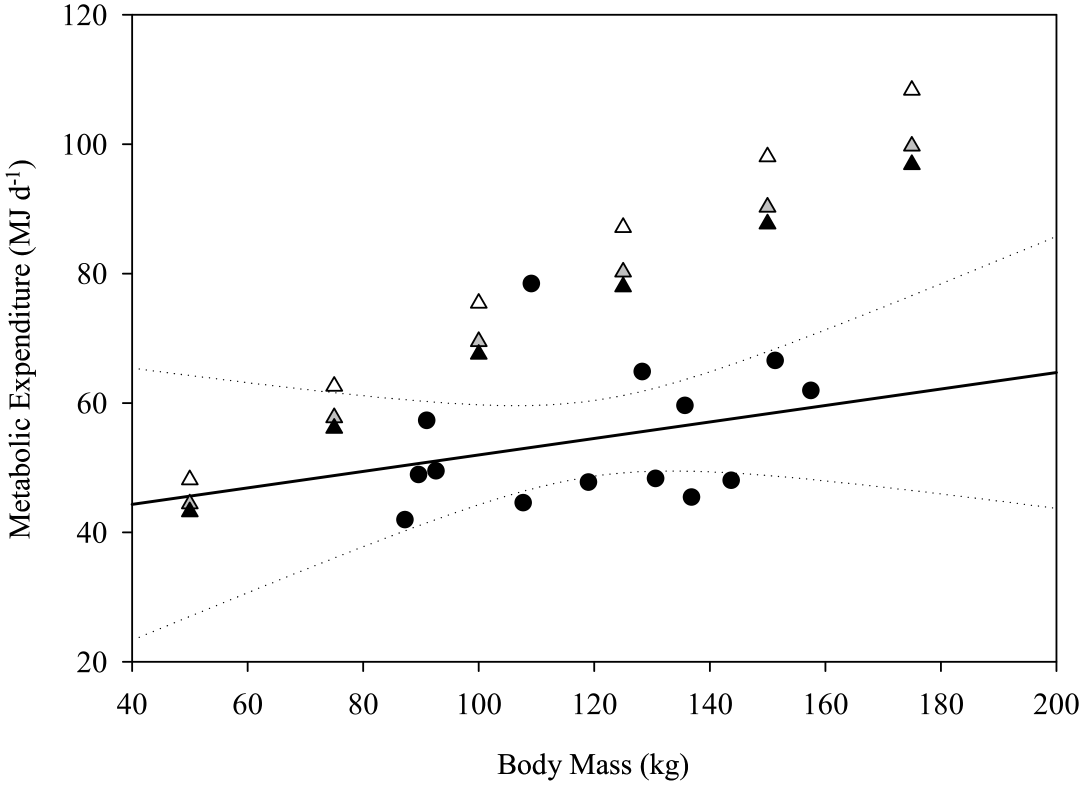

Figure 42. Comparison of the metabolic expenditures (MJ d^{-1}) for captive Steller sea lions (black circles, solid line) in 2 ºC water and predicted results for similar sized sea lions modeled with 1 (white triangles), 2 (grey triangles), and 3 (black triangles) cm of blubber in flowing (1.2 m s^{-1}) water. Dotted lines represent the ±95% confidence intervals around the regression line. The model overestimates metabolic expenditures in flowing water.

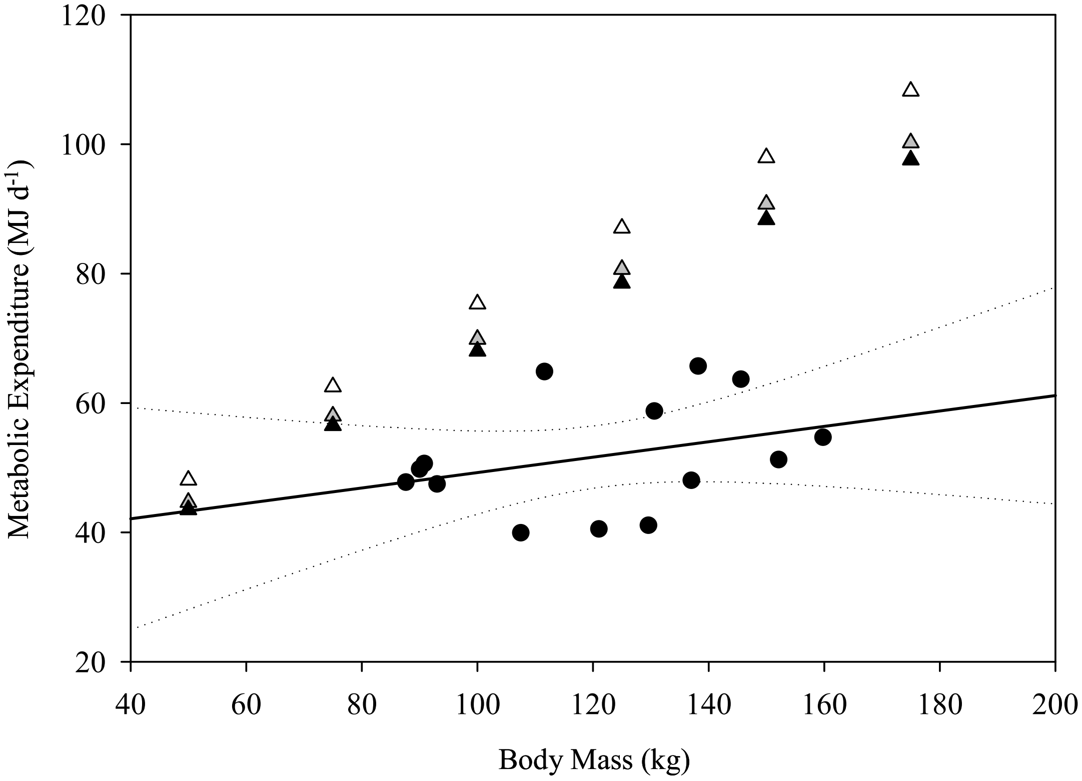

Figure 43. Comparison of the metabolic expenditures (MJ d^{-1}) for captive Steller sea lions (black circles, solid line) in 4 ºC water and predicted results for similar sized sea lions modeled with 1 (white triangles), 2 (grey triangles), and 3 (black triangles) cm of blubber in flowing (1.2 m s^{-1}) water. Dotted lines represent the ±95% confidence intervals around the regression line. The model overestimates metabolic expenditures in flowing water.

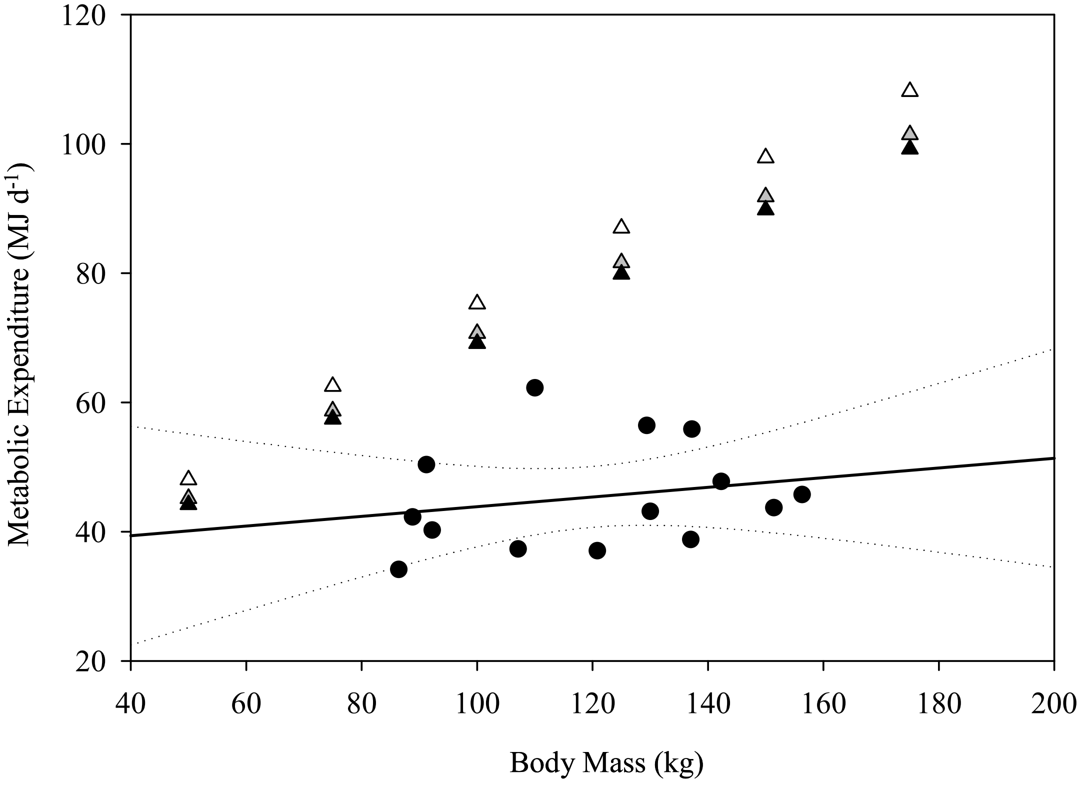

Figure 44. Comparison of the metabolic expenditures (MJ d^{-1}) for captive Steller sea lions (black circles, solid line) in 8 ºC water and predicted results for similar sized sea lions modeled with 1 (white triangles), 2 (grey triangles), and 3 (black triangles) cm of blubber in flowing (1.2 m s^{-1}) water. Dotted lines represent the ±95% confidence intervals around the regression line. The model overestimates metabolic expenditures in flowing water.

