SOUTHERN SEA OTTER (*Enhydra lutris nereis*) FUR MORPHOLOGY, THERMAL FUNCTION, AND BUOYANCY ACROSS ONTOGENY

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ABSTRACT

Southern Sea Otter (*Enhydra lutris nereis*) Fur Morphology, Thermal Function, and Buoyancy Across Ontogeny

Kate Riordan

Sea otters (*Enhydra lutris*) have the densest fur of any animal, and the hairs function to trap a layer of air that is used for insulation. When a sea otter is born, it has a natal pelage (baby fur), and sea otters eventually molt that fur and replace it with a pelt resembling the adult fur. Sea otter fur is composed of 3 types of hairs: underhairs, intermediate hairs, and guard hairs. Sea otters are more susceptible to the negative effects of oiling from oil spills compared to other marine mammal species because they solely rely on fur for insulation. Though numerous studies have investigated the functional morphology of adult sea otter fur and how it is affected by oil, not much is known about the characteristics and insulative abilities of sea otter natal fur.

To better understand the structure and function of sea otter fur, I examined the hair morphology and related it to the thermal function and buoyancy of southern sea otter (*Enhydra lutris nereis*) pelts across different age classes. Guard hair length, hair circularity, guard hair and underhair cuticular scales, and hair density of sea otter pelts were measured across six age classes: neonate, small pup, large pup, juvenile, subadult, and adult. Thermal conductivity and thermal resistance of sea otter pelts were measured under three treatment conditions: in air, in water, and oiled. Pelt buoyant force in water was also measured under three treatment conditions: control (normal), oiled, and washed (with Dawn® dish soap). To scale up to the whole animal and examine differences related to body size, I used these measurements to estimate total heat loss, whole-animal buoyancy force, and mass-specific buoyant force.

I confirmed the timing of the transition between the natal fur and adult fur, which occurs some time in between the small pup and large pup age classes. I observed a consistent pattern in the hair circularity and shape of guard hairs across all age classes. It is important for all sea otters to have the ellipsoid shape of the guard hair so that the hairs can lie flat against the air layer to protect it in water. Comparisons of hair density indicated that sea otters with natal fur (neonate, small pups, and some large pups) have approximately 25-53% lower hair density than older age classes with the adult-type pelage. This thinner hair density may explain why young sea otters are always on their mother’s belly to stay out of the cold water.

When I examined the thermal function of the fur in air, I found that pups with natal fur have less efficient but equally effective thermal insulation. However, when I scaled up the thermal findings using body morphometrics and created a volume-based heat loss model, it was clear that younger sea otters have substantially more heat loss for their body size, compared to older age classes. Values for oiled heat loss demonstrated the vulnerability associated with sea otters relying on fur for insulation, and the large surface area to volume ratio associated with a small body size. It is already a high energy requirement for sea otters to maintain mammalian body temperate in cold water, and when the fur becomes oiled, the severe reduction of their insulation can cost them even more energy.
Across age classes, there was no difference in the buoyant force of the fur when I focused on the pelt samples from the laboratory-based experiments. When I scaled up the pelt buoyant force values to the whole animal using body mass, total body length, and surface area, it became clear that buoyant force varied across sea otter age classes. The smaller body size present in younger sea otter age classes allows for a higher mass-specific fur buoyant force. The larger size and higher hair density in adult sea otters explains the higher whole-animal fur buoyant force, as more individual hairs allow for more air to be trapped within the fur. Oiling of the fur reduces the overall buoyancy, and washing the pelts with Dawn® did not consistently restore the air layer in the fur.

In summary, the results of these studies indicate that the success of sea otters is due to the overall shape and structure of the individual hairs, along with the incredibly high hair density. The different hair types function together to trap the air layer in the fur to provide sufficient insulation. Ultimately, all ages of sea otters are vulnerable to the effects of oiling, with younger sea otters being the most susceptible.

Keywords: buoyant force, development, fur density, hair, insulation, lanugo, natal, oiling, pelage, thermal conductivity, thermal resistance
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GENERAL INTRODUCTION

When terrestrial mammals reinvaded the water and transitioned to aquatic living in the late Eocene (Uhen, 2007), these homeothermic endotherms needed to adapt and adjust insulation types in order to maintain their body temperature in a thermally challenging environment. The presence of fur is a major characteristic of Class Mammalia (Cleveland et al., 2011), and marine mammals evolved and split off into utilizing different types of insulation. Modern-day marine mammals have three different strategies for thermal insulation: fur, blubber, and a combination of fur and blubber. Sea otters and polar bears, the two most recently evolved marine mammals, rely on fur as their primary insulation. Otariids (eared seals) have a combination of fur and blubber, while phocids (true seals), odobenids (walruses), sirenians (manatees and dugongs), and cetaceans (whales, dolphins, porpoises) rely only on blubber for insulation (Pabst et al., 1999; Liwanag et al., 2012a,b; Berta et al., 2015).

Fur provides insulation by trapping a layer of air at the base of an animal’s skin (Sokolov, 1962; Frisch et al., 1974; Reynolds, 1993). Fur is lighter in weight than blubber and a considerably better insulator in air, but the effectiveness of fur as an insulator decreases when wet (Scholander et al., 1950; Hammel, 1955; Johansen, 1962; Ling, 1970; Frisch et al., 1974; Morrison et al., 1974; Doncaster et al., 1990; Fish, 2000; Kvadsheim & Aarseth, 2002; Sharma & Liwanag, 2017). A key difference between the pelt of fur-bearing marine mammals, such as fur seals and sea otters, and that of terrestrial mammals is the higher hair density for marine mammals (Fish, 2000; Liwanag et al., 2012a). The insulative properties of fur depend on the hair density and hair length (Reynolds, 1999), as a higher hair density allows for better retention of air in fur (Kuhn &
Meyer, 2010). Other morphological adaptations have evolved to increase the effectiveness of fur insulation in water, including flattening of the hair shaft and scale patterns on the hair cuticle that facilitate interlocking of the hairs (Liwanag et al., 2012a).

Sea otters (*Enhydra lutris*) face unique thermoregulatory challenges because they rely exclusively on fur for insulation. The coastal water temperatures in which sea otters live ranges from 21 to 38°C below their core body temperature (Williams & Davis, 1995). Due to seawater conducting heat 25 times faster than air at the same temperature, having a high surface area to volume ratio makes sea otters vulnerable to being thermally stressed in the cold waters (Costa & Kooymen, 1982; Yeates & Williams, 2007). Sea otters are born with natal pelage, which they later molt into a pelt resembling the adult pelage. Many studies have described the morphology and thermal function of the adult sea otter pelage (Costa & Kooymen, 1982; Williams et al., 1988; Williams et al., 1992; Dunkin, 2001; Kuhn & Meyer, 2010), but little is known about the functional characteristics of the natal pelage or how well it insulates younger otters.

The southern sea otter (*Enhydra lutris nereis*) population has increased significantly since 1938, when a small raft was first spotted in Big Sur after they were presumed extinct (Bolin, 1938). Sea otters have been able to expand their range along the coast and increase the population size more than 50-fold (Hatfield et al., 2019) due to the conservation efforts by government agencies, nonprofit organizations, and the general public. However, any positive population growth is still limited by their vulnerability to the effects of oil spill events, slow growth rate, and narrow geographic range with high rates of shark bite mortalities at the peripheries (Tinker et al., 2016; Nicolson et al., 2018; Moxley et al., 2019; Miller et al., 2020). The exclusive use of fur for insulation makes sea
otters particularly vulnerable to oil spills because oiling causes the fur to become matted and it can no longer insulate them efficiently (Costa & Kooyman, 1982).

How do oil spills affect sea otters at different ages? Despite past research that investigated the negative effects of oil on adult sea otter fur (Costa & Kooyman, 1982; Williams et al., 1988; Dunkin, 2001; Jessup et al., 2012), not much was known about the natal pelage and how it functions. The natal pelage of sea otter pups is described as “fluffy” and especially buoyant in comparison to the adult pelage (Sandegren et al., 1973; Payne & Jameson, 1984; Yochem & Stewart, 2009). At 13 weeks of age, sea otters have fully shed the natal pelage and replaced it with a coat that is similar to the adult pelage (Payne & Jameson, 1984). However, the morphology, thermal characteristics, and buoyancy of natal pelage have never been empirically measured.

The water repelling tendency of sea otter fur not only provides protection against heat loss, but it also contributes to the otter’s positive buoyancy (Fish et al., 2002). It is known that water infiltration into the air layer and pelage matting can cause decreases in buoyancy, so we anticipate oiling will cause these decreases and other problems such as hypothermia and drowning, regardless of age class (Costa & Kooyman, 1982; McEwan et al., 1974). A female sea otter performs extensive maternal care of her young pup, including prolonged periods of grooming and carrying the pup on her chest (Sandegren et al., 1973; Payne & Jameson, 1984; Thometz et al., 2014). It is generally assumed that both grooming and keeping the pup out of the water are important for preventing hypothermia. However, a pup must float on its own at the water’s surface when its mother embarks on short foraging bouts; this is when the buoyancy of a pup’s pelage becomes especially important.
To better understand the differences in sea otter fur across age classes, I investigated the morphology, thermal function, and buoyancy of sea otter pelts under normal conditions, and after oiling. In Chapter 1, I describe hair length, hair circularity, cuticular scales, and hair density of sea otter pelts across ontogeny. In Chapter 2, I provide measurements of the insulative properties of the pelts (thermal conductivity and thermal resistance) and estimate total heat loss under three treatment conditions: in air, in water, and oiled. In Chapter 3, I calculate the fur buoyant force, whole animal buoyant force, and mass-specific buoyant force of sea otter pelts under three treatment conditions: control (normal), oiled, and washed.
1. ONTOGENETIC CHANGES IN SOUTHERN SEA OTTER (*Enhydra lutris nereis*) FUR MORPHOLOGY

1.1 ABSTRACT

Many animals exhibit morphological changes across ontogeny associated with adaptations to their environment. Sea otters (*Enhydra lutris*) live in cold water environments and rely on dense fur to remain properly insulated, due to morphological adaptations that allow the hairs to trap air when submerged. Sea otter fur is composed of guard hairs, intermediate hairs, and underhairs. Past studies have investigated the morphology and hair density of adult sea otter fur, but these characteristics have not been measured for other age classes, including for the natal pelage. This study quantified ontogenetic changes in hair morphology of southern sea otter (*Enhydra lutris nereis*) pelts. We measured guard hair length and circularity, shape of cuticular scales on guard hairs and underhairs, and overall hair density for sea otter pelts across six age classes: neonate (<1 month), small pup (1-2 months), large pup (3-5 months), juvenile (6 months-1 year), subadult (1-3 years), and adult (4-9 years). Neonate and small pup pelts had significantly longer guard hairs than older age classes (p<0.01). Natal pelage guard hairs were similarly shaped (circularity p=0.864) but smaller in diameter (p<0.001) than adult guard hairs. Hairs of the natal pelage had similar cuticular scale patterns as adult hairs, indicating the importance of this structure for the function of the fur. Natal pelages had a lower hair density than the pelages of older age classes (p<0.001), with the adult pelage exhibiting the highest hair density. Overall, the morphological differences between natal
and adult pelage in sea otters suggest functional differences that may make sea otter pups may be more vulnerable to heat loss.

1.2 INTRODUCTION

One of the most defining characteristics in Class Mammalia is the presence of fur (Cleveland et al., 2011). Mammalian fur functions to aid in camouflage, behavioral signaling, waterproofing, and most importantly insulation (Cleveland et al., 2011). Fur acts as an insulator by trapping a layer of still air between an animal’s skin and the surrounding environment (Scholander et al., 1950; Sokolov, 1962; Frisch et al., 1974; Reynolds, 1993). Mammalian fur consists of two main types of hairs: guard hairs, which are stout and thick, and underhairs, which are thinner and shorter (Tarasoff, 1974). Some mammals also have an intermediate hair type (Erdsack et al., 2015; Liwanag, unpubl. data), but the function and presence/absence of this hair type are not well described in the literature. Mammal fur is organized into bundles that typically consist of one individual guard hair surrounded by varying numbers of underhairs (Scheffer, 1964; Liwanag et al., 2012a). Each hair is composed of a cortex, an outer cuticle, and a central medulla (Williams & Davis, 1995).

Over evolutionary time, some mammals have evolved particular adaptations in the fur to facilitate an aquatic or semi-aquatic lifestyle, including increased hair density, elongated cuticular scales on guard hairs and underhairs, and the flattening and lengthening of guard hairs (Liwanag et al., 2012a). During submergence, the underhairs of aquatic-adapted fur traps an air layer by creating a meshwork of individual fibers resembling an impenetrable mat to water (Williams & Davis, 1995). The shape of the
cuticular scales has been shown to be particularly important for trapping air in the fur because the elongated scales allow individual underhairs to interlock (Swift, 1977; Liwanag et al., 2012a). The wavy nature of the underhairs, the small interstices (intervening spaces) between hairs, and the hydrophobic surface of the cuticle prevent water from penetrating and allow air to remain trapped in the fur (Weisel et al., 2005; Kuhn et al., 2010). The guard hair cross-section has been described as almond-like (Kuhn & Meyer, 2010), or ellipsoid shaped, and the guard hairs function to lay flat over the underhairs to protect the air layer in water (Liwanag et al., 2012b). These morphological adaptations are crucial for the function of mammalian fur as an insulator in water.

Sea otters (Enhydra lutris) are unique among marine mammals because they are small-bodied and they rely solely on fur for insulation (Kenyon, 1969; Morrison et al., 1974; Tarasoff, 1974; Young, 1976; Castellini & Mellish, 2015). Sea otters also have the densest fur of any animal (Tarasoff, 1974; Williams et al., 1992), and that fur exhibits adaptations to aquatic living such as longer, flatter guard hairs, and elongated cuticular scales (Liwanag et al., 2012a). It is presumed that sea otters, like other mammals, have two different types of pelage: natal pelage and mature pelage. Sea otter natal pelage has been described as lighter brown in color and woollier compared to the pelage of adults (Kenyon, 1969). This natal pelage is eventually shed and replaced with a coat resembling the adult pelage at around 13 weeks of age (Payne & Jameson, 1984). The presence of these two different pelage types provides a unique opportunity to examine the key morphological properties of sea otter fur across ontogeny.