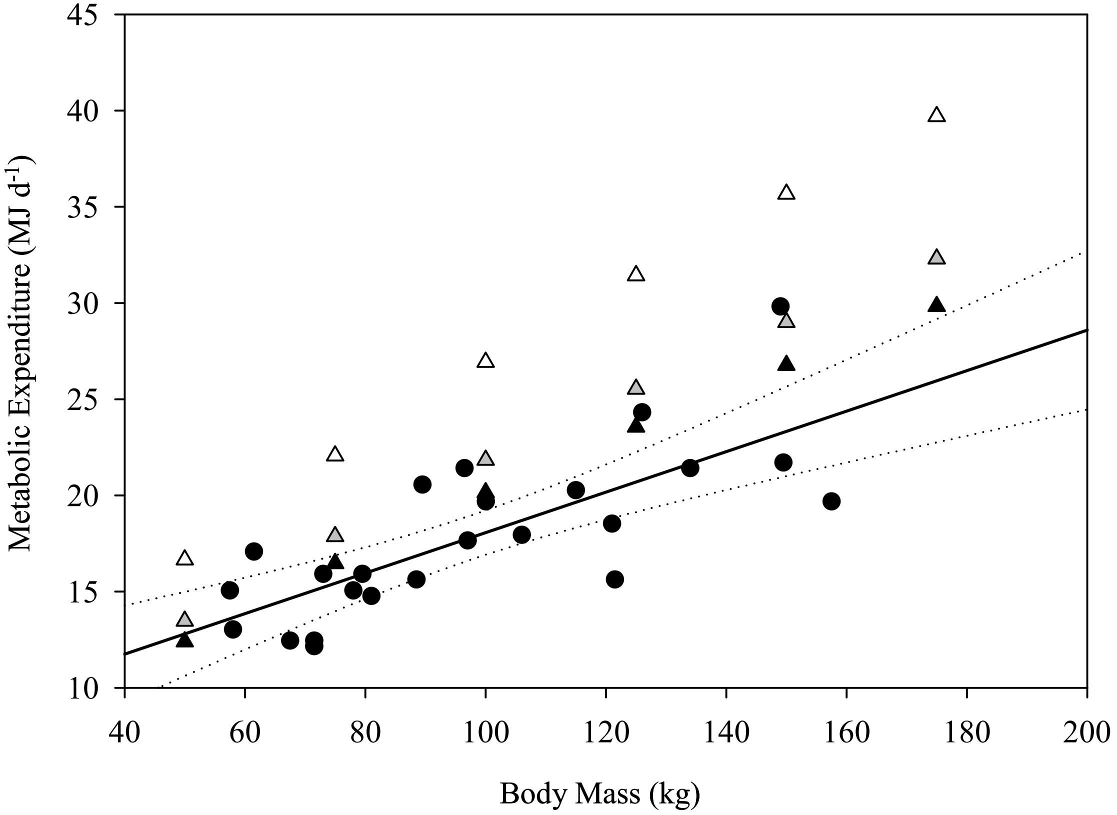

Figure 45. Comparison of the metabolic expenditures (MJ d^{-1}) for free-ranging Steller sea lions (black circles, solid line) in air temperatures between 4 and 10 ºC and predicted results for sea lions modeled with 1 (white triangles), 2 (grey triangles), and 3 cm (black triangles) of blubber in air at 5 ºC (triangles). Dotted lines represent the ±95% confidence intervals around the regression line.

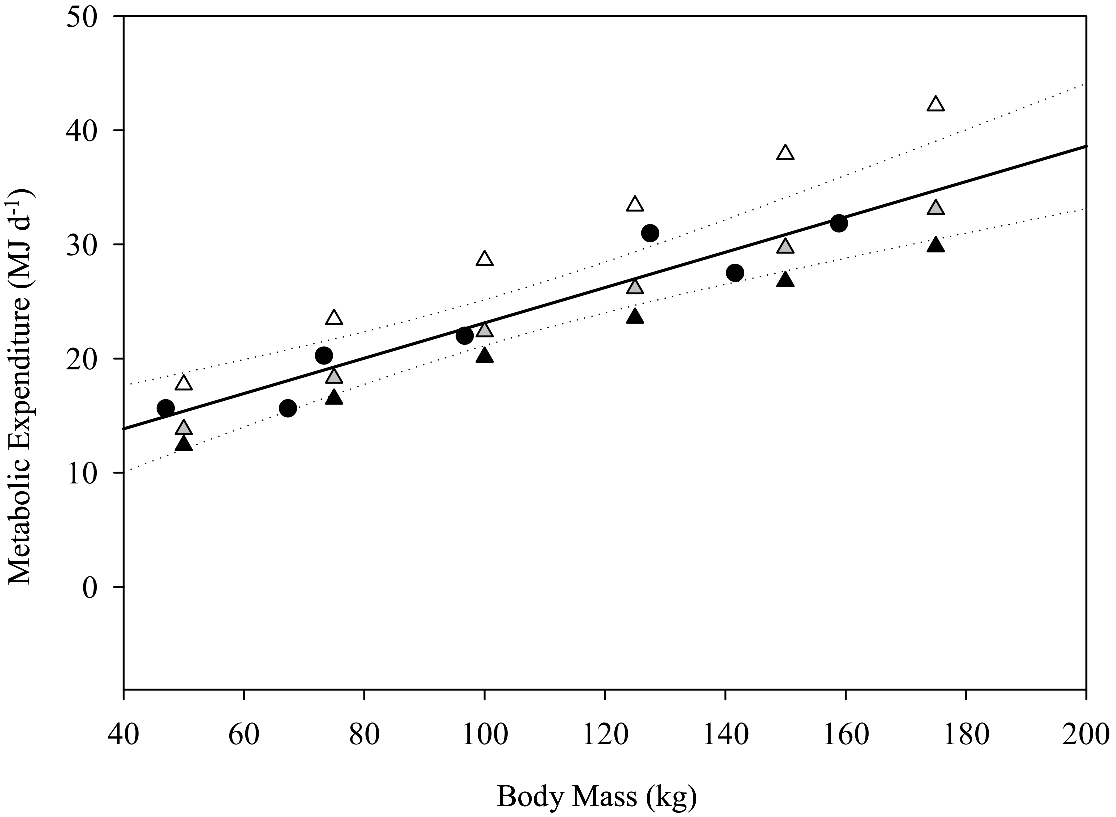

Figure 46. Comparison of the metabolic expenditures (MJ d^{-1}) for free-ranging Steller sea lions (black circles, solid line) in air temperatures between -1 and 1 ºC and predicted results for sea lions modeled with 1 (white triangles), 2 (grey triangles), and 3 (black triangles) cm of blubber in air at 0 ºC. Dotted lines represent the ±95% confidence intervals around the regression line.

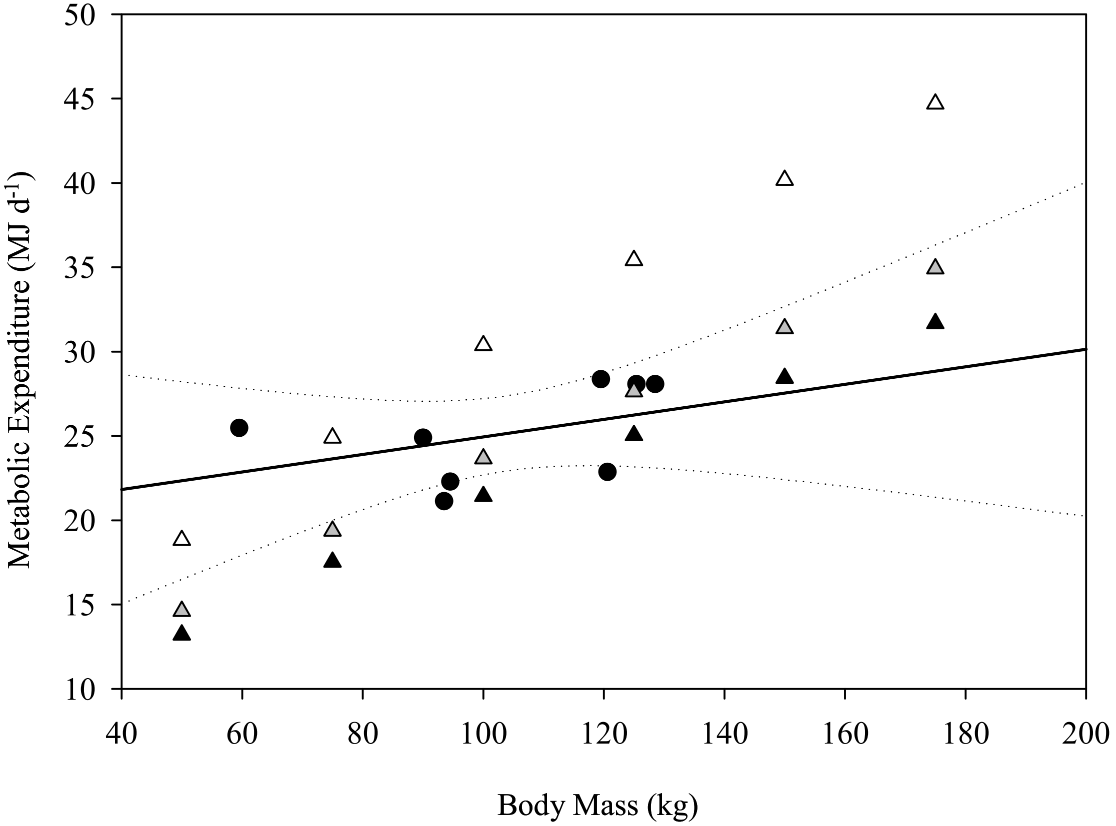

Figure 47. Comparison of the metabolic expenditures (MJ d^{-1}) for free-ranging Steller sea lions (black circles, solid line) in air temperatures between -6 and -3 ºC and predicted results for sea lions modeled with 1 (white triangles), 2 (grey triangles), and 3 (black triangles) cm of blubber in air at -5 ºC. Dotted lines represent the ±95% confidence intervals around the regression line.

vasodilation, which in turn affects the conductivity of the tissue. Blubber conductivity in the original model was set to between 0.18 and 0.20 W m^{-1} ºC^{-1} based on measured values in dead tissue samples (e.g., Worthy, 1991) which do not account for the likelihood of some minimal peripheral blood flow. It was assumed that sea lions at the lower end of their TNZ would have little blood flow in their blubber to reduce heat losses. Under these conditions, blubber conductivity would be similar to that of dead blubber. In order to increase insulative capacity in the model, thus correcting for the overestimation in heat loss, the lowest conductivity value (0.18 W m^{-1} ºC^{-1}) was selected.

Additional avenues for the overestimation of heat loss include the temperature differentials between the blubber/muscle layer and between the blubber/skin interface. The original model set the blubber/muscle layer interface temperature to 32 ºC, 5.5 ºC below core body temperature. When this temperature was decreased to 30 ºC, the model more accurately predicted heat losses. At the blubber/skin interface, Kvadsheim et al. (1997) reported an average difference of 3 ºC between the skin and ambient temperatures of surrounding medium. A difference of 2-4 ºC above ambient was also evident in body surface temperatures of juvenile Steller sea lions measured upon emergence from flowing water (see Chapter III), with average whole body surface temperatures being 3 ºC warmer than ambient water temperatures. Thus, T_s was maintained at 3 ºC above T_a for all environmental conditions. Based on these modifications, the model was re-run for juvenile sea lions in moving water to see if model predictions could better estimate actual metabolic expenditures.

Modifications to the model (blubber conductivity, blubber/muscle interface temperature) produced little change in predicted metabolic expenditures for juvenile sea lions in moving water. Thermal expenditures from the modified model were at most 3% lower than original values, and the modified predictions were still high compared to actual measurements (Figs. 48, 49, 50). It is possible that the model is still overestimating thermal heat loss in flowing water because it assumes that sea lions are stationary in flowing water. This rarely was the case during experimental trials. The model is designed to allow for the substitution of heat generated through locomotion to total heat losses when the animal is swimming. To explore the possibility that sea lions may use the heat generated from activity to offset thermal costs in flowing water, swim speed was modeled at the same velocity as water speed (0.1, 0.5, 1.0, 1.2 m s^{-1}) as a proxy for increased activity and model output was compared to experimental data on captive sea lions. The predicted increase in heat production due to locomotion helped defray total heat loss by 16% and reduce expenditures to within the range of actual measurements in smaller animals (Figs. 48, 49, 50). Even with the reduction in total heat loss, predicted expenditures for larger juveniles still overestimated actual values.

Comparison of Lower Critical Temperatures

Metabolic expenditures in both water and in air are dependent upon whether the environmental conditions under which they are measured are within the animals' TNZ. Determination of the lower critical temperature (T_{lc}) was possible for both the in-water and in-air experimental data sets (see Chapters II, IV). The original model predicted a

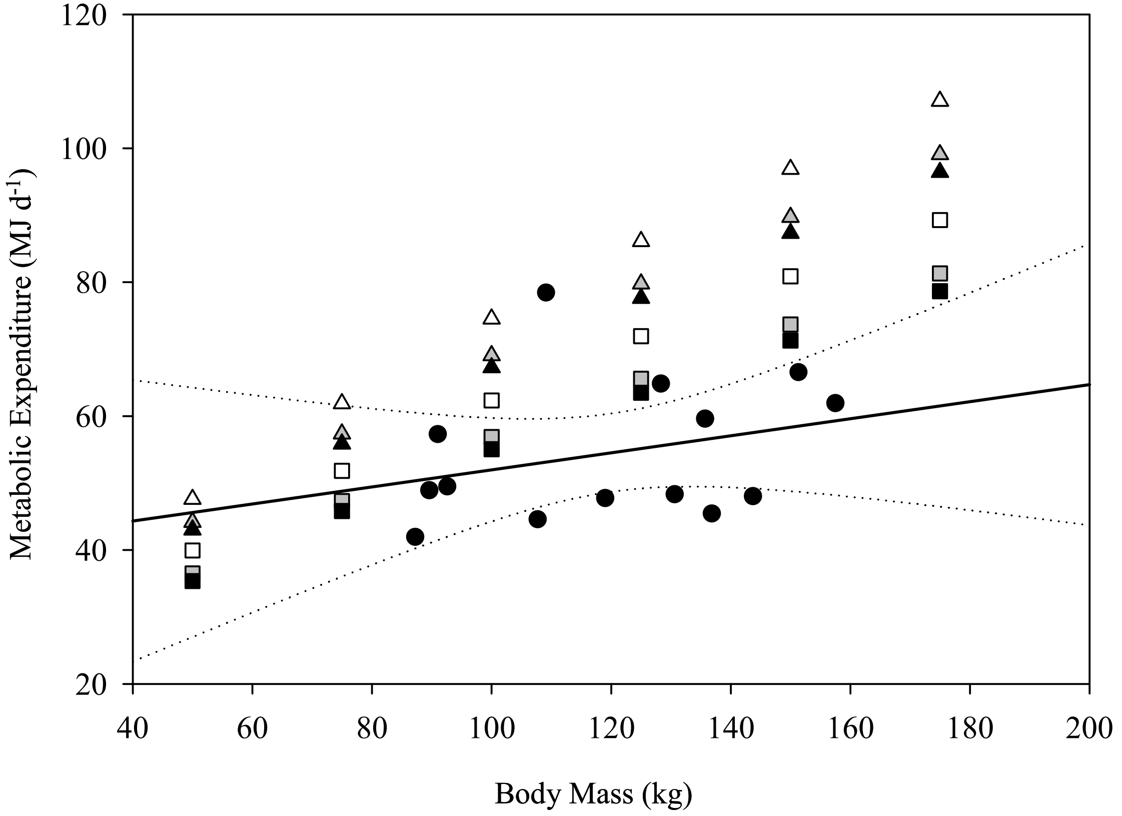

Figure 48. Comparison of the metabolic expenditures (MJ d^{-1}) for captive Steller sea lions (black circles, solid line) in flowing (1.2 m s^{-1}), 2 ºC water and modified model results for sea lions with 1 (white triangles), 2 (grey triangles), and 3 (black triangles) cm of blubber. Predicted results overestimate actual measurements. The predicted reduction in total heat loss due to heat generated from locomotion is plotted for sea lions with 1 (white squares), 2 (grey squares), and 3 (black squares) cm of blubber. Dotted lines represent the ±95% confidence intervals around the regression line.