Past studies have estimated adult sea otter fur density from different parts of the body (Tarasoff et al., 1972; Williams et al., 1992; Fish et al., 2002; Kuhn et al., 2010),
but no studies have investigated the hair density of other age classes such as pups and juveniles. The aims of this study were to (1) confirm when the transition from the natal pelage to the adult pelage occurs in sea otters, and (2) compare sea otter hair morphology across ontogeny. To accomplish this, we measured sea otter guard hair length and circularity, as well as hair density, across six age classes: neonate, small pup, large pup, juvenile, subadult, and adult. We hypothesized that sea otter natal pelage would only be present in neonates and small pups, in accordance with previous evidence that pups shed the natal pelage at around 13 weeks old (Payne & Jameson, 1984). We also hypothesized that the adult pelage would have a higher hair density compared age classes bearing the natal pelage.

1.3 MATERIALS AND METHODS

1.3.1 Sample Collection

In collaboration with California Department of Fish and Wildlife (CDFW), sea otter pelts were collected from San Luis Obispo, Monterey, and Santa Cruz counties from animals that died in the wild or during rehabilitation efforts. In accordance with Section 109(h) of the U.S. Marine Mammal Protection Act (MMPA), the U.S. Fish and Wildlife Service’s regulations implementing the MMPA at 50 CFR 18.22(a), and the U.S. Fish and Wildlife Service’s regulations implementing the U.S. Endangered Species Act at 50 CFR 17.21(c)(3), the samples used to complete this work were collected from fresh, necropsied sea otter carcasses taken from the wild by an official or employee of CDFW in the course of their duties as an official or employee of CDFW. Only pelts considered fresh and in good condition (i.e., not matted or decayed) were used for this study. The
original 24 cm x 20 cm pelt samples were collected from the back (dorsal) region of the animal. The samples were packaged in three layers of plastic food wrap, kept flat (not folded), stored in two-gallon freezer bags, and kept frozen (-20°C to -16°C) until analysis in the various experiments. For guard hair morphology and shape of hair cuticle experiments, we collected hairs from a standard location on the right anterior section of the original sample, using tweezers. To clean the sample, each hair was placed in a 70% ethanol (FisherSci) bath prior to analysis for one week. A total of 38 samples represented six age classes, including neonate pups (<1 month, N=9), small pups (1-2 months, N=5), large pups (3-5 months, N=5), juveniles (6 months-1 year, N=6), subadults (1-3 years, N=6), and adults (4-9 years, N=7), with varying sample sizes dependent on the experiment performed (detailed below). No aged adult sea otter pelt samples were used in this study. Sea otter pelts were categorized into age classes by CDFW employees based on well-established sea otter stranding age estimation protocols that use total body length and tooth development data as identifiers.

1.3.2 Guard Hair Length andCircularity

Following the methods described in Liwanag et al. (2012a), we measured guard hair length by using three guard hairs from an area of the pelt unused in other experiments. All 38 sea otter pelts were included. We measured wet hair length by pipetting 1 mL of deionized water onto the hair using a plastic transfer pipette and then straightening the hair with a blunt dissecting probe on a microscope slide. Wet hair length was measured to the nearest 0.01 mm using digital calipers (Absolute Digimatic Caliper Series 500, Mitutoyo®, Aurora, IL, USA).
The same guard hair used to measure hair length was also used to measure hair circularity, using the minimum and maximum diameters, following the methods of Liwanag et al. (2012a). To measure the guard hair minor and major diameters, we cut the hair at the widest point of the hair shaft under a dissecting microscope (American Optical Stereo Star 659) to produce a cross-section perpendicular to the length of the hair. Next, we transferred the cross-section to a compound microscope (Leica ICC50 HD) with the x 40 objective (x 400 total magnification). To calculate hair circularity, we used an ocular micrometer to measure the minor diameter and major diameter to the nearest 0.01 μm as follows:

\[
Hair \text{ circularity} = \frac{\text{minor diameter}}{\text{major diameter}}
\]  

We used the average values from three hairs to produce a single value per individual for analysis of guard hair length and guard hair circularity.

1.3.3 Cuticular Scales

To mount the hairs, we placed one individual hair on a conductive carbon tab (PELCO Tabs™, Ted Pella, Inc., Redding, CA, USA) mounted on a pin stub mount (Ted Pella, Inc.) using tweezers. We used a Phillips FEI Quanta 200 scanning electron microscope (SEM) on the low vacuum setting to analyze the cuticular scales on the hairs. We qualitatively assessed the SEM images to compare morphological features between natal and mature pelage underhairs and guard hairs. We tried to match the magnifications as closely as possible, although they were ultimately determined by the focal window of the SEM. We visualized scale patterns at the base of each hair. Original SEM images were modified to reorient the sample and remove the debris from the background, using
Adobe Photoshop. The individual hairs and cuticular scale patterns were not modified in the images.

1.3.4 Hair Density

We estimated sea otter hair density following the methods of Liwanag et al. (2012a) and Kuhn (2009) for a subset of the pelt samples (n=18 total): neonates (N=3), small pups (N=3), large pups (N=3), juveniles (N=3), subadults (N=3), and adults (N=3). Pelt samples were trimmed into 2.5 cm x 2.5 cm squares from the original samples. Next, we shaved the subsamples before fixing them in formalin (Buffered 10%; FisherSci) for four weeks in individual glass jars at room temperature. Once a week, we changed out the formalin solutions and flipped the samples to aid in complete tissue penetration. The remaining steps of the histological process were completed by laboratory assistants at the Western Diagnostic Services Laboratory (WDSL) in Santa Maria, CA, USA. Tissue samples were cut into 2.5 x 2.5 mm blocks and processed for paraffin embedding. Paraffin blocks were cut longitudinally at 4.5-5.0 μm, parallel to the skin surface, to visualize cross sections of the hair follicles. Sections were mounted onto slides and stained with hematoxylin and eosin by an autostainer (Leica ST5020 Autostainer; Leica Biosystems©). The microscope slide images were captured using a slide scanner (Aperio GT 450 slide scanner; Leica Biosystems©). WDSL uploaded the digitized microscope slide images to Aperio eSlide Manager (Leica Biosystems© 2006-2020, Version 12.4.4.5015), where we captured the images for manual analysis at 15x magnification (Fig. 1).
To calculate the number of hairs per unit area, we used ImageJ Software (National Institute of Health) to draw a rectangular area (approx. 1,800 μm by 1,000 μm) on each tissue section image. We digitally marked the individual underhairs, intermediate hairs, and guard hairs within the rectangle, using differently sized marks for each hair type. We then used ImageJ to isolate and count the marks (Liwanag et al., 2014). We determined the type of hair based on follicle size within a bundle, using the methods of Erdsack et al. (2015), who described intermediate hairs as being half the size of guard hairs and three times the size of underhairs. Using the known rectangle area and the number of hairs present, we calculated the number of hairs per unit area for each sample. For each sample, we averaged the counts from three microscope slide images originating from similar depths in the skin to produce a single value per individual.

1.3.5 Statistical Analyses

All statistical analyses were performed in R Studio Software (Version 2023.03.0+386). We compared guard hair length, guard hair circularity, and guard hair minor and major diameters among age classes using a one-way ANOVA, followed by a Tukey honestly significant difference test (TukeyHSD). Using the Tukey outputs, we made visualizations of paired comparisons for different age classes (multcompView package). The number of underhairs, intermediate hairs, guard hairs, and hair density were compared using a linear mixed effects model (lmerTest package). The model included age class as a main effect and sea otter sample ID as a random effect. To make pairwise comparisons, we computed estimated marginal means for age class combinations in our models (emmeans package).
1.4 RESULTS

Guard hair length differed significantly across age classes ($F_{5,32}=17.05$, $p<0.001$; Fig. 2). Neonate and small pup pelts had significantly longer guard hairs than adult (p<0.001), subadult (p<0.001), juvenile (p<0.001), and large pup pelts (p=0.004, p<0.001). There was no significant difference in guard hair length between neonate and small pup pelts (p=0.849). Additionally, there was no significant difference in guard hair length among large pup, juvenile, subadult, and adult pelts (p range: 0.514-0.999 for all comparisons).

There was no significant difference in guard hair circularity across ontogeny ($F_{5,32}=0.37$, p=0.864; Table 1). However, there was a significant difference in guard hair minor diameter across age classes ($F_{5,32}=6.17$, $p<0.001$; Table 1). The minor diameter of neonate guard hairs was significantly smaller than the minor diameter of subadult (p=0.038) and adult (p=0.001) guard hairs. Similarly, the minor diameter of small pup guard hairs was significantly smaller than the minor diameter of subadult (p=0.046) and adult (p=0.003) guard hairs. The minor diameter of large pup and juvenile guard hairs was significantly smaller than the minor diameter of adult guard hairs (p=0.03 for both comparisons). Guard hair major diameter was significantly different across age classes ($F_{5,32}=6.06$, $p<0.001$; Table 1). The major diameters of neonate (p=0.0028), large pup (p=0.034), and juvenile (p=0.028) guard hairs were significantly smaller than the major diameter of adult guard hairs but did not significantly differ from subadult. The major diameter of small pup guard hairs was significantly smaller than the major diameter of subadult (p=0.038) and adult (p=0.0022) guard hairs. Across sea otter ontogeny, the
minor and major diameters increased in tandem to generate a similar ellipsoid shape (Fig. 3).

There was no visual difference in cuticular scales between the neonate and adult underhairs (Fig. 4). Both guard hairs and underhairs had similar cuticular scale patterns, for both natal pelage and adult pelage. In particular, the geometric scale patterning of the underhairs appeared qualitatively identical, and the hairs similar in size. The adult guard hair appears to have more cuticular scales total, as the hair is larger in diameter and thus has a larger surface area.

There were visual differences in sea otter hair density across age classes in our longitudinal histological skin sections (Fig. 1). Guard hairs, intermediate hairs, and underhairs were present across all age classes. We observed that hairs were organized into bundles of hair follicles separated by interstitial space, but not every bundle was associated with a guard hair or intermediate hair. The number of underhair follicles appeared to increase across age classes, from neonates through adults (Fig. 1).

Hair density differed significantly across age classes ($F_{5,12}=18.24$, $p<0.001$; Table 2; Fig. 5). Neonate pelts had a significantly lower hair density than juvenile ($p=0.0023$), subadult ($p=0.0013$), and adult ($p<0.0001$) pelts. Small pup, large pup, and juvenile pelts had a significantly lower hair density than adult pelts ($p$ range: 0.0003-0.0474). Small pups also had a significantly lower hair density than subadult pelts ($p=0.0427$). There were no significant differences in hair density among neonates, small pups, and large pups ($p$ range: 0.0745-0.9116), and there were no significant differences among any other age classes ($p$ range: 0.0784-0.9989; Fig. 5).
There was no significant difference in the number of guard hairs present across ontogeny ($F_{5,12}=1.13, p=0.397$). Similarly, there was no significant difference in the number of intermediate hairs present across all age classes ($F_{5,12}=2.54, p=0.085$). However, there was a significant difference in the number of underhairs present across ontogeny ($F_{5,12}=20.33, p<0.001$; Table 2). Adult sea otter pelts had more underhairs compared to all other age classes ($p$ range: $<0.0001-0.0200$). Neonate pelts had significantly fewer underhairs than juvenile ($p=0.0015$), subadult ($p=0.0027$), and adult ($p<0.0001$) pelts. There was no significant difference in the number of underhairs across all other age classes ($p$ range: $0.0594-0.9987$).
Figure 1. Neonate (A), small pup (B), large pup (C), juvenile (D), subadult (E), and adult (F) southern sea otter (*Enhydra lutris nereis*) longitudinal sections from the skin of the dorsal region of the body, visualized on hematoxylin and eosin-stained slides. Arrows and labels in (A) designate a specific type of hair follicle; UH = underhair follicle, IH = intermediate hair follicle, GH = guard hair follicle, IS = interstitial space. All images were taken at 15x magnification.
Table 1. Minor and major diameter (μm) and hair circularity (minor diameter / major diameter) for sea otter guard hairs across ontogeny.

<table>
<thead>
<tr>
<th>Age class</th>
<th>N</th>
<th>Minor diameter (μm)</th>
<th>Major diameter (μm)</th>
<th>Hair circularity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonate</td>
<td>9</td>
<td>24.96 ± 2.49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>86.27 ± 8.01&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.2919 ± 0.038</td>
</tr>
<tr>
<td>Small pup</td>
<td>5</td>
<td>24.13 ± 3.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>81.22 ± 9.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.2969 ± 0.038</td>
</tr>
<tr>
<td>Large pup</td>
<td>5</td>
<td>26.50 ± 4.13&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>89.23 ± 14.07&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.2978 ± 0.036</td>
</tr>
<tr>
<td>Juvenile</td>
<td>6</td>
<td>26.83 ± 3.04&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>89.81 ± 6.96&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.3012 ± 0.050</td>
</tr>
<tr>
<td>Subadult</td>
<td>6</td>
<td>29.61 ± 8.11&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>98.11 ± 24.20&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.2947 ± 0.037</td>
</tr>
<tr>
<td>Adult</td>
<td>7</td>
<td>35.27 ± 5.81&lt;sup&gt;c&lt;/sup&gt;</td>
<td>113.29 ± 12.93&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.3144 ± 0.049</td>
</tr>
</tbody>
</table>

Values are presented as mean ± 1SD. N represents the number of individual pelt samples for each age class. Different superscript letters represent significantly different means among age classes for that measurement. There were no significant differences among age classes for hair circularity.
Figure 2. Sea otter guard hair length (mm) across ontogeny. Bar heights indicate means and error bars indicate standard error for the associated age class: neonate (N=9), small pup (N=5), large pup (N=5), juvenile (N=6), subadult (N=6), and adult (N=7). Different letters above the bars indicate statistically significant differences among means.
Figure 3. Sea otter guard hair cross section across age classes: neonate (N=9), small pup (N=5), large pup (N=5), juvenile (N=6), subadult (N=6), and adult (N=7). Ellipses represent the shape formed by the mean minor diameter (solid arrow) and major diameter (dashed arrow) for each age class at the widest point of the hair shaft. Values next to the arrows for minor and major represent the mean diameters for neonates.
Figure 4. Scanning electron micrographs of sea otter hairs: (A) guard hair from the pelt of a neonate, (B) guard hair from the pelt of an adult, (C) underhair from the pelt of a neonate, and (D) underhair from the pelt of an adult. These images show the cuticular scale patterns on individual hairs. Magnifications differ slightly, as indicated on each micrograph. Magnifications were matched as closely as possible, within the limitations of the microscope.
Figure 5. Sea otter hair density (hairs/mm²) across age classes: neonate (N=3), small pup (N=3), large pup (N=3), juvenile (N=3), subadult (N=3), and adult (N=3). The horizontal line within each box indicates the median value of the interquartile range, and the box boundaries indicate the upper and lower interquartile range. Vertical lines indicate minimum and maximum values within 1.5 times the interquartile range. Individual points are outlier values >1.5 times and <3 times the interquartile range. Diamonds within boxplots indicate the mean values for each age class. Different letters above the boxes indicate statistically significant differences among means.
Table 2. Number of underhairs, intermediate hairs, and guard hairs, and overall hair density (hairs/mm$^2$) for sea otter pelts across ontogeny.