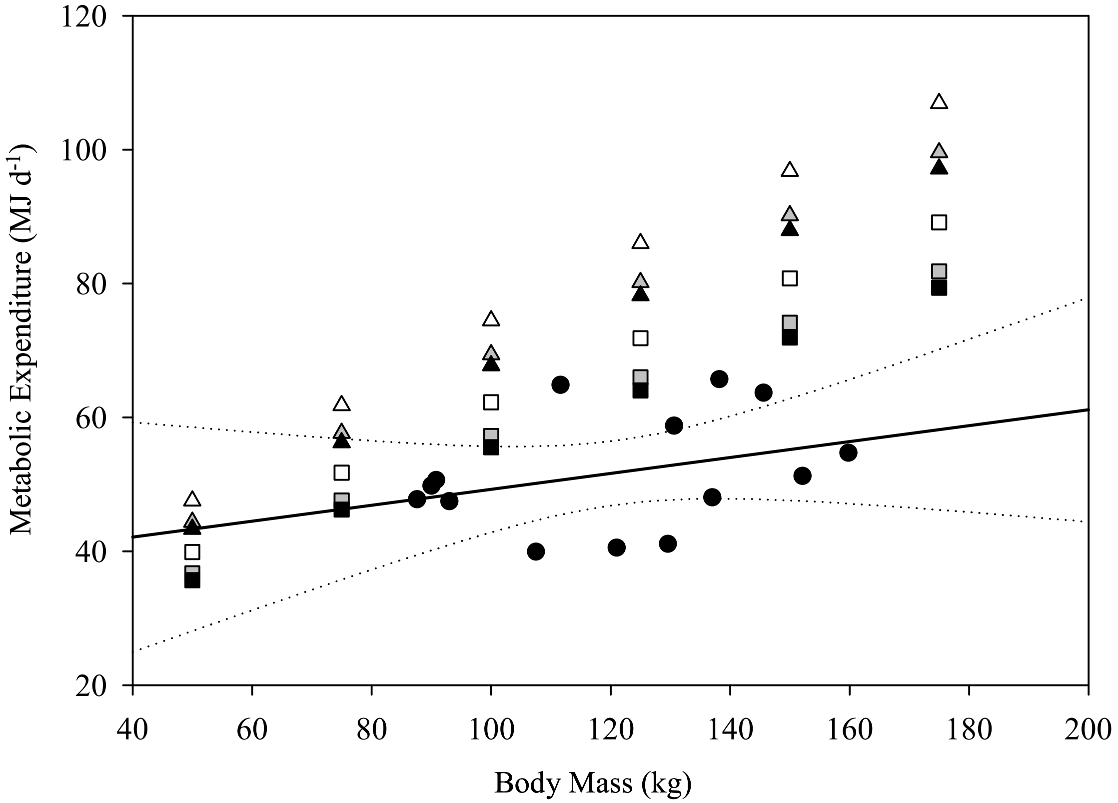

Figure 49. Comparison of the metabolic expenditures (MJ d^{-1}) for captive Steller sea lions (black circles, solid line) in flowing (1.2 m s^{-1}), 4 ºC water and modified model results for sea lions with 1 (white triangles), 2 (grey triangles), and 3 (black triangles) cm of blubber. Predicted results overestimate actual measurements. The predicted reduction in total heat loss due to heat generated from locomotion is plotted for sea lions with 1 (white squares), 2 (grey squares), and 3 (black squares) cm of blubber. Dotted lines represent the ±95% confidence intervals around the regression line.

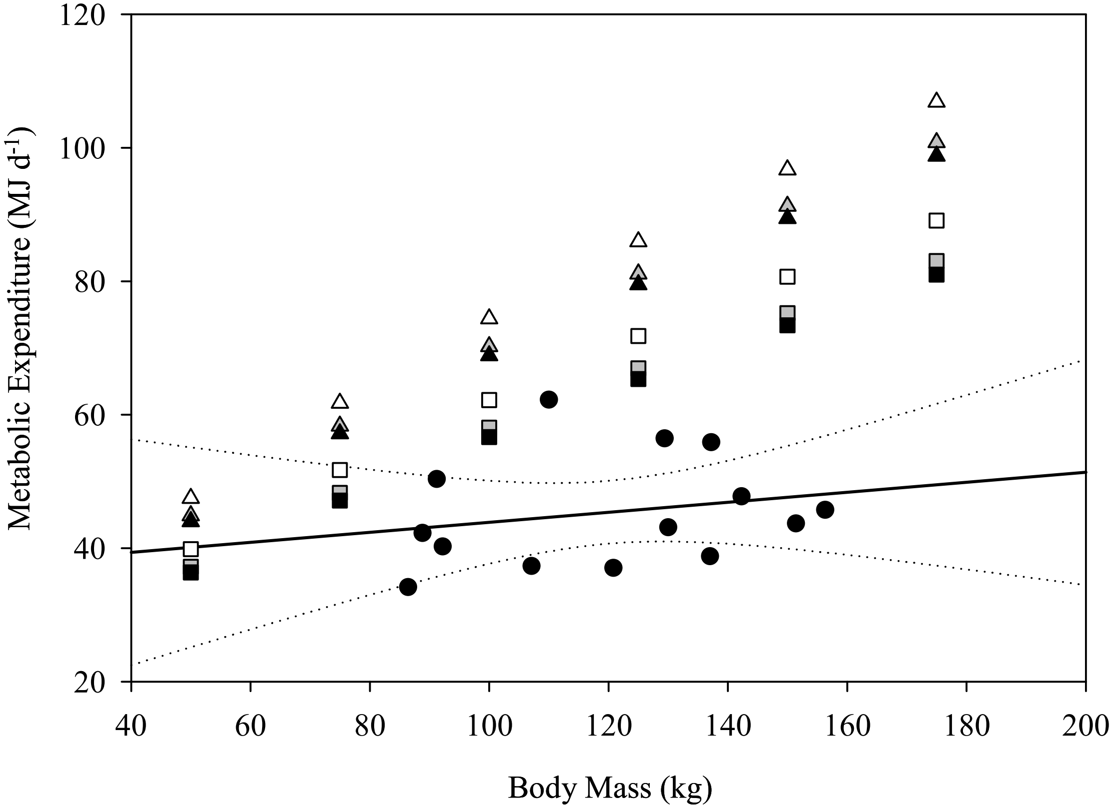

Figure 50. Comparison of the metabolic expenditures (MJ d^{-1}) for captive Steller sea lions (black circles, solid line) in flowing (1.2 m s^{-1}), 8 ºC water and modified model results for sea lions with 1 (white triangles), 2 (grey triangles), and 3 (black triangles) cm of blubber. Predicted results overestimate actual measurements. The predicted reduction in total heat loss due to heat generated from locomotion is plotted for sea lions with 1 (white squares), 2 (grey squares), and 3 (black squares) cm of blubber. Dotted lines represent the ±95% confidence intervals around the regression line.

T_{lc} in water of 6 ºC for 100 kg animals. A T_{lc} was not determined under environmental conditions at night in air for any sized sea lion in the original model (Roscow, 2001). The model was modified to include smaller sea lions masses (50, 100, 150 kg) and predicted expenditures were plotted over a range of water and air temperatures. Three patterns of skin temperature change were explored. Based on thermography results with captive sea lions (see Chapter III) T_s was first assumed to consistently be 3 ºC above ambient conditions. Second, a graded response to T_s with changing ambient conditions was modeled. Here, T_s was assumed to be similar to T_a in warmer conditions (0 ºC differential). Under colder conditions, the model assumed that sea lions would defend T_s, thus minimizing heat loss from the surface, and a maximum temperature differential of 3 ºC was used. Ambient temperatures in between showed a graded response to T_s. Finally, near their T_{lc}, sea lions should be minimizing peripheral blood perfusion through the blubber and skin to prevent heat loss. Under these conditions it is reasonable to assume that T_s is the same as T_a. Model predictions of this scenario will provide T_{lc} values for juvenile animals based on an extreme response in T_s to eliminate metabolic heat loss.

In the original model, predicted expenditures were subtracted from a multiple of BMR (determined by Kleiber, 1975), leaving the increase in metabolism as a result of thermoregulation (MJ d^{-1}). Estimates of BMR equivalent to 2 and 3 times the value predicted by Kleiber (1975) were chosen for comparison based on measured metabolic rates for juvenile sea lions (see Chapter II, IV). If output values were positive, a thermal cost was incurred at that temperature, while negative values indicated no additional cost (a savings) due to thermoregulation. The lowest temperature at which thermal energetic costs are negligible (negative) is considered to be the predicted T_{lc}.

Assuming juvenile sea lions were operating at a metabolic rate comparable to 2 times Kleiber, the model predicts that sea lions weighing between 50 and 150 kg (all blubber depths) would never be thermoneutral in water (-2 to 30 ºC) when skin temperatures are held consistently 3 ºC warmer than ambient conditions. If juvenile sea lions with 1 cm of blubber have a metabolic rate of 3 times Kleiber, the model predicts a T_{lc} in water of 11 ºC for 50 kg animals, 1 ºC for 100 kg animals, and 150 kg animals are thermoneutral at the coldest ocean temperatures (-2 ºC) (Fig. 51). Sea lions with greater blubber depths were thermoneutral at -2 ºC. If we assume that skin temperature is not consistently higher than ambient conditions, but rather shows a graded response to ambient temperature such that there is a larger differential at colder temperatures (3 ºC) and no differential at warmer temperatures (0 ºC), then a very different thermal pattern emerges. Here, the model predicted a T_{lc} for 50 kg animals at 1 ºC, for 100 kg animals near 0 ºC, and for 150 kg animals at -1 ºC when metabolism was assumed to be 2 times Kleiber and blubber depth was 1 cm (Fig. 52). Sea lions with thicker blubber were thermoneutral at -2 ºC. At 3 times Kleiber, all age classes at all blubber depths (1-3 cm) were thermoneutral at -2 ºC water.

In air, the model predicts a T_{lc} of 5 ºC for 50 kg sea lions, 2 ºC for a 100 kg sea lions, and 0 ºC for a 150 kg sea lion at an assumed basal metabolism equilavent to 3 times Kleiber (Fig. 53). These lower critical temperatures did not change regardless of the skin temperature profiles that were used in the model. When the model was run with

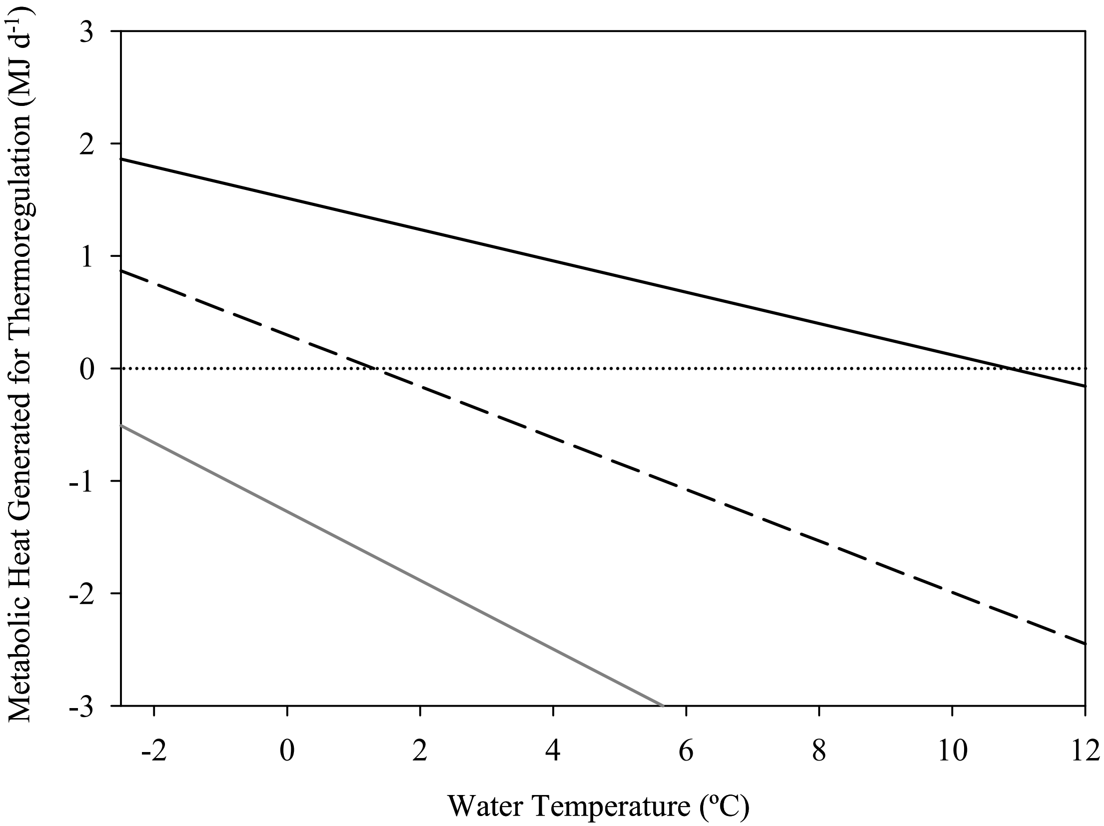

Figure 51. Predicted metabolic expenditures (MJ d^{-1}) for 50 (solid black line), 100 (dashed black line), and 150 (solid grey line) kg sea lions with 1 cm of blubber operating at a basal metabolism 3 times Kleiber in water. Skin temperatures were assumed to be 3 ºC above ambient conditions. The predicted lower critical temperature is the temperature at which heat losses become positive, indicating a need for the animal to increase metabolism to compensate.

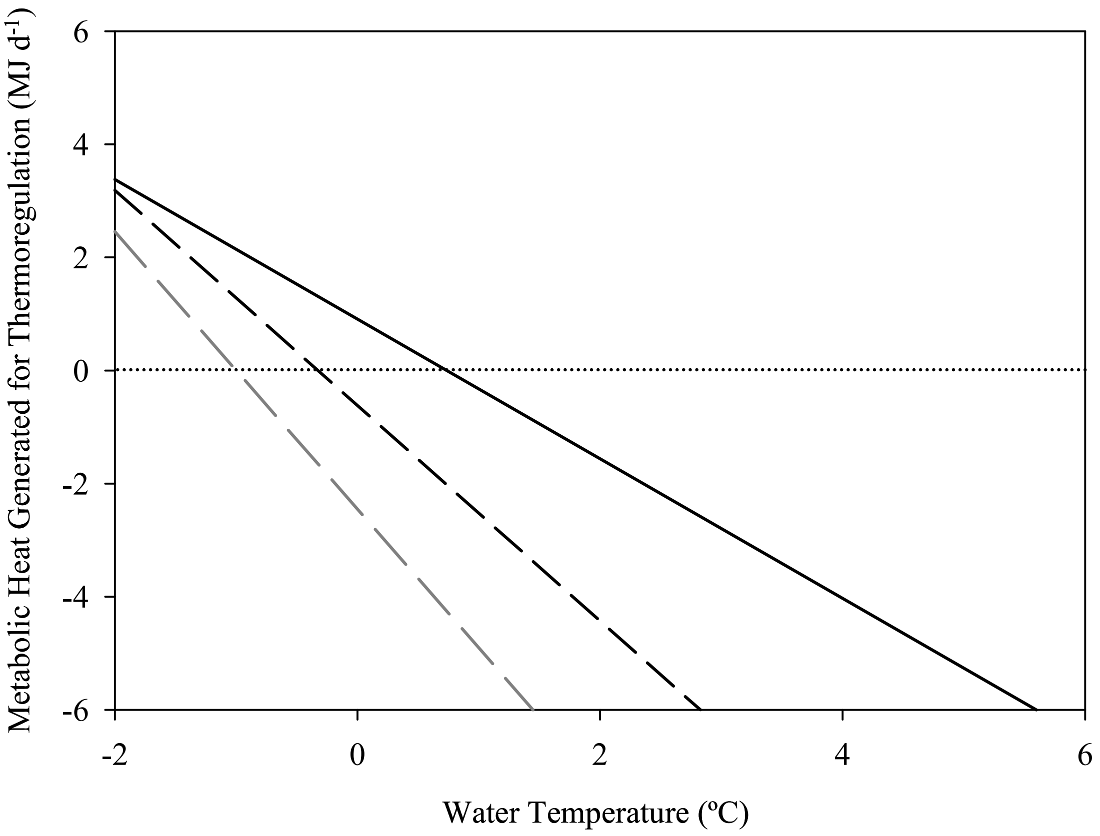

Figure 52. Predicted metabolic expenditures (MJ d^{-1}) for 50 (solid black line), 100 (dashed black line), and 150 (solid grey line) kg sea lions with 1 cm of blubber operating at a basal metabolism 2 times Kleiber in water. Skin temperatures were assumed to show a graded response to ambient conditions. The predicted lower critical temperature is the temperature at which heat losses become positive, indicating a need for the animal to increase metabolism to compensate.