<table>
<thead>
<tr>
<th>Age class</th>
<th>N</th>
<th>Number of underhairs</th>
<th>Number of intermediate hairs</th>
<th>Number of guard hairs</th>
<th>Hair density (hairs/mm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonate</td>
<td>3</td>
<td>665.33 ± 123.14$^a$</td>
<td>9.78 ± 2.73</td>
<td>4.11 ± 1.36</td>
<td>371.65 ± 53.00$^a$</td>
</tr>
<tr>
<td>Small pup</td>
<td>3</td>
<td>1,274.67 ± 203.61$^{ab}$</td>
<td>16.00 ± 5.15</td>
<td>7.56 ± 5.15</td>
<td>637.99 ± 87.45$^{ab}$</td>
</tr>
<tr>
<td>Large pup</td>
<td>3</td>
<td>1,430.38 ± 344.00$^{ab}$</td>
<td>16.50 ± 3.51</td>
<td>3.50 ± 1.77</td>
<td>767.25 ± 191.94$^{ab}$</td>
</tr>
<tr>
<td>Juvenile</td>
<td>3</td>
<td>1,904.88 ± 340.53$^b$</td>
<td>8.38 ± 2.07</td>
<td>5.75 ± 2.19</td>
<td>995.31 ± 188.84$^b$</td>
</tr>
<tr>
<td>Subadult</td>
<td>3</td>
<td>1,890.13 ± 272.14$^b$</td>
<td>19.13 ± 8.90</td>
<td>6.00 ± 1.69</td>
<td>1,079.59 ± 137.05$^{bc}$</td>
</tr>
<tr>
<td>Adult</td>
<td>3</td>
<td>2,863.33 ± 296.87$^c$</td>
<td>20.56 ± 5.79</td>
<td>6.78 ± 0.97</td>
<td>1,449.69 ± 152.03$^c$</td>
</tr>
</tbody>
</table>

Values are presented as mean ± 1SD. N represents the number of individual pelt samples for each age class. Different superscript letters represent significantly different means among age classes for that measurement. There were no significant differences among age classes for the number of intermediate hairs or guard hairs.
Figure 6. Photo of large pup (left) and mother sea otter (right). Note the differences in wet pelage appearance. The natal pelage of the pup is lighter in color, with longer guard hairs that come to a point. The pelage of the mother is more streamlined, and the white oval emphasizes where the dry underhairs at the skin are visible beneath the wet guard hairs on the mother. Photo by Mike Baird, used with permission.
1.5 DISCUSSION

Sea otter fur represents a highly derived form of mammalian fur, as a product of strong selective pressure for the fur to function as an insulator in water. Evolutionary patterns associated with the adaptation of terrestrial mammal fur for aquatic living include the elongation and flattening of guard hairs, the development of cuticular scale patterns that allow underhairs to interlock and trap air, and an increase in hair density (Liwanag et al., 2012a). These patterns are evident in both mustelids (weasels and otters) and pinnipeds (i.e., fur seals), but these adaptations were lost in those pinnipeds (i.e., sea lions and phocids) that switched to blubber as their primary form of insulation in water (Liwanag et al., 2012a,b). The morphological adaptations evident in sea otter fur thus provide a unique insight into the ways evolution can shape an ancestral, synapomorphic trait of mammals.

Despite numerous studies on adult sea otter fur, very little was previously known about the natal pelage and the pelages of intermediate age classes. Previous studies have analyzed sea otter guard hair length for adult sea otters (Tarasoff et al., 1972; Williams et al., 1992; Zagrebelny, 1998; Fish et al., 2002; Kuhn & Meyer, 2010; Liwanag et al., 2012b), but none have investigated the guard hair length of the natal pelage or attempted to approximate when it begins the transition to the adult pelage. In this study, we demonstrated a clear timeline of the transition of sea otter pelage types using guard hair length as a parameter (Fig. 2). Our results suggest that the natal pelage is shed for the adult pelage between the small pup (1-2 months old) and large pup (3-5 months old) age classes (Fig. 2); this is consistent with the estimated time of the molt proposed by previous studies based on behavioral observations of wild otters (Payne & Jameson,
1984; Zagrebelny, 1998). The higher variability in guard hair length for large pups suggests animals in this age class may still have some hairs from the natal pelage (Fig. 2). The contrast between the natal pelage and adult pelage in sea otters is visually apparent (Fig. 6). The longer guard hairs in the natal pelage can be seen coming together to a point, or spike, that corresponds with the top of the dry underfur, and the tip is made up of wet guard hairs (Fig. 6). In contrast, the wet adult pelage is more streamlined (Fig. 6), and the wet tips of the guard hairs lie flat, covering the dry underfur (Kenyon, 1969). If a sea otter grooms sufficiently, the distinction between the dry, lighter gray colored underhairs below the wet, brown guard hairs on both natal and other pelage types is often visible (Fig. 6).

Across ontogeny, the conserved flatness or almond-like shape of sea otter guard hairs in cross section is important for the protection of the air layer (Fig. 3). Our findings are consistent with previously reported morphology values for adult sea otter guard hairs (Williams et al., 1992; Kuhn & Meyer, 2010; Table S1). Sea otters require the guard hairs to lie flat over the underhairs to protect the air layer, which is why the ellipsoid shape of the hair shaft is beneficial across all ages. In individual guard hairs, the cortex elongates as a sea otter ages, and previous studies noted that the shape is consistent across all regions of the pelt (Williams et al., 1992). The absence of arrector pili muscles in sea otters may also play a role in the ability of guard hairs to lie flat over the air layer during submersion in a streamlined way (Kenyon, 1969). For all aquatic mammals using fur as the primary insulator, the flatness of the guard hairs is important for maintaining the air layer (Kuhn & Meyer, 2010; Liwanag et al., 2012a), and the morphology of the hairs allows the fur to function as an insulator in water (Sharma & Liwanag, 2017). The
protective function of guard hairs can also be seen in previous studies that noted mechanical damage to the cuticular scales of river otter guard hairs, while the thin and wispy underhairs had no visible damage (Weisel et al., 2005).

A past study described the cuticular scales of sea otter guard hairs as mosaic and the underhairs as lanceolate (Zagrebelny, 1998). It was unknown whether the natal pelage hairs would exhibit similar patterns to those found on the adult pelage. Using SEM, we found that the cuticular scales on the underhairs of sea otter natal pelage exhibit the same geometric patterns that facilitate interlocking of the underhairs for adult sea otters (Fig. 4). The underhairs themselves and the cuticular scales were similarly sized in both the natal pelage and adult pelage (Fig. 4). The elongated scale patterns of the guard hairs were also similar. On the neonate and adult guard hairs, the scales were similar in size. The adult guard hairs were larger in diameter, allowing for more scales because the total surface area was larger in comparison to the neonate guard hair. This is the first description of this particular adaptation in sea otter natal pelage, which demonstrates that this waterproofing adaptation is present from birth. This makes sense, as even non-aquatic adult mustelids exhibit elongation of the cuticular scales on their hairs (Liwanag et al., 2012a); but it is a novel finding that this structural component is present in the natal coat.

The presence of intermediate hairs has not yet been studied in sea otters. Past research has noted the presence (Liwanag, unpubl. data) and size characteristics (Erdsack et al., 2015) of intermediate hairs in other mammalian species, but no study has estimated the number of intermediate hairs. Our samples had intermediate hairs present across all age classes (Fig. 1; Table 2). Some hair bundles had one intermediate hair present, and
some had one guard hair and one intermediate hair. Future research should investigate the prevalence and function of intermediate hairs in mammals. We also noted that not all hair bundles had an intermediate hair and/or guard hair present (Fig. 1), which is in contrast to previous descriptions of the organization of mammalian hair bundles (Scheffer, 1964; Kuhn et al., 2010; Liwanag et al., 2012a).

The hair density for non-adult sea otters has never been previously estimated. Historically, only adult sea otter hair density values have been estimated, and our results align with previously reported values (Sokolov, 1962; Kenyon, 1969; Tarasoff, 1972; Tarasoff, 1974; Williams et al., 1992; Fish et al., 2002; Kuhn et al., 2010; Table S2). We hypothesized that the adult pelage would have a higher hair density compared to age classes bearing the natal pelage, and that hypothesis was supported by our data (Fig. 5). It appears that the difference in hair density among age classes is driven primarily by a change in the number of underhairs (Table 2). When examining guard hair lengths, we saw a clear transition from the natal to adult pelage between small and large pup pelts (Fig. 2). However, our hair density findings demonstrate that the timeline of the transition from natal to an adult-type pelage may not occur as abruptly as our guard hair length results imply. There is a gradual increase in hair density, driven by increases in the number of underhairs, as a sea otter ages (Fig. 5); this may allow for better waterproofing and insulation in older age classes.

Interestingly, we did not observe a decrease in hair density associated with an increase in body size in sea otters with natal pelage (i.e., from neonate to small pup). This is in contrast with what has been shown for phocid seals, which have a clear transition from lanugo (natal pelage) to the adult-like pelage; lanugo-bearing seal pups have a
decrease in hair density associated with an increase in body size, concomitant with a switch from reliance on fur to a reliance on blubber as the primary insulator (Pearson et al., 2014; Gmuca et al., 2015). Perhaps the lack of a blubber layer necessitates a different ontogenetic pattern of fur density in sea otters. It appears that sea otters are continuing to add more hair follicles as they age, even prior to what we consider the “molt” from the natal pelage to a more mature pelage type. Past observational studies of sea otters have noted a complete change from the natal pelage to an adult-like pelage at 13 weeks of age (Payne & Jameson, 1984). More recent studies have found that the natal pelage begins to shed at 3-11 weeks of age, and the pelage takes several weeks to fully shed (Nicholson et al., 2023). In our study, 11 weeks of age is categorized under the large pup age class. The large pup age class has a guard hair length more similar to that of the adult pelage rather than the guard hairs of the natal pelage. The guard hairs of the natal coat are shed and replaced with hairs more like the guard hairs of adults, and there is a gradual increase in the number of underhairs from birth through to adulthood (Table 2). Therefore, there is no fixed age to describe when sea otter fur transitions to an older pelage type.

Our longitudinal histological samples, which were ideal for determining hair density, did not allow us to examine the presence of sebaceous glands, which have been shown to be important for water repulsion and thermoregulation in an experimental model (Dahlhoff et al., 2016). Sea otter sebum is primarily composed of squalene, which acts to protect the skin and may assist in the water-resisting abilities of the fur in water, in addition to the morphological adaptations described here (Williams et al., 1992; Castellini, 2009). In adult sea otter fur, each guard hair is associated with a sebaceous gland (Williams et al., 1992). Given that the density of guard hairs did not increase with
ontogeny (Table 2), it is likely that the density of sebaceous glands does not change across sea otter age classes. Future research should investigate the role of sebum for sea otters across pelage types.

Overall, these findings shed light on the ontogenetic development of the pelt of the furriest mammal, the sea otter. As sea otter pups have a lower hair density compared to adults, they may be more vulnerable to heat loss; this may explain why sea otter mothers typically keep their younger pups on their bellies and out of the cold water. Alternatively, the longer guard hairs, conserved flatness of the guard hairs, and cuticular structure of the underhairs may thermally compensate for the lower hair density. An increased understanding of the form of sea otter fur is key to assessing the thermal function of the sea otter’s only form of insulation. Future work should examine how these differences in structure affect sea otter fur function.
1.6 ACKNOWLEDGMENTS

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2. EFFECTS OF ONTOGENY AND OILING ON THE THERMAL FUNCTION
SOUTHERN SEA OTTER (*Enhydra lutris nereis*) FUR

2.1 ABSTRACT

For most marine mammals, the use of fur as an insulator has been replaced with more streamlined blubber. Yet the sea otter (*Enhydra lutris*) relies on a dense pelage with an air layer for insulation. Adult sea otters have a different type of pelage than younger sea otters that possess a natal pelage, and these pelage types differ in morphology. In this study, we investigated the ontogenetic changes in thermal function of southern sea otter (*Enhydra lutris nereis*) pelts in air, in water, and when oiled with crude oil. Pelt thermal conductivity, pelt thickness, and thermal resistance were measured for six age classes: neonate (<1 month), small pup (1-2 months), large pup (3-5 months), juvenile (6 months-1 year), subadult (1-3 years), and adult (4-9 years). Pelt conductivity was significantly higher for pelts in air than in water, with oiled pelts having the highest conductivities (p<0.001). Oiled pelts had the lowest thermal resistance which suggests that regardless of age, all sea otters are vulnerable to the effects of oiling (p<0.001). To scale up our laboratory findings, we used a volume-specific geometric model of conductive heat transfer for a simplified sea otter body across age classes and treatments. Neonate, small pup, and large pups are more vulnerable to the effects of oiling compared to older age classes (p<0.0001) due to a higher surface area to volume ratio. The results are consistent with the known thermal conductance values for adult sea otter pelts, yet this is the first time such thermal differences have been demonstrated in nonadult otters. Overall, body
size and age play a more important role in the thermal abilities of sea otters than previously thought.