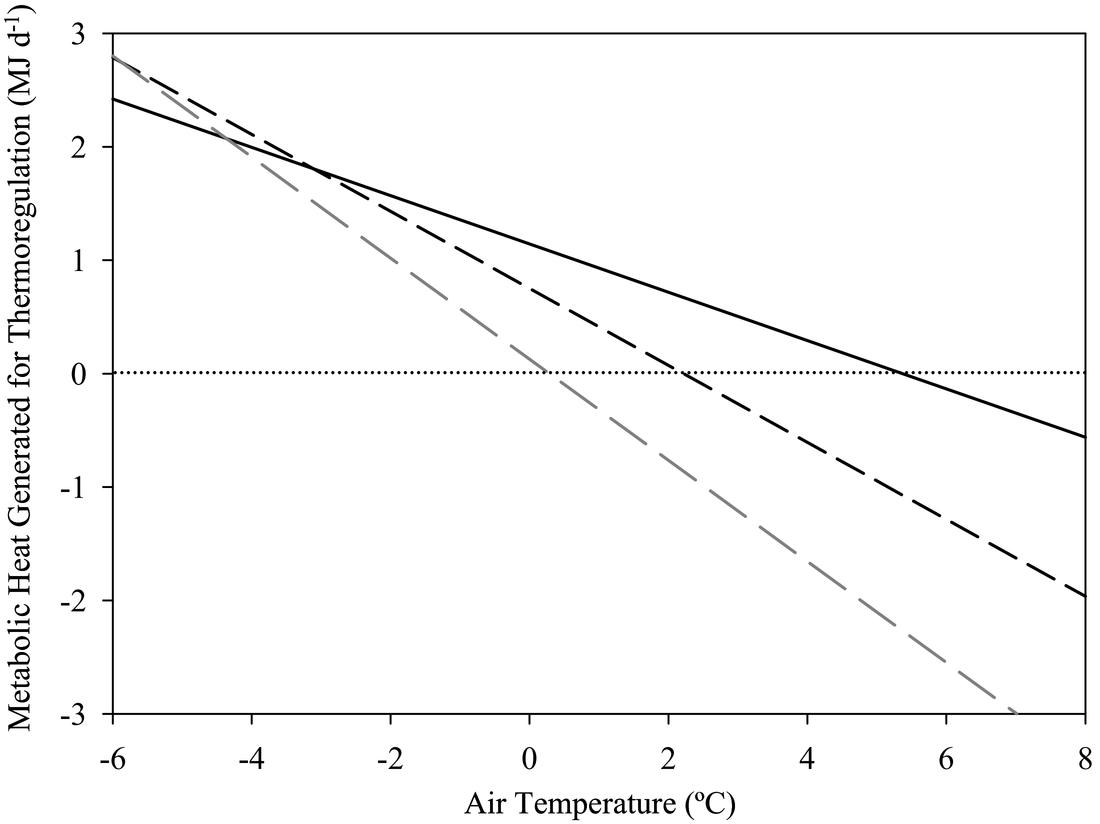

Figure 53. Predicted metabolic expenditures (MJ d^{-1}) for 50 (solid black line), 100 (dashed black line), and 150 (solid grey line) kg sea lions with 1 cm of blubber operating at a basal metabolism 3 times Kleiber in air. The predicted lower critical temperature is the temperature at which heat losses become positive, indicating a need for the animal to increase metabolism to compensate. Lower critical temperatures were the same regardless of which skin temperatures were chosen.

sea lions at 2 times Kleiber, heat losses were predicted over the entire range of air temperatures (-5 to 28 ºC) for all sizes of sea lions, and no T_{lc} was identified.

Discussion

Roscow's (2001) thermal energetics model predicts total heat loss under a variety of environmental conditions for both the aquatic and terrestrial habitats that Steller sea lions commonly occupy. This model, is a simplified representation of what in reality is a complex physiological system. However, the value of models is that they allow for the conceptualization of these complex systems and allow for predictions to be made about real world situations that are difficult to measure. Understanding the thermal energetics of juvenile Steller sea lions is an example of such a system, especially in light the potential for nutritional deficiencies to impact the fate of the blubber layer which has the conflicting role of both insulator and fuel source. Before the model can have any value at predicting thermal reality in Steller sea lions, model validation using actual measurements from juvenile Steller sea lions was required.

Model predictions were within the range of actual metabolic measurements collected from captive and wild Steller sea lions at rest both in water and in air. The original model was designed to compare total heat loss values to BMR (determined by Kleiber, 1975) to gauge whether maintenance requirements were enough to offset heat loss or whether the animal was required to increase metabolism to prevent the heat loss. It is clear from the experimental data that Steller sea lions, like most other otariids are not operating at metabolic rates comparable to what the Kleiber equation would predict, but at some multiple of it. In water this averaged 3.1 times Kleiber (individual range 2.4 to 4.0 times predicted) and in air sea lions averaged 2.2 times Kleiber (individual range 1.5 to 4.0 times predicted). This higher level of metabolism is commonly seen in juvenile pinnipeds and it is unclear as to whether this is a thermoregulatory response (Oftedal et al., 1987; Thompson et al., 1987; Whittow, 1987) or is a function of the added cost of growth (McNab, 1980). Regardless, the use of Kleiber's (1975) relationship for adult terrestrial mammals to estimate BMR in Steller sea lions overestimates the need for sea lions to increase their metabolic rate to cope with heat losses and suggests that the original model may have predicted additional thermoregulatory costs where none need exist. Previous models have used multipliers of Kleiber's equation to account higher metabolic rates in immature growing animals (e.g., Malavear, 2002; Winship et al., 2002) and this likely provides better estimates of maintenance requirements.

Comparisons between predicted and actual thermal expenditures suggest that the original model may be overestimating heat loss for juvenile sea lions in flowing water. Since heat transfer mechanisms operating externally on the sea lion in water (mostly conduction and convection) were determined by standard thermal equations and the properties of the medium in which they are modeled, it seems that the overestimation was likely attributable to the parameters which dictate internal heat production and transfer. Blubber conductivity, blubber/muscle interface temperature, and skin temperatures were altered in the model to attempt to model heat losses in flowing water within the range of actual measurements. Changes in blubber conductivity (with the range measured for dead tissue) and blubber/muscle interface temperature produced little change (3%) in predicted values. Changing skin temperature drastically altered the model output. This is not surprising given the complex vascular network in the subcutaneous blubber layer which can bypass or shunt heat to the outer body surface depending on thermal demands on the animal. Patterns of blood perfusion and vasoconstriction/vasodilation are not well understood in the skin and blubber layer of marine mammals, but obviously serve an important physiological role in maintaining thermoregulation. Studies measuring skin temperatures in otariids are limited and measurements from free-ranging fur seals show large fluctuations in T_s during diving and swimming in cold water, and animals tended not to minimize skin temperatures as would be expected to minimize heat losses in cold water (Boyd, 2000). While captive sea lions showed relatively constant differences (2-4 ºC difference) between surface temperatures (which were assumed to be equivalent to T_s) and ambient water temperatures, this appears to be an oversimplification since the model produced predictions that still overestimated actual expenditures. Further study examining the environmental and physiological mechanisms which influence skin temperature are necessary in order to increase the predictive power of the model.

Another possibility for the disparity in predicted and actual metabolic expenditure values for juvenile sea lions in flowing water was the observation that at highest flow speeds, sea lions increased their activity level in the swim flume (L. Hoopes, personal observation). The model assumes that sea lions are stationary in flowing water, when in reality this was rarely the case. It is possible that these animals were increasing their activity level to help defray thermal losses, especially at the colder water temperatures. While the model does not have an activity component, the substitution of heat generated from locomotion was examined. When water flow is greater than 0.1 m s^{-1}, the model assumes that the animal is swimming and the heat due to locomotion is subtracted from total heat loss. Predicted output for juvenile sea lions generating heat from locomotion was within the range of measured values in captive sea lions. This possibility likely explains the overestimation in the model output and evidence of the substitution of heat generated through activity was observed at the higher flow speeds in the experimental data set (see Chapter II).

Captive sea lions were also observed shivering at the colder water temperatures and higher flow speeds (L. Hoopes, personal observation). Shivering is a common mechanism by which heat is generated for thermoregulation due to muscular contractions that generate metabolic heat. The contribution of shivering thermogenesis to overall heat balance has been examined in relation to thermal acclimation, typically in small, terrestrial mammals (e.g., Bockler and Heldmaier, 1983; Nespolo et al., 1999). The contribution of shivering in overall heat production in pinnipeds is not known, but is probably small. The model did not take into account heat production via shivering thermogenesis.