2.2 INTRODUCTION

The southern sea otter (*Enhydra lutris nereis*) population has increased significantly since 1938, when a small raft of 30-50 otters were first spotted in Big Sur, California after they were presumed extinct (Bolin, 1938). Thanks to determined conservation efforts by government agencies, nonprofit organizations, and the general public, sea otters have been able to expand their range along the coast and the population has increased more than 50-fold (Hatfield et al., 2019). Despite sea otters’ recent success, any substantial population growth is still handicapped by their limited geographic distribution, slow growth rate, and vulnerability to oil spills. The largest threat to sea otters is the potential for a major oil spill event, and this threat is one of the main reasons sea otters were listed as threatened on the Federal Endangered Species Act (Greenwalt, 1977; Van Blaricom & Jameson, 1982).

Sea otters are unique because they are endothermic homeotherms living in cold water (1-16°C), where they are constantly thermally challenged (Costa & Kooyman, 1984; Reidman & Estes, 1990). Seawater conducts heat 25 times faster than air at the same temperature, and a high surface area to volume ratio puts otters at risk of being thermally stressed in the ocean, including the cold waters of the California current (Costa & Kooyman, 1982). Because they rely exclusively on fur for their insulation, sea otters exhibit particular morphological adaptations to their pelage that enable the fur to function as an insulator in water (Liwanag et al., 2012a,b). Fur functions as an insulator by
trapping air between the skin and the surrounding environment (Tregear, 1965; Korhonen & Harri, 1986; Reynolds, 1993; Russell & Tumlison, 1996). Sea otters have the densest fur of any mammal, and the air layer supplies 70% of the overall thermal insulation (Kenyon, 1969; Tarasoff, 1974; Williams et al., 1988; Williams et al., 1992). To maintain the insulative properties of sea otter fur, frequent grooming is necessary (Reynolds & Rommel, 1999). Grooming the pelage serves to clean the fur, maintain the hair loft and air layer, and redistribute the natural oils over the skin and hairs (Williams et al., 1988; Williams & Davis, 1995; Yochem & Stewart, 2009). The unique structure and function of sea otter fur makes sea otters particularly vulnerable to the effects of oil spills. When the fur becomes oiled, it is no longer able to trap air effectively, and the pelt loses its water-repelling qualities (Costa & Kooymen, 1982; Jessup et al., 2012). Previous studies investigated the thermal conductance of adult sea otter pelts and found that oiling of sea otter fur causes up to a 70% decrease in thermal insulation, potentially leading to hypothermia or hyperthermia (Davis et al., 1988; Williams et al., 1988). Despite a wealth of knowledge regarding the functional morphology of adult sea otter pelage and the negative effects of oiling, until recently very little was known about the characteristics of sea otter natal pelage, or the pelage of other age classes (juveniles and subadults).

Sea otters are born with a natal pelage, which they gradually molt into a more adult-like adult pelage by 13 weeks of age (Payne & Jameson, 1984; Chapter 1). Recent research has described the morphological differences between the natal pelage and the adult pelage (Chapter 1). Natal pelage-bearing age classes have longer guard hairs that increase pelt thickness, but their hair density is one-fourth that of adult pelts (Chapter 1). Given that these differences in pelt morphology could affect its thermal function, I
hypothesized that sea otters with natal pelage could be more vulnerable to heat loss, particularly in water and when oiled. The aims of this study were to: (1) compare the thermal function of sea otter fur across ontogeny, in air and in water, (2) examine the effects of oiling on the thermal function of sea otter pelts across ontogeny, and (3) calculate the scaled volumetric heat loss for sea otters across ontogeny in various treatment conditions (in air, in water, oiled). Here, I provide measures of sea otter thermal function and pelt thickness, describe whole-animal heat loss, examine the potential drivers for the differences in insulation effectiveness across age classes, and determine whether certain age classes of sea otters are more vulnerable to the effects of oiling.

2.3 MATERIALS AND METHODS

2.3.1 Sample Collection

In collaboration with California Department of Fish and Wildlife (CDFW), sea otter pelts were collected from San Luis Obispo, Monterey, and Santa Cruz counties from animals that died in the wild or during rehabilitation efforts. In accordance with Section 109(h) of the U.S. Marine Mammal Protection Act (MMPA), the U.S. Fish and Wildlife Service’s regulations implementing the MMPA at 50 CFR 18.22(a), and the U.S. Fish and Wildlife Service’s regulations implementing the U.S. Endangered Species Act at 50 CFR 17.21(c)(3), the samples used to complete this work were collected from fresh, necropsied sea otter carcasses taken from the wild by an official or employee of CDFW in the course of their duties as an official or employee of CDFW. Only pelts considered fresh and in good condition (i.e., not matted or decayed) were used for this study. The original 24 cm x 20 cm pelt samples were collected from the back (dorsal) region of the
animal. The samples were packaged in three layers of plastic food wrap, kept flat (not folded), stored in two-gallon freezer bags, and kept frozen (-20°C to -16°C) until analysis in the various experiments. A total of 44 samples represented six age classes, including neonate pups (<1 month, N=9), small pups (1-2 months, N=5) with the natal pelage, and large pups (3-5 months, N=5), juveniles (6 months-1 year, N=6), subadults (1-3 years, N=10), and adults (4-9 years, N=9) with a mature pelage type, with varying sample sizes dependent on the experiment condition (detailed below). No aged adult sea otter pelt samples were used in this study. Sea otter pelts were categorized into age classes by CDFW employees based on well-established sea otter stranding age estimation protocols that use total body length and tooth development data as identifiers.

2.3.2 Thermal Conductivity and Thermal Resistance

To evaluate thermal function, we measured thermal conductivity ($k_i$) and pelt thickness ($L_i$), and we then calculated thermal resistance ($R$) for each pelt. Thermal conductivity (W·m⁻¹·°C⁻¹) is a material property that describes how easily heat moves through a particular substance, independent of thickness (Kvadsheim et al., 1994). Thermal resistance (m²·°C·W⁻¹), also called thermal insulance, describes how well a material resists the flow of heat, and it incorporates both the thermal conductivity and the thickness of the material. Thermal resistance is the inverse of thermal conductance ($c$), or heat transfer (Scholander et al., 1950). Note that thermal conductivity is sometimes confused with thermal conductance, but the latter varies with the thickness of the material whereas the former is independent of thickness (Kvadsheim et al., 1994). Because of this,
we used thermal conductivity to compare the efficiency of the pelts, and we calculated thermal resistance to compare their overall effectiveness.

We measured thermal conductivity of each pelt in air, in water, and after application of crude oil to the fur, using the standard material method (Kvadsheim et al., 2002; Liwanag et al., 2012a,b; Sharma & Liwanag, 2017). Original pelt samples were trimmed into 15 cm x 15 cm squares from the original dorsum pelt swatches. We washed each pelt in cold running water to remove any sand or dirt. We gently dabbed the fur with paper towels to help dry the pelt samples. We then used a hair dryer (Trezero® 2200-Watt ceramic tourmaline blow dryer) on the cool setting to dry the pelt, as it has been found that blow drying will help restore the insulating air layer in the pelage (Williams & Davis, 1995). Most in-air, in-water, and crude oil trials were performed on the same day for each pelt, to reduce the effects of freeze-thaw cycles on the pelt. For in-air trials, all 44 sea otter pelts were included: neonates (N=9), small pups (N=5), large pups (N=5), juveniles (N=6), subadults (N=10), and adults (N=9). Because the condition of some of the pelts were deemed unusable due to deterioration of the pelt quality after in-air trials, we had varying sample sizes for in-water and oiled trials. A total of 35 sea otter pelts were used for in-water trials: neonates (N=8), small pups (N=5), large pups (N=5), juveniles (N=5), subadults (N=7), and adults (N=5). A total of 35 sea otter pelts were used for oiled trials: neonates (N=7), small pups (N=5), large pups (N=5), juveniles (N=5), subadults (N=8), and adults (N=5).

To measure pelt thickness ($L_i$), we took measurements of the skin and the fur loft to the nearest 0.01 mm from the right, left, and posterior sides of the pelt (three measurements on each side, nine total) using digital calipers (Absolute Digimatic Caliper
Mean pelt thickness values were used in calculations. We calculated dry pelt thickness as the skin thickness plus the fur loft (to the top of the guard hairs). To calculate wet pelt thickness for in-water and oiled trials, we measured fur loft using a blunt probe and a ruler while the pelt was set up in the apparatus with water on top of the air layer; this ensured we recorded the true thickness of the pelt during submergence. We estimated wet pelt thickness (for both in-water and oiled trials) by summing skin thickness (measured outside the apparatus) with the fur loft measured in the apparatus.

To collect thermal conductivity measurements, we used a heat flux chamber with a highly insulated lower compartment and chilled upper compartment, described in Pearson et al. (2014). The lower insulated compartment contained a heat source of a sealed aluminum box with heated water to mimic mammalian body temperature (37°C), maintained with a circulating water bath (model SD07R-20; PolyScience, Niles, IL, USA). The chilled upper compartment contained ice packs to produce a consistent thermal gradient in the apparatus. We used an elastomer (Plastisol vinyl; Carolina Biological Supply, Burlington, NC, USA) as the standard material ($k_{\text{standard}} = 0.099231361 \pm 0.01459027 \text{ W}\cdot\text{m}^{-1}\cdot\text{°C}^{-1}$, $L_{\text{standard}} = 0.00790478 \pm 0.00014422 \text{ m}$). We placed the standard material on top of the heat source, and placed each pelt sample on top of the standard material (Pearson et al., 2014). To measure temperature, we arranged copper-constantin (Type T) thermocouples (Physitemp Instruments, Inc., Clifton, NJ, USA) at the different interfaces. We placed three thermocouples between the surface of the heat source and the standard material, three thermocouples between the standard material and the pelt sample, and three thermocouples directly on top of the fur (Pearson et al., 2014).
Additionally, we placed two thermocouples on the surface of the skin, at the base of the fur. To measure the heat flux through the sample, we placed a heat flux disc (Thermonetics Inc., San Diego, CA, USA) between the pelt and the standard material, and another heat flux disc on top of the pelt. We recorded the outputs for all thermocouples every 6 s onto a desktop computer, using a Fluke Hydra data logger (model 2625A; Fluke Inc., Everett, WA, USA). Trials proceeded for a minimum of 2 hours to confirm that the apparatus reached a steady equilibrium state, and data were analyzed for the final 30 min of each trial.

Using the Fourier equation, we calculated thermal conductivity across the pelt (Kreith, 1958):

\[ H = k_i \cdot A \cdot \Delta T \cdot L_i^{-1} \]  

(2)

where \( H \) is heat transfer (J·s\(^{-1}\)), \( k_i \) is thermal conductivity (W·m\(^{-1}\)·°C\(^{-1}\)), \( A \) is area through which the heat is dissipating (m\(^2\)), \( \Delta T \) is the temperature differential (°C) across the pelt, and \( L_i \) is pelt thickness (m), i.e., the sum of skin thickness and hair loft, for a given treatment (in-air, in-water, oiled). Heat transfer was assumed to be to be equal across the standard material and pelt sample, and the equations were set equal to solve for pelt thermal conductivity (Scholander et al., 1942; Scholander et al., 1950; Hart & Irving, 1959; Bryden, 1964; Worthy, 1991; Kvadsheim et al., 1994). To account for changes in insulation due to changes in the insulation of the fur with development, we calculated thermal resistance (\( R \); m\(^2\)·°C·W\(^{-1}\)) for each pelt using the equation:

\[ R = L_i \cdot k_i^{-1} \]  

(3)

For in-water trials, we gently poured 40 mL of freshwater at 10°C on top of the pelt, and then placed three thermocouples just on top of the visible, silvery air layer. We
used freshwater to ensure consistency in the thermal conductivity of the water for comparison across samples; thermal conductivity is inversely related to the salinity of water (IAPWS, 2015). For these trials, we only used the heat flux disc between the pelt and the standard material. We did not place a heat flux disc on top of the pelt for in-water and oiled treatments to avoid disturbing the air layer on top of the pelt. For crude oil trials, we placed plastic food wrap and sealant (Loctite® clear silicone waterproof sealant, Westlake, OH, USA) underneath the pelt to ensure no crude oil seeped onto the thermocouples beneath (Pearson et al., 2019); we cut out a 5 cm x 5 cm square in the plastic food wrap to ensure the thermocouples were in contact with the underside of the skin. We applied 10 mL of Scott Well unrefined crude oil (Texas Raw Crude©, Midland, TX, USA) at 25°C to the pelt using a syringe, and gently massaged the oil into the fur for 30 seconds, similar to the grooming movements performed by a sea otter, ensuring the oil was evenly distributed across the pelt sample. Next, we placed 40-60 mL of water at 10°C on top of the oiled fur using a plastic transfer pipette. We placed three thermocouples on top of the wet, flattened fur, similar to the in-water treatment.

Once an oiled trial was completed, we washed the pelt using Dawn® dishwashing detergent (Dawn® Ultra Dishwashing Liquid, Proctor & Gamble, Cincinnati, OH, USA). All crude oil hazardous waste was collected and properly disposed using PPE (gloves, goggles, facial coverings). We applied Dawn® to the oiled pelt using a syringe, and we gently massaged small amounts of the detergent into the pelt. We then used cold running water to remove the detergent, and repeated adding small amounts of Dawn® at a time followed by rinsing. Time spent washing and the amount of Dawn® needed to wash the pelt were recorded. Washed pelts were considered clean when no oil or soap residue was
visible and the water coming off the pelt was clear. We conducted additional visual inspections of the pelt after the pelt was patted dry with paper towels, to ensure all oil and soap was removed before blow-drying the pelt.

2.3.3 Heat Loss Model

To scale our laboratory-based thermal conductivity findings to the whole animal level, we estimated whole-body conductive heat loss through the pelt in air and in water following the methods in Pearson et al. (2019). We calculated total conductive heat transfer \( \text{Heat}_{\text{tot}}; \text{W/m}^3 \) for sea otters as heat loss through a layer of insulation surrounding a body divided up into 3 parts: head (sphere), trunk (cylinder), tail (cone).