The model performed well in predicting energetic expenditures in air both within and outside of the TNZ (see Chapter IV) when compared to experimental data from free-ranging sea lions. The model that simulated nighttime conditions was selected in an effort to minimize the effect of solar radiation on total heat loss given that the metabolic chamber was kept shaded. Solar radiation can certainly influence thermoregulation in the metabolic chamber (Porter, 1969) and during daylight hours, the model predicts significant reductions in heat loss during clear sunny days, even at very cold (-20 ºC) air temperatures (Roscow, 2001).

Model estimates of T_{lc} in water were variable and depended largely on how variations in T_s were defined. Measurements of T_s for pinnipeds in water are scarce (e.g., Worthy, 1985; Boyd, 2000; Willis et al., 2005) and comparisons can be difficult due to differing measurement techniques and confusion over what is actually being measured (e.g., skin/blubber interface temperature vs. surface temperature). The three modeled variations in T_s with changing T_a were based on patterns observed in other studies or assumptions made in other thermal models (Worthy, 1985; Lavigne et al., 1990; Ryg et al., 1993; Boily, 1995; Hansen and Lavigne, 1997a; Hind and Gurney, 1997; Kvadsheim et al., 1997). Skin temperature, which is regulated by the delivery of heat from the core to the surface, has been regarded as one of the most important physiological mechanisms by which endotherms regulate metabolic rate in cold water (Boyd, 2000). Yet, patterns of T_s change with immersion are still poorly understood. Direct measurements of T_s can only increase of peripheral blood flow and the control of heat from the core to the surface.

Captive work with juvenile Steller sea lions suggests a T_{lc} in water between 2 and 4 ºC for smaller sea lions having undergone reductions in total body mass (and potentially lipid mass). However, when body mass was recovered, an inflection in the data was no longer evident (see Chapter II). The range of experimental water temperatures that were tested was constrained by how cold the chiller unit could keep the water given the mild ambient air conditions in Vancouver, British Columbia. Therefore, it was hard to say which of model predictions were more accurate. In the original model, sensitivity analysis revealed that T_s in the water model was one of the most sensitive parameters to change with as little as a 1 ºC change in temperature influencing the heat production for thermoregulation by as much as 140% (Roscow, 2001). This reinforces for need for accurate skin temperature measurements over a range of ambient conditions in order to improve the predictive ability of the model.

While predictions of T_{lc} in water seem to be sensitive to changes in T_s, estimates of T_{lc} in air showed little sensitivity to this parameter. This was confirmed by results of the sensitivity analysis in the original model (Roscow, 2001). However, model predictions of T_{lc} at 2 ºC for 100 kg sea lions may be too low. Data from free-ranging juvenile Steller sea lions suggest that the T_{lc} in air may actually be around 4 ºC (see Chapter IV). While this temperature was determined over a range of differing sized sea lions, the majority of sea lions were near 100 kg in size. The high thermal losses predicted by the in-air model may be a function of the simulation of night time conditions and the absence of solar radiation. Predicted output (at 2 times Kleiber) suggests that thermal heat losses remain high over a range of air temperatures that are likely well within the TNZ, and may even be approaching the upper critical temperature (T_{uc}). This overestimation of the model to actual heat losses is a common feature of thermal models in marine mammals and has been attributed to errors in determining surface area both in the body and body core and in the calculation of the distribution of blubber along the body surface (Kvadsheim et al., 1997).

Thermal models can be a useful tool in examining how abiotic changes in the habitat of juvenile Steller sea lions influence the cost of thermoregulation, but the model is only as good as the quality of information used in its construction. Understanding how pinnipeds change their thermal conductance through the regulation of skin temperature is probably one of the most important, and least understood, mechanisms for determining total heat loss across the body surface. As more information becomes available, the model was designed such that changes in parameter inputs could easily be accomplished. However, given the available physiological information on Steller sea lions, the thermal model appears to reasonably predict thermal costs in young animals. While the model was created specifically for Steller sea lions, it could easily be modified and tested for other pinniped species in which more experimental data may be available for experimental validation.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Maintaining thermoregulatory balance is critical for marine mammals, especially for pinnipeds that must maintain the ability to regulate body temperature over a variety of ambient temperatures and habitats as part of their natural life history. The ability to maintain thermal balance, in spite of potentially greater heat losses in water, can affect submergence times, dive depth, and dive duration during foraging. Young pinnipeds are especially vulnerable given their immature physiology and inexperience and the added energetic cost of needing to acquire new tissue for growth. Given that juvenile life history stages are typically subject to enormous environmental (abiotic) and physiological pressures, it is expected that changes in the quality and/or quantity of prey resources may greatly impact survivability. If a nutritional deficiency is occurring in juvenile Steller sea lions as hypothesized, then body condition, and subsequently thermoregulatory homeostasis, might be compromised beyond sustainable limits.

Body condition was shown to have a significant effect on thermoregulatory ability in Steller sea lions. Sea lion body size, water temperature, and water velocity also influenced energetic expenditures. Smaller sea lions were particularly affected as suggested by the shift in T_{lc} to colder temperatures (outside the range tested) when total body mass was recovered. The present study was the first to examine how body condition influences thermoregulatory abilities in juvenile Steller sea lions. This information is essential for understanding basic energetic requirements in this species and for creating realistic bioenergetic models, which have previously assumed or ignored these types of relationships.

While small changes in total body mass (0-6%) and hence, composition, had less of an impact on energetic expenditure than large changes in total body mass (10-15%), it is not clear how seasonal changes in body mass and metabolism would impact an animal's ability to remain thermally neutral in water, and over what range of temperatures. This is an area of research that warrants further investigation. Results from the present study suggest that the T_{lc} for juvenile Steller sea lions in water is below 2 ºC. Expanding the range of water temperatures at the lower limit in juvenile Steller sea lions would be the next step in examining the thermal constraints on juvenile Steller sea lions which ultimately dictate how these animals operate in their environment.

One parameter that was not feasible to measure in the captive Steller sea lions was core body temperature. Core body temperature in pinnipeds is indirectly inferred through either rectal temperature or stomach temperature, neither of which was feasible in this study due to logistical constraints. Including these types of measurements in future studies would allow for discussions of whether or not the sea lion was thermally defending a smaller core at the colder water temperatures or if all heat losses were occurring peripherally.

While this work fills a significant gap in our understanding of the thermal energetics in Steller sea lions, it is recognized that caution should be exercised when inferring results from captive animals to those in the wild. In the present study, captive sea lions were considerably smaller (in total mass) and had thinner blubber layers than similarly aged free-ranging Steller sea lions. This largely was a function of early weaning experienced by captive sea lions and the fact these animals were long-term research subjects involved in diet manipulations. In attempting to discuss the potential impacts of changing body condition on thermoregulation in wild Steller sea lions, the focus was kept to general processes and trends. While application of studies with captive animals should be cautiously applied to their wild cohorts, it should be noted that the captive setting allowed for precisely controlled experimentation of metabolic responses to changing water temperature and water flow that would otherwise have been impossible to measure.

Thermography was used to assess patterns of vasoconstriction in captive Steller sea lions after emergence from water by measuring surface temperature as a proxy for skin temperature. Thermal images were also taken serially to assess patterns of warming in air and monitor development of thermal windows along the body trunk. After emergence from the water, the fore and hind flippers tended to be the coldest regions of the body, suggesting peripheral constriction in these regions to maintain heat in the body trunk. This trend was more pronounced in cold water temperatures and in smaller sea lions. Once in air, sea lions quickly warmed and within 15 minutes some animals had developed thermal windows along the region of the shoulder and hips to allow for heat dissipation. By 60 minutes, individual thermal windows had merged to cover large portions of the body at relatively high temperatures in some of the larger sea lions.

This study allowed for inferences to be made about peripheral blood flow control for sea lions while in cold flowing water and provided a first look at patterns of surface warming in air. The time scale at which the thermal images were taken turned out to be too broad to assess individual thermal window development. Examining the spatial development of thermal windows on the trunk of Steller sea lions at a finer temporal scale will allow for better interpretation of the physiological mechanism of heat dissipation in these animals. Further work in this area is already planned for future studies in Vancouver.

The rate of heat flow to the environment is dependent on a number of factors, including activity. Pinnipeds must compromise between remaining on land with lower thermal costs, resulting in a lack of opportunity to increase energy intake, or entering the water to forage, which allows for energy intake, but at the cost of increased energy expenditure for thermoregulation. Heat generated during swimming and diving may actually offset some of these energetic costs for sea lions in water; however, decreased metabolic rates observed during diving may make the need for heat substitution less likely. Understanding patterns of warming and the role of thermoregulation in sea lions post-dive might help elucidate whether such compromises occur during foraging.