We followed Eq. 27 from Mathewson and Porter (2013) and modelled heat flux \( Q; \text{W} \) for each body region (Fig. 7; see Supplemental Tables). The heat flux of a sphere \( Q_{\text{sphere}}; \text{W} \) was calculated as:

\[
Q_{\text{sphere}} = \frac{T_{MB} - T_A}{\frac{1}{4\pi k_i} \left( \frac{1}{R_{hc}} - \frac{1}{R_{ht}} \right)}
\]

where \( T_{MB} \) is the temperature at the muscle-skin interface (°C), \( T_A \) is the ambient temperature (set to 16°C for air and 13°C in water), \( k_i \) is the thermal conductivity (W·m\(^{-1}\)·°C\(^{-1}\)) for a given treatment (in-air, in-water, oiled), \( R_{hc} \) is the head core radius (m), and \( R_{ht} \) is the total head radius (m) including separate pelt thickness for each treatment (\( L_i; \text{m} \)).

The pelt thickness values were collected from the back region of the pelts. To account for known differences in pelt thickness along the body, we multiplied the pelt thickness by \( \frac{3}{4} \) to account for the decrease in pelt thickness on the head and tail. We used the subcutaneous temperature \( T_{sq} \) value (36.5°C) from Costa and Kooyman (1982) as \( T_{MB} \). We averaged head girth values from data taken during routine CDFW live captures of
adult (N=10), subadult (N=10), and large pup (N=1) sea otters collected in November 2022. For the neonate and small pup age classes, we estimated the head girth using the difference between juvenile and large pup age classes and subtracted that difference. We used the original head radius from the head girth values to estimate \( R_{hc} \) and \( R_{ht} \). To calculate \( R_{hc} \), we used the head radius and subtracted the wet pelt thickness. We estimated all core radius values because the hair loft was flattened during girth data collection, and we subtracted the wet pelt thickness from each shapes’ core radius. The heat flux of a cylinder (\( Q_{cyl} \); W) was calculated as:

\[
Q_{cyl} = 2\pi \cdot H_{cyl} \cdot k_i \left[ \frac{T_{MB} - T_A}{ln\left(\frac{R_{ac}}{R_{at}}\right)} \right]
\]

where \( H_{cyl} \) is the cylinder height (m) or trunk length, \( R_{ac} \) is the axillary core radius (m), and \( R_{at} \) is the total axillary radius (m) which includes pelt thickness. To calculate \( H_{cyl} \), we used the total body length (\( L_{body} \); m) and subtracted cone height (\( H_{cone} \); m) and head diameter. We used \( L_{body} \) and tail length values from the animals from which the pelts were collected in the present study, from CDFW live sea otter captures, and live neonate (N=22), small pup (N=21), and large pup (N=12) rehabilitation patients under the Sea Otter Research and Conservation (SORAC) program in Monterey Bay Aquarium in Monterey, CA. To calculate \( R_{ac} \), we used axillary girth values from CDFW live otter captures, and live sea otter data collected from SORAC patients. The heat flux of a cone (\( Q_{cone} \); W) was calculated as:

\[
Q_{cone} = \left[ \frac{\pi k_i (T_{MB} - T_A)}{H_{cone}} \right] (R_{tc} \cdot R_{tt})
\]

where \( H_{cone} \) is the cone height (m) or tail length, \( R_{tc} \) is the tail core radius (m), and \( R_{tt} \) is the total tail radius (m) which includes pelt thickness. To calculate \( R_{tc} \), we made a ratio of
the known axillary girth and known tail girth from an adult otter to estimate \( R_{tc} \) for other age classes. For \( H_{cone} \), we used tail length values collected from the present study pelts. If any values were missing, we averaged the values of that parameter by age class. To calculate the volume of each shape, we used the known volume equations of a sphere, cylinder, and cone. To calculate the total heat loss for each treatment, we summed all the heat flux values from a sphere, cylinder, and cone, and divided by the sum of the volumes of a sphere, cylinder, and cone.

2.3.4 Statistical Analyses

All statistical analyses were performed in R Studio Software (Version 2023.03.0+386). Thermal conductivity, thermal resistance, pelt thickness, and heat transfer were compared using a linear mixed model (lmerTest package). The model included treatments (in air, in water, oiled) and age classes as main effects, along with all possible interactions, and sea otter sample ID as a random effect. To make pairwise comparisons, we computed estimated marginal means for treatment and age class combinations in our models (emmeans package). To compare for differences across age classes in the amount of Dawn\textsuperscript{®} needed to clean each pelt and the amount of cleaning time after the crude oil treatment, we used a one-way ANOVA followed by a Tukey honestly significant difference test. To investigate the correlation between sea otter hair density and thermal conductivity, we ran a linear regression model. Hair density values were from a previous study on the same pelt samples, and values were matched by individual (Chapter 1).
Figure 7. Geometric representations of sea otter body shape used to model heat loss across ontogeny. All of the models simulate a body core surrounded by a pelt layer. (A) The three shapes (sphere, cylinder, cone) used to estimate heat loss with total body length ($L_{\text{body}}$). (B) Spheroid model used to represent the head of the otter, as described in Eq. 4. (C) Conical model of heat loss used to represent the tail, as described in Eq. 6. (D) Cylindrical model of heat loss used to represent the trunk, as described in Eq. 5.
2.4 RESULTS

Thermal Conductivity and Thermal Resistance

There was a significant difference in sea otter pelt thermal conductivity across age classes (F_{5.30}=3.04, p=0.025; Fig. 8), treatment (F_{2.58}=210.77, p<0.001), and for the age class and treatment interaction (F_{10.58}=3.91, p<0.001). There was a significant difference among age classes within the in-air treatment, such that neonate pelt thermal conductivity in air was significantly higher than juvenile (p=0.006) and subadult (p=0.001) pelt conductivities in air. There was no significant difference in pelt thermal conductivity among age classes for the in-water treatment (p range: 0.9936-1.000), or for the oiled treatment (p range: 0.0732-1.000).

Pelt thermal conductivities were significantly lower in water than in air across all age classes (p<0.001; Fig. 8). Additionally, thermal conductivity of pelts in water was significantly lower than for oiled pelts across all age classes (p<0.001). Subadult pelt thermal conductivity in air was higher than that for all other age classes in water (p range: <0.0001-0.0483) except for adult pelt thermal conductivity in water (p=0.0921). Juvenile pelts had a higher thermal conductivity in air than subadult (p=0.006) and neonate (p=0.001) pelt thermal conductivities in water. Adult, large pup, small pup, and neonate pelt thermal conductivities in air were significantly higher than that for all other age classes in water (p<0.001 for all comparisons). Within each age class, there was no significant difference between in-air and oiled thermal conductivity values (p range: 0.0647-1.000). Adult pelt thermal conductivity in air was significantly higher than large pup (p=0.0425) oiled pelt thermal conductivity. Subadult and juvenile pelt thermal conductivities in air were significantly higher than adult (p=0.013, p=0.03), large
pup \((p<0.001, p<0.001)\), and small pup \((p=0.004, p=0.012)\) oiled pelt thermal conductivities. Large pup, small pup, and neonate pelt thermal conductivities in air were not significantly different from any oiled pelt thermal conductivities across age classes \((p \text{ range: } 0.4662-1.000)\).

There was no significant difference in thermal resistance across age classes \((F_{5,35}=0.91, p=0.487; \text{Fig. 9})\) and no significant interaction between age class and treatment \((F_{10,61}=1.04, p=0.423)\). However, there was a significant difference in thermal resistance across treatments \((F_{2,61}=307.65, p<0.001)\). The thermal resistance of pelts in air was significantly higher than that of pelts in water \((p<0.001)\) and that of oiled pelts \((p<0.001)\), and the thermal resistance of pelts in water was significantly higher than that of oiled pelts \((p<0.001)\).

There was a significant interaction between age class and treatment for pelt thickness \((F_{10,64}=13.42, p<0.001; \text{Table 3})\). There was a significant difference in pelt thickness across age classes \((F_{5,33}=9.72, p<0.001)\) and among treatments \((F_{2,64}=1,252.65, p<0.001; \text{Table 3})\). Overall, pelt thickness decreased significantly when submerged and after oil application \((p<0.001 \text{ for all comparisons})\). Neonate and small pup pelts \((p<0.001 \text{ for all comparisons})\) had significantly greater dry pelt thickness compared to all older age classes.

There was no significant difference in the amount of Dawn® used to clean the pelts after the oiled treatment across age classes \((F_{5,29}=0.54, p=0.741; \text{Table 4})\). However, there was a significant difference in the amount of time necessary to fully rid the pelts of crude oil \((F_{5,29}=3.48, p=0.0138)\). The amount of cleaning time for neonate \((p=0.0181)\)
and small pup (p=0.0161) pelts was significantly less than the time required to clean subadult pelts (Table 4).

Heat Loss Model

There were significant differences in the heat loss model across age class (F_{5,30}=35.44, p<0.001; Fig. 10), treatment (F_{2,66}=424.07, p<0.001), and for the age class and treatment interaction (F_{10,66}=25.06, p<0.001). Total heat loss was significantly higher when oiled than in air (p range: <0.0001-0.0004) and in water (p range: <0.0001-0.0140) across all age classes. Neonate, small pup, and large pup heat loss when oiled was significantly higher than that of all older age classes when oiled (p<0.0001 for all comparisons). For both in air and in water, neonates had significantly more heat loss than juveniles (p=0.003, p=0.0467), subadults (p<0.0001, p<0.0010), and adults in air and in water (p<0.0001, p=0.0269). In water, neonates and small pups had significantly more heat loss than juveniles, subadults and adults (p range: 0.0001-0.0091). There was no significant difference for heat loss in air and in water across all age classes (p range: 0.6157-1.0000).

The regression analysis revealed that sea otter thermal conductivity was negatively correlated with hair density (F_{1,40}=13.87, r^2=0.2389, p=0.0006; Fig. 11), according to the equation k = -0.00004434(hair density) - 0.1877.
Table 3. Thicknesses (mm) of sea otter pelts (fur and skin) when dry, when submerged, and when treated with crude oil.

<table>
<thead>
<tr>
<th>Age class</th>
<th>N dry</th>
<th>Dry pelt thickness (mm)</th>
<th>N submerged</th>
<th>Submerged pelt thickness (mm)</th>
<th>N oiled</th>
<th>Oiled pelt thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonate</td>
<td>9</td>
<td>42.05 ± 2.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8</td>
<td>8.11 ± 2.17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7</td>
<td>7.32 ± 1.12&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Small pup</td>
<td>5</td>
<td>43.12 ± 6.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5</td>
<td>9.12 ± 2.58&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5</td>
<td>8.24 ± 2.24&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Large pup</td>
<td>5</td>
<td>33.80 ± 6.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5</td>
<td>7.81 ± 2.66&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5</td>
<td>7.58 ± 1.30&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Juvenile</td>
<td>7</td>
<td>30.04 ± 2.38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5</td>
<td>7.99 ± 1.54&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5</td>
<td>7.37 ± 1.39&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Subadult</td>
<td>9</td>
<td>28.85 ± 2.47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7</td>
<td>8.88 ± 2.46&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8</td>
<td>8.55 ± 2.01&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Adult</td>
<td>9</td>
<td>30.86 ± 2.77&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5</td>
<td>9.33 ± 0.81&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5</td>
<td>8.16 ± 1.50&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values (mean ± 1SD) are provided for each age class. N represents the number of individual pelt samples for each age class classified by treatment. Pelt thickness decreased significantly when wet and when oiled, compared to the dry pelt, for all age classes. Different superscript letters represent statistically significantly different means among age classes, within a treatment.
Table 4. The amount of Dawn® (mL) required to remove crude oil from the pelts, and the amount of time (min) needed to clean the pelts.

<table>
<thead>
<tr>
<th>Age class</th>
<th>N</th>
<th>Amount of Dawn® (mL)</th>
<th>Clean time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonate</td>
<td>7</td>
<td>9.86 ± 2.73</td>
<td>6.50 ± 1.89</td>
</tr>
<tr>
<td>Small pup</td>
<td>5</td>
<td>11.40 ± 5.55</td>
<td>6.20 ± 1.10</td>
</tr>
<tr>
<td>Large pup</td>
<td>5</td>
<td>12.80 ± 4.55</td>
<td>7.60 ± 0.89</td>
</tr>
<tr>
<td>Juvenile</td>
<td>5</td>
<td>10.60 ± 2.79</td>
<td>7.30 ± 0.57</td>
</tr>
<tr>
<td>Subadult</td>
<td>8</td>
<td>13.00 ± 5.13</td>
<td>9.06 ± 1.57</td>
</tr>
<tr>
<td>Adult</td>
<td>5</td>
<td>12.40 ± 4.77</td>
<td>7.60 ± 1.64</td>
</tr>
</tbody>
</table>

Values (mean ± 1SD) are provided for pelt samples used in oiled thermal conductivity trials. N represents the number of individual pelt samples for each age class. Different superscript letters represent statistically significantly different means among age classes.
Figure 8. Thermal conductivity (W·m⁻¹·°C⁻¹) of sea otter pelts across ontogeny in air (gray), in water (blue), and after oiling with crude oil (brown). Heights of the bars and lines indicate means and standard errors for associated treatment and age class: neonate (N=9 in air, N=8 in water, N=7 oiled), small pup (N=5 for all treatments), large pup (N=5 for all treatments), juvenile (N=6 in air, N=5 in water, N=5 oiled), subadult (N=10 in air, N=7 in water, N=8 oiled), and adult (N=9 in air, N=5 in water, N=5 oiled). Different letters above the bars indicate statistically significant differences among means.
Figure 9. Thermal resistance ($m^2 \cdot ^\circ C \cdot W^{-1}$) of sea otter pelts across ontogeny in air (gray), in water (blue), and after oiling with crude oil (brown). Heights of the bars and lines indicate means and standard errors for the associated treatment and age class: neonate (N=9 in air, N=8 in water, N=7 oiled), small pup (N=5 for all treatments), large pup (N=5 for all treatments), juvenile (N=6 in air, N=5 in water, N=5 oiled), subadult (N=10 in air, N=7 in water, N=8 oiled), and adult (N=9 in air, N=5 in water, N=5 oiled). Different letters above the bars indicate statistically significant differences among means.
Figure 10. Heat loss per unit volume (W/m$^3$) of sea otter pelts across ontogeny in air (gray), in water (blue), and after oiling with crude oil (brown). The horizontal line within each box indicates the median value and the box boundaries indicate the upper and lower interquartile range. Vertical lines indicate minimum and maximum values within 1.5 times the interquartile range. Individual points are outlier values >1.5 times and <3 times the interquartile range. Diamonds within boxplots indicate the mean values for each age class. Different letters above the boxes indicate statistically significant differences among means.
Figure 11. Correlation between hair density (hairs·mm\(^{-2}\)) and thermal conductivity (W·m\(^{-1}\)·°C\(^{-1}\)) of sea otter pelts in air (p=0.0006). Each sample is represented by a single symbol, and different symbols and colors represent age classes. Line represents the best-fit linear regression.
2.5 DISCUSSION

Thermal Function of Sea Otter Pelts

This study compared the insulative properties of southern sea otter pelage across ontogeny in three different treatments: in air, in water, and oiled. The higher conductivity of neonate pelts compared to juvenile and subadult pelts suggests that the natal pelage is a less efficient insulator in air compared to the pelts of older age classes (Fig. 8). This increased conductivity is due to the lower hair density of the natal pelage compared to adult pelage (Chapter 1), as thermal conductivity is inversely related to hair density (Fig. 11). However, the greater loft of the natal pelage (Table 3) resulted in similar thermal resistance, and thus similar insulative effectiveness, across all age classes (Fig. 9). The longer guard hairs present in neonate and small pup pelts provide a thicker insulating layer of air in the fur. The fluffy nature of sea otter natal pelage makes the overall insulation equivalent to the adult pelage, even with a lower hair density.