It is also unclear how changes in insulation affect thermal flow in pinnipeds. In theory, decreased insulation would lead to increased heat flow, perhaps due to higher rates of flow at specific sites and the size of thermal windows. Conversely, if heat flow is effectively mediated by circulatory adjustments (vasoconstriction), then substantial changes in lipid stores might occur before there was an observed effect on thermal heat loss. This hypothesis is supported by suggestions that the hypodermal blubber layer of otariids under normal conditions is greater than solely required for thermoregulatory considerations.

While this study measured surface temperatures in captive sea lions that had recovered their body mass in the spring, planned future studies in Vancouver will utilize thermal imaging to assess the development of thermal windows in juvenile Steller sea lions under different seasonal and body condition states in order to better understand the physiological mechanisms of heat transfer in a species of concern and how these mechanisms are influenced by the nutritional and environmental fluctuations. It is hoped that thermography will also be useful in measuring post-diving surface temperature in sea lions to see if thermal mechanisms during foraging/diving can be inferred.

While previous studies were focused on how young Steller sea lions would physiologically respond to theoretical changes in their prey resources (assessed through changes in body condition), the study with free-ranging Steller sea lions was designed to see if evidence of such a nutritional impact could be detected in wild populations. Animals experiencing periods of fasting or undernutrition would be expected to limit energy expenditures, usually by lowering metabolic rate. Current population trends of Steller sea lions in Alaska indicate that western populations are continuing to decline, while eastern populations are stable or increasing. Resting metabolic rates of juvenile sea lions were compared across three geographical regions and results suggested no evidence of differing metabolic rates. Ambient air temperature played a significant role in determining metabolic rate and estimations of T_{lc} were near 4 ºC. While there is evidence of a shift in the TNZ of some pinnipeds with maturity, measurement of metabolism over an incomplete range of ambient air temperatures prevented this from being analyzed. Further metabolic studies over a larger range of ambient conditions and sizes of free-ranging Steller sea lions would help define how the T_{lc} changes with maturity or ontogeny.

Results from the current study suggest that while short-term changes in body mass and body composition (as might be experienced due to nutritional stress) have the potential to significantly impact the thermoregulatory ability of captive juvenile Steller sea lions; still, evidence from free-ranging Steller sea lions showed no differences in energy expenditure at rest between juveniles in eastern and western regions. This is consistent with recent studies which have failed to detect physiological and behavioral differences in eastern and western populations that would be consistent with the presence of nutritional stress in juvenile sea lions. Contrary to predictions, pup masses, growth rates, and estimated body fat levels from the region of decline were greater than in stable pup populations (Merrick et al., 1995; Rea et al., 1998; Brandon, 2000; Rea et al., 2003). Adult females were larger in the west and perinatal period and time spent nursing were also greater (Brandon, 2000; Milette and Trites, 2003). Measures of blood metabolites (ketone bodies, blood urea nitrogen, and fatty acids) as indicators of nutritional stress indicate that a higher proportion of pups were engaging in short-term fasting in the western stock, however, given the larger and fatter animals in the west, this increased fasting was apparently not influencing body condition (Rivera et al., 2006). Lastly, no differences in thyroid levels were detected between sea lions from eastern and western regions (Myers et al., 2006). Taken together, these studies provide a distinct lack of evidence that nutritional stress may be influencing continued declines in western juvenile Steller sea lions. Model results by Holmes and York (2003) suggest that while Steller sea lions declines in the early 1980s were associated with low juvenile survivorship, declines in the 1990s were attributed to low fecundity. Combined with high levels of organochlorine contaminants, polychlorinated biphenyls, and diphenyl-trichloroethane (which are known to influence reproduction in vertebrates) in the tissues of Steller sea lions (Lee et al., 1996; Beckmen et al., 2004), it seems that research efforts should be redirected to other life history stages (e.g., reproductive females).

Significant ecosystem changes are occurring in the North Pacific. Not only have Steller sea lions shown dramatic declines across the Bering Sea and Gulf of Alaska, but other marine mammals such as northern fur seals in the Pribilof Islands, harbor seals in western Alaska, and sea otters in the Aleutian Islands have also experienced population declines. Population declines in this region have occurred across other taxonomic groups and include reductions in seabird (kittiwakes, least auklets, murres) numbers and fish assemblages. Declines on this scale suggest a physical change in the ecosystem that likely cannot be pinpointed to one specific mechanism. Rather declines are probably linked to complex climatic, oceanographic, and biological interactions. While support for long-term research in this region is essential to understanding the interactions of these mechanisms this should be coupled with an understanding the physiological processes which dictate how a species deals with its changing environment.

Recently, effort has shifted from trying to identify a single mechanism that explains the decline and the species' subsequent failure to recover since receiving protection under the US Endangered Species Act, to looking at the combined effects of several mechanisms, such as fishing, predation, competition, and ocean productivity (e.g., Cornick et al., 2006; Guénette et al., 2006; e.g., Trites et al., 2007). These alternate hypotheses include the suggestion that large-scale fisheries in the Gulf of Alaska modified the ecosystem structure to the detriment of sea lions (e.g., Alverson, 1992), that sea lions have been predated on by transient killer whales, *Orcinus orca* (e.g., Springer et al., 2003; Williams et al., 2004a), that sea lions are unsuccessfully competing for resources with other species, and that shifts in ocean climate in the 1970's have impacted primary production and thus the food web structure that sea lions depended on (e.g., Merrick et al., 1997; Anderson and Piatt, 1999; Benson and Trites, 2002; Trites et al., 2007). Modeling results suggest that ocean climate change (e.g., higher sea surface temperatures) in the Bering Sea ecosystem occurred in the late 1970's and this may have caused bottom-up effects through several trophic levels which had the potential to affect the distribution of Steller sea lion prey (Guénette et al., 2006; Trites et al., 2007). The effects of fishing and predation by killer whales appears to be secondary in determining sea lion abundance and likely contributed to declines once populations were already depressed (Cornick et al., 2006; Guénette et al., 2006). Trites et al. (2007) maintain that support for the ocean climate hypothesis does not discount other hypotheses to explain the decline in Steller sea lions, such as the nutritional stress hypothesis. Rather, they maintain that the ocean climate hypothesis provides a 'holistic' framework within which each of the alternate hypotheses can be explained.

Complex physiological systems lend themselves well to exercises in modeling, where it is often difficult to collect whole or partial components of the model in a natural setting. Predictions from a thermal energetics model created for Steller sea lions based on the principles of thermodynamics were within the range of actual energetic expenditures for juvenile sea lions in water and in air. The model tended to overestimate expenditures in flowing water, and this was attributed to uncertainty in how skin temperatures fluctuate with varying environmental conditions to regulate heat flux to the surface of the sea lion. While a model is only as good as the reliability of information used to construct it, the model did allow for the manipulation of various limiting physiological and environmental variables that would otherwise be impossible to study.

While the model was constructed specifically for Steller sea lions, it relies on basic thermal equations that are applicable to any pinniped and could easily be modified to examine similar questions on thermal constraints. Additionally, the thermal model could serve as a component of a larger bioenergetics model for Steller sea lions since most pinniped models tend to ignore thermoregulation by assuming animals are within their TNZ. Research with otariids has shown that this is not the case, and in fact many species are living near the lower limit of their TNZ.

This model is static in that it estimates thermal expenditures at a given point in time, and it might be interesting to develop a more dynamic model that changes with season, or development of the sea lion. This could include seasonal changes in body composition and metabolism and how they might impinge thermoregulation during periods of undernutrition. With ontogeny and an increase in size, sea lions gain greater thermoregulatory tolerances and lower metabolic rates. Body composition changes greatly at independence and this transitional period is likely a difficult period of thermal adjustment. The addition of these types of parameters into the model might provide clues to important physiological milestones in the life history of juvenile Steller sea lions.

This is the first study to measure resting metabolism in free-ranging juvenile Steller sea lions, a basic physiological parameter which is essential to understanding how this species uses and stores energy. Metabolic rate forms the basis for many energetic calculations and forms the foundation for discussions of thermoregulatory capabilities, locomotory costs, diving limitations, and prey consumption rates in marine mammals. This is also the first study to explore thermal constraints on Steller sea lions and how thermoregulatory capacity can change with changes in insulation. Understanding the energetics and thermoregulatory capabilities of juvenile Steller sea lions is especially critical given precipitous population declines over past three decades which are thought to be attributed to changes in quantity and/or quality of prey availability. Basic physiological measurements combined with the predictive power of modeling will allow for the exploration of the effects of environmental and physiological interactions on individual sea lions, thus pinpointing potential avenues that warrant further study.

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