Living in water is thermally challenging due to the higher thermal capacity and conductivity of water compared to air. Sea otter pelage plays an important role in insulation, helping to minimize contact between the cold ocean water and skin. Surprisingly, sea otter pelt thermal conductivity in freshwater was lower than in air across age classes (Fig. 8). Inside the thermal conductivity apparatus, the temperature-reading thermocouples were submerged and placed directly against the flattened air layer after cold water was applied. Sea otter fur maintains a stagnant layer of air, even when underwater, to prevent heat loss (Hammel, 1955; Ling, 1970; Kvadsheim & Aarseth, 2002). When sea otter fur is submerged, it becomes compressed and lies flat against the body (Table 3). The individual hairs overlap to prevent water penetration because the
interstitial space between the underhairs decreases, and water has a high surface tension (Davis, 2019). Our results suggest that the intricate interlocking nature of the underhairs is capable of trapping warm air at the base of the skin for prolonged periods of time if the air layer is not disturbed. When the pelts were submerged in water, the air layer was compressed but still maintained a similar thermal gradient across that smaller distance (16°C in air vs. 13°C while submerged, on average); this resulted in lower pelt thermal conductivity values in water (Fig. 8).

Due to the lower hair density of the natal pelage, we originally predicted that sea otter pelts with the natal pelage would be less insulative compared to mature pelts. Instead, there was no difference in sea otter pelt thermal resistance in water across ontogeny. The difference in the pelt thickness for dry versus wet fur causes the fur to be less effective underwater (Fig. 9). The air layer is still trapping enough air to sustain the natural water repelling qualities of the fur even under compression at the surface. Note, however, that the hydrostatic pressure imposed on the pelt during diving would cause the air layer to become further compressed, and at least some of the air would likely bubble out of the fur during the ascent of a dive (Liwanag et al., 2012a; Nankey et al., 2021). The compression at depth and the loss of air upon ascent would necessitate grooming to restore the air layer and the insulative properties of the pelt after a dive.

*Effects of Oiling on Sea Otter Pelts*

The air layer of the fur must be preserved and well-groomed to minimize heat loss in water (Hammel, 1955). When sea otter fur becomes fouled by crude oil (or other petroleum products), there is a detrimental loss in the insulative properties of the fur.
Previous studies found that oiling of sea otter fur causes a 70% decrease in thermal insulation, potentially leading to hypothermia (Davis et al., 1988; Williams et al., 1988). Our findings are consistent with previous research (Williams et al., 1988) that found sea otter pelts have poorer thermal capabilities when oiled compared to intact pelts in air and in water (Fig. 9), demonstrating that the presence of oil in the fur reduces the ability to maintain the air layer and the natural waterproofing feature of the pelt. We predicted that the oiled natal pelage would be less efficient at insulating compared to oiled mature pelts. The application of crude oil removed the air layer from the fur and caused a 5-fold reduction in the thermal resistance compared to pelts in air (Fig. 9). This suggests that all sea otters may be vulnerable to the effects of oiling, regardless of age.

In addition, the amount of insulation provided by hairs varies with pelt thickness and hair density (Davis, 2019). Crude oil ruins the fur’s waterproofing and insulation abilities. Oiled northern sea otters (*Enhydra lutris kenyoni*) are more likely to haul out of the water to reduce body heat loss due to the reduction in insulatory abilities caused by the cold seawater and oil contamination (Costa & Kooyman, 1982). If there is an oil spill event in the range of southern sea otter populations, the ambient air temperature could cause hypothermia to oiled otters. To compensate for heat loss, sea otters will consequently need to increase metabolic rates (Siniff et al., 1982). This is difficult due to the fact that sea otters are already thermally compromised because of their increased lower critical temperature of 20°C (Costa & Kooyman, 1982), and high thermal liability (Liwanag, 2010). The present study only focused on the external effects of oil to sea otter fur in a laboratory-based setting. Crude oil exposure can cause severe physiological
issues to marine wildlife, and we did not investigate any internal toxicological impacts on sea otters.

Previous research has demonstrated the benefits of cleaning oiled sea otter pelts with Dawn® dish soap (Williams et al., 1998; Jessup et al., 2012), and the use of Dawn® for cleaning oiled wildlife has become standard practice for wildlife rehabilitators (Newman et al., 2003; Jessup et al., 2012; Tseng and Ziccardi, 2020). We found that a similar amount of Dawn® allowed us to fully clean the natal pelage of oil in less time than required to fully clean the more mature pelage (Table 2), likely due to the lower hair density of the natal pelage (Riordan et al., 2023). It is important to note that Dawn® would also remove the natural oils from the pelt, and those natural oils are likely important for fully restoring the waterproofing properties to the pelt; this requires otters in rehabilitation to exhibit their natural grooming behaviors in order to restore their insulation (Jessup et al., 2012).

**Scaling Up: Whole Body Insulation**

Protection from heat loss is an important concern for sea otters, as endotherms living in water. Water has a high thermal conductivity compared to air, and this creates a dynamic thermal challenge for sea otters to maintain a body temperature of 38.1°C (averaged from values in Morrison et al., 1974; Costa & Kooyman, 1982; Davis et al., 1988). It is difficult for smaller animals to maintain a body temperature higher than that of their environment, due to a high surface area to volume ratio (SA:V) (Schmidt-Nielsen, 1990). Although our thermal resistance results were similar across age classes, these measurements in the laboratory are for pelt samples of the same surface area, and
therefore do not account for differences in SA:V when scaled up to a whole animal. Using our heat loss model, we scaled our laboratory-based results to the whole animal (Fig. 7). Our findings have conservation implications, as they indicate younger sea otters are more vulnerable to the negative effects of oiling due to their large SA:V (Fig. 10). Previous research noted that sea otters transition from the natal pelage to a more adult-like pelage sometime between the small pup and large pup age classes (Chapter 1). However, we still see that large pups are experiencing similar heat loss values to younger age classes (Fig. 10), even with pelage morphology that resembles the adult pelage (Chapter 1). The body size of large pups is still much smaller than that of older age classes, and the higher SA:V in large pups increases their total heat loss. Thus, sea otter body size apparently contributes more to their overall rates of heat loss than the hair density of the pelt. Indeed, sea otters have a larger body size compared to their semi-aquatic and terrestrial relatives, reducing their SA:V and thus heat loss (Pabst et al., 1999).

For adult sea otters, a smaller SA:V is beneficial because it decreases the amount of heat loss to the environment (Worthy & Edwards, 1990; Pabst et al., 1999). Our thermal findings may explain why sea otter mothers keep small-bodied pups on their bellies and out of the water, because the fur is a better insulator when dry (Fig. 9). Past observations have noted that sea otter mothers carry their pups high on their chest, out of the water while resting and swimming (Kenyon, 1969), and they spend a substantial amount of time grooming the pup’s fur (Sandegren et al., 1973) potentially confirming the importance of keeping the pup’s fur as dry as possible.
Implications

Despite the physiological challenges associated with the use of fur as the primary insulator in water, sea otters have remained successful due to the morphology and thermal function of their unique pelage. The body size of sea otters plays an important role in the amount of heat loss to their environment, making young sea otters more prone to heat loss. Regardless of age class and pelage type, all sea otters are susceptible to negative anthropogenic events like oil spills that can cause detrimental fouling of the fur. This is because the maintenance of air trapped in the fur is critical for thermal insulation and survival. As a result of the infamous Exxon Valdez oil spill, sea otters experienced the highest direct mortality of any mammal, primarily due to their inability to effectively thermoregulate when oiled (Garrott et al., 1993; Williams and Davis, 1995). Within the last fifty years, there have been nine large-scale oils spills (≥5,574,000 gal) in the Pacific Ocean that have affected the U.S. (California Coastal Commission). Of those nine oil spills, eight oil spills have been in the vicinity of the southern sea otter range, and there are multiple oil platforms in proximity to the population range off Point Conception (Fig. S1). Although all sea otters are more vulnerable and more likely to suffer negative consequences compared to older sea otters in the event of an oil spill due to their higher SA:V and overall rates of heat loss.
2.6 ACKNOWLEDGMENTS

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3. A NOVEL COMPARISON OF SOUTHERN SEA OTTER (*Enhydra lutris nereis*)

FUR BUOYANCY ACROSS ONTOGENY

3.1 ABSTRACT

Aquatic animals have developed various buoyancy mechanisms to facilitate controlled maneuverability in the water. Sea otters are extremely positively buoyant and spend most of their time resting at the water surface. It is understood that some of this buoyancy comes from the air layer that otters maintain in their pelage, with the lungs providing an additional source of positive buoyancy. Past studies have investigated the fur buoyant force in adult otters; however, not much is known about the fur buoyant force in younger sea otter age classes, which have a different pelage type compared to adults. This study compared ontogenetic changes in the fur buoyant force of southern sea otter (*Enhydra lutris nereis*) pelage. We measured the fur buoyant force of pelt samples, scaled that to the whole animal, and calculated mass-specific fur buoyant force for six age classes: neonates (<1 month, N=9), small pups (1-2 months, N=5), large pups (3-5 months, N=5), juveniles (6 months-1 year, N=6), subadults (1-3 years, N=6), and adults (4-9 years, N=6). Each pelt sample was measured under three treatment conditions: control, oiled, and washed with Dawn® dish soap. Oiled and washed pelts had a lower fur buoyant force compared to the control pelts across all age classes (p<0.001). When oil fouls the fur, the air layer of the otter pelt is ruined and no longer provides sufficient positive buoyancy or thermal insulation for the animal. Pelts washed with Dawn® had higher variability in buoyant force compared to other conditions, and the washed treatment did not restore the air layer consistently. When we scaled up to mass-specific fur buoyant force, we found
that younger age classes are more buoyant due to their larger surface area to volume ratio. These differences in buoyancy may underlie variations in energetic costs and also behavior among sea otters across development.

3.2 INTRODUCTION

The regulation of buoyancy is important for animals to effectively navigate the aquatic environment. Aquatic animals have evolved different physiological mechanisms and/or anatomical structures to regulate their buoyancy (Beck et al., 2000). For example, teleost fish have a gas-filled swim bladder, some cephalopod shells have gas-filled chambers, and sharks have hydrofoils or squalene to aid in buoyancy regulation (Alexander, 1982; Alexander, 1990). In the water column, a positive buoyant force acts on animals in a vertical direction (i.e., toward the surface of the water), and this force can be generated by the animal’s body volume or weight, tissue composition, and respiratory systems (Williams, 2001). The buoyancy of aquatic animals can also affect locomotory strategies, such as glide duration or stroke frequency (Williams et al., 2000; Adachi et al., 2014).

Marine mammals face unique challenges in water compared to other aquatic species, as they must return to the surface to breathe (Williams, 2001). Buoyancy plays an important role in reducing energetic costs for marine mammals during swimming and diving (Castellini & Mellish, 2015). Deep-diving mammals can control their buoyancy to their advantage; for example, some marine mammals can decrease energetic costs associated with diving and overall total body volume by collapsing respiratory structures to allow for effortless, prolonged gliding during descents (Williams et al., 2000).
The physical forces that affect the buoyancy of deep-diving marine mammal species contrasts significantly with marine mammals that spend a majority of their time at the surface, like sea otters (Williams, 2001). Sea otters face unusual challenges in the aquatic environment due to their extremely dense pelage, large lung size, and small body size (Cashman, 2002; Fish et al., 2002; Thometz et al., 2015; Zellmar et al., 2021). Other marine mammal species may be neutrally buoyant, and some exhale before diving (Williams, 1989; Cashman, 2002; Zellmar et al., 2021). Sea otters rarely achieve neutral buoyancy and they inhale before diving, which means otters are constantly fighting against their own buoyant force while foraging (Williams, 1989; Cashman, 2002). This positive buoyancy is generally beneficial for sea otters, as they use a majority of their time at the surface for resting and grooming (Hanson et al., 1993). Sea otter mothers also rely on this positive buoyancy to safely leave their young pups at the surface while they forage (Payne & Jameson, 1984; Thometz et al., 2015).

The natal pelage of sea otter pups has been described as fluffy and especially buoyant in comparison to the adult pelage, and it is hypothesized that sea otter natal pelage is so buoyant that young pups cannot dive for the first few months of life (Thometz et al., 2015). However, the buoyancy of sea otter natal pelage has never been empirically measured. Past research compared the overall buoyancy of pups compared to adult southern sea otters (Thometz et al., 2015), but due to a lack of available data it was assumed that pup and adult pelts had similar buoyant properties, which we believe may not have accurately reflected the true buoyancy of pups. The objectives of this study were to (1) empirically measure the buoyant force of the sea otter pelt across ontogeny, under normal conditions and after oiling, and (2) estimate the whole-animal fur buoyant force
and scaled mass-specific fur buoyant force for sea otters across age classes, from pelt measurements. We predicted sea otter pups would have the largest mass-specific fur buoyant force, as pups are assumed to be especially buoyant. We also predicted sea otter pups would have the largest change in fur buoyant force when oil is applied, due their lower fur density compared to older age classes (Chapter 1).

3.3 MATERIALS AND METHODS

3.3.1 Sample Collection

In collaboration with California Department of Fish and Wildlife (CDFW), southern sea otter pelts (*Enhydra lutris nereis*) were collected from San Luis Obispo, Monterey, and Santa Cruz counties from animals that died in the wild or during rehabilitation efforts. In accordance with Section 109(h) of the U.S. Marine Mammal Protection Act (MMPA), the U.S. Fish and Wildlife Service’s regulations implementing the MMPA at 50 CFR 18.22(a), and the U.S. Fish and Wildlife Service’s regulations implementing the U.S. Endangered Species Act at 50 CFR 17.21(c)(3), the samples used to complete this work were collected from fresh, necropsied sea otter carcasses taken from the wild by an official or employee of CDFW in the course of their duties as an official or employee of CDFW. Only pelts considered fresh and in good condition (i.e., not matted or decayed) were used for this study. The original 24 cm x 20 cm pelt samples were collected from the back (dorsal) region of the animal. The samples were packaged in three layers of plastic food wrap, kept flat (not folded), stored in two-gallon freezer bags, and kept frozen (-20°C to -16°C) until analysis in the various experiments. We analyzed a total of 37 sea otter pelt samples across six age classes: neonates (<1 month,
N=9), small pups (1-2 months, N=5), large pups (3-5 months, N=5), juveniles (6 months-1 year, N=6), subadults (1-3 years, N=6), and adults (4-9 years, N=6). No aged adult sea otter pelt samples were used in this study. Sea otter pelts were categorized into age classes by CDFW employees based on the well-established sea otter stranding age estimation protocols that use total body length and tooth development data as identifiers. Each pelt sample was measured under three treatment conditions: control, oiled, and washed.

3.3.2 Fur Buoyancy

We measured the buoyancy of pelts in freshwater using an apparatus similar to that described in Fish et al. (2002), in a 10-gallon glass tank (Fig. 12). For these measurements, we used 5 x 5 cm pelt pieces cut from the original pelt samples. We washed each pelt in cold running water to remove any sand or dirt. We gently dabbed the fur with paper towels to help dry the pelt samples. We then used a hair dryer (Trezero® 2200-Watt ceramic tourmaline blow dryer) on the cool setting to dry the pelt, as it has been found that blow drying will help restore the insulating air layer in the pelage (Williams & Davis, 1995).

The control condition consisted of a normal pelt with the air layer present. To ensure the pelt did not float upon submersion, we adhered the pelt to a round glass plate (13 cm diameter) using sealant (Loctite® clear silicone waterproof sealant, Westlake, OH, USA) and then flattened the pelt using a smooth plastic tube (5 in long, 1.5 in diameter) as a roller to remove any air bubbles between the pelt and the glass plate. After the control trial, we applied 10 mL of Scott Well unrefined crude oil (Texas Raw Crude©,
Midland, TX, USA) at 25°C to the pelt using a syringe, and gently massaged the oil into the fur for 30 seconds, similar to the grooming movements performed by a sea otter, ensuring the oil was evenly distributed across the pelt sample. We measured the buoyancy of the oiled pelt using the same methodology. We collected and properly disposed of all crude oil hazardous waste using PPE (gloves, goggles, facial coverings). Once an oiled trial was completed (approx. 5-10 min), we washed the pelt using Dawn® dishwashing detergent (Dawn® Ultra Dishwashing Liquid, Proctor & Gamble, Cincinnati, OH, USA). We applied 0.5-1 mL of Dawn® to the oiled pelt using a syringe, and we gently massaged small amounts of the detergent into the pelt. We then used cold running water to remove the detergent, and repeated adding 0.5-1 mL of Dawn®, followed by rinsing, until the pelt was fully clean. Washed pelts were considered clean when no oil or soap residue was visible and the water coming off the pelt was clear. We conducted additional visual inspections of the pelt after the pelt was patted dry with paper towels, to ensure all oil and soap was removed before blow-drying the pelt. We recorded the time spent washing and the total amount of Dawn® needed to fully wash the pelt. Before measuring buoyancy after washing with Dawn®, we attempted to restore the air layer via blow drying, and then we reattached each pelt sample with sealant.

During treatment conditions, we weighed each sample twice: once when dry (in air) and once when submerged (in water). We measured mass of the pelage in air \( m_{af} \) to the nearest 0.01 g using an electronic balance (Ohaus® Electronic Balance SPX222, Parsippany, NJ, USA). We measured pelage mass in water \( m_{aw} \) by submerging the secured pelt on a glass plate. The submerged glass plate rested on a 3-D printed holder that hung from the scale, which rested on a plexiglass sheet covering half the tank (Fig.
We attached a 100-g ballast weight to the bottom of the holder to provide sufficient negative buoyancy to fully submerge the sample. This process was repeated on the same pelt for all three treatment conditions. After the Dawn® trials, we cut down the hairs using sharp, haircutting shears, and we then used men’s facial razors to shave the hairs as close to the skin as possible. We recorded the time spent shaving the pelts.

To calculate the pelt buoyant force \( F_b \) (N) of the air layer in the fur (Fish et al., 2002), we used the mass with the fur intact in air \( m_{af} \) and in water \( m_{wf} \) for all three treatment conditions, as well as the mass with the fur shaved in air \( m_{as} \) and in water \( m_{ws} \), according to the equation:

\[
F_b = g(m_{af} - m_{wf} - m_{as} - m_{ws})
\]

where \( g \) is gravitational acceleration (9.8 m/s\(^2\)), \( m_{af} \) is pelage mass (kg) in air, \( m_{wf} \) is pelage mass (kg) in water, \( m_{as} \) is skin mass (kg) in air with hair removed, and \( m_{ws} \) is skin mass in water with hair removed.

### 3.3.3 Scaled Fur Buoyancy

To scale up \( F_b \) to the whole animal, we estimated the whole-animal fur buoyant force \( F_{wa} \) (N) using the following equation:

\[
F_{wa} = \frac{F_b}{A_p} \cdot S
\]

where \( F_b \) is pelt buoyant force (N), \( S \) is estimated total body surface area (m\(^2\)), and \( A_p \) is the pelt sample area (m\(^2\)). To estimate the \( S \) for each sea otter sample, we used the following equation:

\[
S = 0.087 \cdot BM^{0.67}
\]
where 0.087 is the area constant determined in Costa and Kooyman (1982), BM is the body mass (kg), and 0.67 is the constant for the 2/3 rule of surface area to volume scaling. We used BM for the individual animals from which the pelts were collected. To calculate the scaled mass-specific fur buoyant force ($F_{ms}$; N/kg), we divided whole-animal buoyant force by the body mass, using the following equation:

$$F_{ms} = \frac{F_{wa}}{BM}$$ (10)

3.3.4 Statistical Analyses

All statistical analyses were performed in R Studio Software (Version 2023.03.0+386). We compared pelt buoyant force, whole-animal fur buoyant force, and scaled mass-specific fur buoyant force using linear mixed effects models (lmerTest package). Each model included treatment condition (control, oiled, washed) and age class as main effects, along with all possible interactions, and sea otter sample ID as a random effect. To make pairwise comparisons, we computed estimated marginal means for treatment and age class combinations in our models (emmeans package). We compared differences across age classes in the amount of Dawn® needed to clean each pelt, and the amount of cleaning time and shaving time after the fur crude oil treatments, using a one-way ANOVA followed by a Tukey honestly significant difference test. To investigate the correlation between sea otter hair density and buoyancy, we ran linear regression models for pelt buoyant force, whole-animal fur buoyant force, and mass-specific fur buoyant force. Hair density values were from a previous study on the same pelt samples (Chapter 1).
Figure 12. Diagram of the fur buoyancy apparatus setup for a submerged pelt (left), and photo of the buoyancy system with an oiled pelt in situ (right). Methods adapted from Fish et al. (2002).
3.4 RESULTS

There was no significant difference in pelt buoyant force ($F_b$) across age classes ($F_{5,31}=2.14, p=0.060$; Table 5; Fig. 13), and no significant interaction between age class and condition ($F_{10,62}=1.89, p=0.063$). The mean $F_b$ for all age classes was $0.311 \pm 0.083$ N for control pelts, $0.145 \pm 0.031$ N for oiled pelts, and $0.197 \pm 0.078$ N for washed pelts. There was a significant difference in $F_b$ across conditions ($F_{2,62}=64.95, p<0.001$; Fig. 13). The $F_b$ was significantly higher in the control condition compared to oiled pelts ($p<0.001$) and washed pelts ($p<0.001$). Washed pelts had significantly higher $F_b$ than oiled pelts ($p=0.003$). There was no significant difference in the amount of Dawn® required to fully clean the sea otter pelts ($F_{5,31}=2.04, p=0.1$), the amount of time spent cleaning the pelts ($F_{5,31}=1.063, p=0.4$), or the amount of time spent shaving the pelts ($F_{5,31}=1.76, p=0.15$) across all age classes (Table 6).

There was a significant interaction between age class and condition for whole-animal fur buoyant force ($F_{wa}$) ($F_{10,62}=4.31, p<0.001$; Fig. 14), and there was a significant difference in sea otter $F_{wa}$ across age classes ($F_{5,31}=15.14, p<0.001$) and conditions ($F_{2,62}=42.38, p<0.001$). Across all age classes, $F_{wa}$ was highest in the control condition, lowest for oiled pelts and intermediate for washed pelts ($p<0.0001$ for all comparisons; Table 5; Fig. 14). $F_{wa}$ was significantly higher for adults in the control condition, compared to all other age classes and conditions ($p$ range: <0.0001-0.0360). With the exception of the adults, there was no significant difference in $F_{wa}$ between the control and washed condition across age classes ($p$ range: 0.1652-1.0000). There was no significant difference in $F_{wa}$ across age classes in the washed condition ($p$ range: 0.0700-1.0000).
There was a significant difference for the scaled mass-specific fur buoyant force ($F_{ms}$) across age classes ($F_{5,31}=6.66$, $p<0.001$) and for all conditions ($F_{2,62}=59.00$, $p<0.001$), but there was no significant interaction between age class and condition ($F_{10,62}=1.77$, $p=0.086$; Fig. 15). $F_{ms}$ was highest in the control condition, lowest for the oiled condition, and intermediate in the washed condition for all age classes (p range: <0.0001-0.0018; Table 5). For age classes overall, $F_{ms}$ was significantly higher for neonates ($p=0.0059$), small pups ($p=0.0005$), and juveniles ($p=0.0212$) compared to adults. Additionally, $F_{ms}$ was significantly higher for small pups compared to subadults ($p=0.0052$).

There was no significant relationship between hair density and pelt buoyant force ($F_{1,35}=2.73$, $r^2=0.0459$, $p=0.1073$), according to the equation $F_b = 0.00006101($hair density$) - 25.06$. Also, there was no relationship between hair density and mass-specific fur buoyant force ($F_{1,35}=3.66$, $r^2=0.06871$, $p=0.0641$), according to the equation $F_{ms} = -0.001638($hair density$) + 7.43065$ (Fig. 16). However, there was a significant positive relationship between hair density and whole-animal fur buoyant force ($F_{1,35}=54.32$, $r^2=0.5969$, $p<0.0001$), according to the equation $F_{wa} = 0.05533($hair density$) + 6.47382$ (Fig. 16).
Table 5. Average sea otter body mass ($BM$; kg), pelt buoyant force ($F_b$; N), whole-animal fur buoyant force ($F_{wa}$; N), and mass-specific fur buoyant force ($F_{ms}$; N/kg), across age classes and conditions.

<table>
<thead>
<tr>
<th>Age class</th>
<th>N</th>
<th>$BM$ (kg)</th>
<th>$F_b$ (N) control</th>
<th>$F_b$ (N) oiled</th>
<th>$F_b$ (N) washed</th>
<th>$F_{wa}$ (N) control</th>
<th>$F_{wa}$ (N) oiled</th>
<th>$F_{wa}$ (N) washed</th>
<th>$F_{ms}$ (N/kg) control</th>
<th>$F_{ms}$ (N/kg) oiled</th>
<th>$F_{ms}$ (N/kg) washed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonate</td>
<td>9</td>
<td>± 1.22</td>
<td>± 0.08</td>
<td>± 0.03</td>
<td>± 0.11</td>
<td>± 8.20</td>
<td>± 3.72</td>
<td>± 8.82</td>
<td>± 1.93</td>
<td>± 0.92</td>
<td>± 2.75</td>
</tr>
<tr>
<td>Small pup</td>
<td>5</td>
<td>± 0.91</td>
<td>± 0.09</td>
<td>± 0.04</td>
<td>± 0.08</td>
<td>± 8.84</td>
<td>± 4.31</td>
<td>± 4.72</td>
<td>± 2.42</td>
<td>± 0.90</td>
<td>± 2.24</td>
</tr>
<tr>
<td>Large pup</td>
<td>5</td>
<td>± 1.78</td>
<td>± 0.06</td>
<td>± 0.05</td>
<td>± 0.05</td>
<td>± 8.85</td>
<td>± 8.20</td>
<td>± 7.04</td>
<td>± 1.46</td>
<td>± 0.69</td>
<td>± 1.01</td>
</tr>
<tr>
<td>Juvenile</td>
<td>6</td>
<td>± 2.39</td>
<td>± 0.36</td>
<td>± 0.01</td>
<td>± 0.10</td>
<td>± 9.72</td>
<td>± 4.42</td>
<td>± 15.64</td>
<td>± 1.68</td>
<td>± 0.41</td>
<td>± 2.04</td>
</tr>
<tr>
<td>Subadult</td>
<td>6</td>
<td>± 3.09</td>
<td>± 0.14</td>
<td>± 0.03</td>
<td>± 0.09</td>
<td>± 33.86</td>
<td>± 6.39</td>
<td>± 19.74</td>
<td>± 1.87</td>
<td>± 0.41</td>
<td>± 1.45</td>
</tr>
<tr>
<td>Adult</td>
<td>6</td>
<td>± 4.83</td>
<td>± 0.08</td>
<td>± 0.03</td>
<td>± 0.03</td>
<td>± 27.30</td>
<td>± 10.90</td>
<td>± 8.37</td>
<td>± 0.95</td>
<td>± 0.32</td>
<td>± 0.46</td>
</tr>
</tbody>
</table>

Values (mean ± 1SD) are provided for samples used in fur buoyancy trials. N represents the number of individual pelt samples for each age class.
Table 6. The amount of Dawn® (mL) required to remove crude oil from the pelts, the amount of time (min) needed to wash the pelts, and the amount of time (min) it took to shave sea otter pelts, across age classes.

<table>
<thead>
<tr>
<th>Age class</th>
<th>N</th>
<th>Amount of Dawn® (mL)</th>
<th>Clean time (min)</th>
<th>Shave time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonate</td>
<td>9</td>
<td>8.72 ± 4.06</td>
<td>8.28 ± 2.74</td>
<td>52.33 ± 32.40</td>
</tr>
<tr>
<td>Small pup</td>
<td>5</td>
<td>11.40 ± 2.61</td>
<td>8.20 ± 0.84</td>
<td>58.00 ± 20.49</td>
</tr>
<tr>
<td>Large pup</td>
<td>5</td>
<td>9.60 ± 1.52</td>
<td>7.70 ± 0.84</td>
<td>65.00 ± 32.02</td>
</tr>
<tr>
<td>Juvenile</td>
<td>6</td>
<td>8.33 ± 1.03</td>
<td>6.17 ± 1.13</td>
<td>44.17 ± 12.81</td>
</tr>
<tr>
<td>Subadult</td>
<td>6</td>
<td>12.50 ± 3.94</td>
<td>8.50 ± 2.88</td>
<td>85.83 ± 33.53</td>
</tr>
<tr>
<td>Adult</td>
<td>6</td>
<td>11.67 ± 2.66</td>
<td>8.08 ± 1.80</td>
<td>79.17 ± 36.93</td>
</tr>
</tbody>
</table>

Values (mean ± 1SD) are provided for samples used in fur buoyancy trials. N represents the number of individual pelt samples for each age class.
Figure 13. Pelt buoyant force ($F_b$; N) of sea otter pelt samples across ontogeny and by condition: control (gray), after oiling with crude oil (brown), and washed with Dawn® (blue). The horizontal line within each box indicates the median value and the box boundaries indicate the upper and lower interquartile range. Vertical lines indicate minimum and maximum values within 1.5 times the interquartile range. Individual points are outlier values >1.5 times and <3 times the interquartile range. Diamonds within boxplots indicate the mean values. Diamonds represent the mean for each age class. Different letters above the boxes indicate statistically significant differences among age class means. There were no significant differences in $F_b$ across age classes within a condition. Across all age classes, $F_b$ was significantly higher for the control pelts than for pelts washed with Dawn®, which in turn was significantly higher than $F_b$ after oiling.
Figure 14. Whole-animal fur buoyant force ($F_{wu}$; N) of sea otters across ontogeny: neonate (N=9), small pup (N=5), large pup (N=5), juvenile (N=6), subadult (N=6), and adult (N=6), and by condition: control (gray), after oiling with crude oil (brown), and washed with Dawn® (blue). The horizontal line within each box indicates the median value and the box boundaries indicate the upper and lower interquartile range. Vertical lines indicate minimum and maximum values within 1.5 times the interquartile range. Individual points are outlier values >1.5 times and <3 times the interquartile range. Diamonds within boxplots indicate the mean values. Diamonds represent the mean for each age class. Different letters above the boxes indicate statistically significant differences among means. Compared to the control condition, the oiled condition showed reduced $F_{wu}$ values. Washed $F_{wu}$ values are not consistent due to the successful or unsuccessful reintroduction of the air layer.
Figure 15. Mass-specific fur buoyant force ($F_{ms}$; N/kg) of sea otters across ontogeny: neonate (N=9), small pup (N=5), large pup (N=5), juvenile (N=6), subadult (N=6), and adult (N=6), and by condition: control (gray), after oiling with crude oil (brown), and washed Dawn® (blue). The horizontal line within each box indicates the median value and the box boundaries indicate the upper and lower interquartile range. Vertical lines indicate minimum and maximum values within 1.5 times the interquartile range. Individual points are outlier values >1.5 times and <3 times the interquartile range. Diamonds within boxplots indicate the mean values. Diamonds represent the mean for each age class. Different letters above the boxes indicate statistically significant differences among age class means. $F_{ms}$ was highest in the control condition. $F_{ms}$ decreases with age and as well with oiling, there are variable results when washed.
Figure 16. (A) Relationship between the whole-animal fur buoyant force ($F_{wa}$; N) and hair density (hairs·mm$^{-2}$). There was a significant positive correlation between $F_{wa}$ and hair density ($p<0.0001$). (B) Relationship between mass-specific fur buoyant force ($F_{ms}$; N/kg) and hair density (hairs·mm$^{-2}$). There was no significant correlation between $F_{ms}$ and hair density ($p=0.0641$). For both analyses, each sample is represented by a single symbol, and different symbols and colors represent age classes. Lines represent the best-fit linear regression. Hair density values were used from a previous study (Chapter 1).
3.5 DISCUSSION

Our control condition pelt buoyant force ($F_b$) findings are consistent with previous research on adult sea otter pelts (Fish et al., 2002) (Table 5). We originally predicted we would see differences in pelt buoyancy between the natal pelage (neonates, small pups, some large pups) and the adult pelage (all other age classes) (Chapter 1). However, we found no differences in $F_b$ across ontogeny for our sea otter pelt samples (Table 5; Fig. 13), likely because the pelt samples were similarly sized and trapped a similar amount of air within the fur. However, we did observe differences across age classes when we scaled the buoyant force up to the whole animal ($F_{wa}$) and accounted for body size ($F_{ms}$). Whole-animal fur buoyant force increased with age (Fig. 14), because larger animals have more pelage overall and therefore more trapped air to provide buoyancy. Indeed, we saw a positive relationship between whole-animal fur buoyant force and hair density, which increases with age in sea otters (Chapter 1; Fig. 16).

The relative amount of buoyancy provided by fur decreases as body size increases (Fish et al., 2002). Mass-specific positive buoyant forces of neonate sea otters were previously estimated to be twice that of the adults (Cashman, 2002; Thometz et al., 2015). Our scaled mass-specific findings are consistent with those estimates, and these results demonstrate the importance of a larger surface area to volume ratio in neonates and small pups, as it allows them to generate more buoyant force relative to their smaller body mass (Fig. 15). A pup must float on its own at the water’s surface when its mother embarks on short foraging bouts; this is when the buoyancy of a pup becomes especially important (Payne & Jameson, 1984; Thometz et al., 2015). Pups begin attempting to dive at around 5 weeks of age, but the pups are not successful in diving until around 10 weeks
of age (Hanson et al., 1993). The trade-off with the high relative buoyancy seen in young
sea otter pups is that they can’t dive yet, but they can float very well at the surface.

In animals that have an air layer in the fur, the pelage is more susceptible to
fouling and water infiltration (Costa & Kooymen, 1982; Webb & King, 1984; Kruuk &
Balharry, 1990; Loughlin, 1994; Fish, 2000). If the air layer becomes disturbed from
oiling, the fur loses its buoyant properties (Fig. 13). Past studies have investigated the
effects of oiling on adult sea otter thermal function (Costa & Kooymen, 1982; Williams
et al., 1988; Dunkin, 2001; Chapter 2), but no previous study has researched the effect
of oiling on sea otter buoyancy and made direct comparisons across ontogeny. In general,
oiling reduces the buoyant force of the pelt relative to intact pelage across all age classes
(Fig. 14). This suggests that regardless of age, all sea otters are vulnerable to the
disruption of their buoyancy when oiled, similar to what we found for sea otter thermal
function in Chapter 2. Our study only focused on the physical effects of crude oil on the
fur in a laboratory-based setting. In marine wildlife, exposure to crude oil can cause
severe physiological impacts, and we did not investigate any internal toxicological
impacts on sea otters.

The close proximity of southern sea otter populations to oil platforms off the coast
of California makes sea otters are vulnerable to the internal and external effects of oil
spills (Fig. S1). Special consideration should be made to ensure all the oil is removed
from the fur, and Dawn® has been shown to be efficient at ridding the fur of oil when
using proper wash techniques (Williams et al., 1988; Jessup et al., 2012). Washing the
pelts with Dawn® sometimes restored the buoyant force, but the results were not
consistent. Although the primary purpose of Dawn® is to clean the fur, this suggests that
washing with Dawn® may not fully restore the air layer of the fur (Jessup et al., 2012). However, the sea otter pelt buoyant force values used to estimate $F_{wa}$ and $F_{ms}$ were from pelts in a laboratory setting, not for live animals, which have natural oils and groom consistently for proper fur hygiene and air layer maintenance (Davis et al., 1988; Williams et al., 1988). It is likely that some detergent was not fully removed from the sample, and it might take longer to completely rid the fur of soap due to the higher hair density present in the adult pelage (Chapter 1). It is important that wildlife networks and rehabilitators carefully remove all detergent residue from the fur when washing oiled otters. Additionally, the amount of oil added to the pelts in our study mimics a heavily oiled sea otter, and it could require two wash cycles to fully clean the pelt in an actual rehabilitation scenario. Another possible limitation to our laboratory-based experiment could be that we used freshwater to clean the pelts. Our findings can still improve oiled sea otter care, as it supports efforts to clean the oil from the fur using a cleaning detergent like Dawn®. Surprisingly, there were no differences in the amount of Dawn® used to clean pelts and the clean time across age classes for our sea otter pelt samples (Table 6). Adult sea otter pelts have a higher hair density (Chapter 1), and it may be expected to take longer and require a larger amount of Dawn® to thoroughly clean pelts with denser pelage. In the event of an oil spill, washing sea otter fur with Dawn® is likely to work to restore the air layer, partnered with extended time in rehabilitation to allow for the natural oils to be produced and for the otters to groom themselves.

Diving for sea otters is energetically costly due to their inefficient swimming mode, large lungs, buoyant fur, and the high cost of thermoregulating at depth (Denison & Kooyman, 1973; Kooyman, 1973; Yeates & Williams, 2007; Ponganis, 2011). Sea
otters typically forage at depths less than 25 m, and spend most of their time grooming, resting, and eating at the surface (Riedman & Estes, 1990; Cashman, 2002). The behavior of sea otters may explain why these large quantities of air in the lungs and fur may be beneficial. An increased understanding of the relationship of sea otter body and lung size is key to assessing the overall effects of buoyancy across ontogeny. Future work should examine how these size differences affect sea otter buoyancy.

3.6 ACKNOWLEDGMENTS

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REFERENCES


Uhen, M. D. (2007). Evolution of marine mammals: back to the sea after 300 million years. The Anatomical Record: Advances in Integrative Anatomy and Evolutionary Biology: Advances in Integrative Anatomy and Evolutionary Biology, 290(6), 514-522.


APPENDICES

A. SUPPLEMENTAL FIGURES

Figure 17. Site-scale map of the distribution of southern sea otter populations along the coast relative to crude oil pipelines, refineries, and platforms. California sea otter range data retrieved from USGS 2019 Population Census Results, Spring 2019 Data. Oil data received from the U.S. Energy Information Administration. Map Credit: Kate Riordan and Emily Levin (ArcMap).
### B. SUPPLEMENTAL TABLES

Table 7. Guard hair length (mm) for adult sea otters from the present study and published sources.

<table>
<thead>
<tr>
<th>Guard hair length (mm)</th>
<th>Guard hair circularity</th>
<th>Guard hair major diameter (mm)</th>
<th>Guard hair minor diameter (mm)</th>
<th>N</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>28.33 ± 3.82</td>
<td>0.31 ± 0.040</td>
<td>0.11 ± 0.014</td>
<td>0.035 ± 0.006</td>
<td>7</td>
<td>This study</td>
</tr>
<tr>
<td>31.36 ± 0.401</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>Liwanag et al. (2012a)</td>
</tr>
<tr>
<td>29.00 ± 0.20</td>
<td>-</td>
<td>0.117 ± 0.011</td>
<td>-</td>
<td>5</td>
<td>Kuhn &amp; Meyer (2010)</td>
</tr>
<tr>
<td>27.4 ± 0.9</td>
<td>-</td>
<td>0.079 ± 0.01</td>
<td>-</td>
<td>3</td>
<td>Fish et al. (2002)</td>
</tr>
<tr>
<td>31.0 ± 0.26</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7</td>
<td>Zagrebelny (1998)</td>
</tr>
<tr>
<td>26.9 ± 0.76</td>
<td>-</td>
<td>0.044 ± 0.007</td>
<td>-</td>
<td>10</td>
<td>Williams et al. (1992)</td>
</tr>
</tbody>
</table>

Values are presented as means ± 1 SD. Dashes indicate missing values. N = number of individuals examined in that study.
Table 8. Hair density (hairs/mm^2) for adult sea otters from the present study and published sources.

<table>
<thead>
<tr>
<th>Hair density (hairs/mm^2)</th>
<th>N</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,449.69 ± 152.03</td>
<td>3</td>
<td>This study</td>
</tr>
<tr>
<td>1,315 ± 78</td>
<td>2</td>
<td>Kuhn et al. (2010)</td>
</tr>
<tr>
<td>1,188.77 ± 70.37</td>
<td>3</td>
<td>Fish et al. (2002)</td>
</tr>
<tr>
<td>775.26</td>
<td>1</td>
<td>Williams et al. (1992)</td>
</tr>
<tr>
<td>1,253.33</td>
<td>?</td>
<td>Tarasoff (1974)</td>
</tr>
<tr>
<td>1,340.52</td>
<td>?</td>
<td>Tarasoff (1972)</td>
</tr>
<tr>
<td>1,008.00</td>
<td>?</td>
<td>Kenyon (1969)</td>
</tr>
<tr>
<td>328.75</td>
<td>?</td>
<td>Sokolov (1962)</td>
</tr>
</tbody>
</table>

Values are presented as means ± 1 SD. N = number of individuals examined.
Table 9. Model parameters used to estimate heat loss that were consistent across treatments.

<table>
<thead>
<tr>
<th>Age class</th>
<th>Body length (m) [L_{body}]</th>
<th>Head girth (m)</th>
<th>Head core radius (m) [R_{hc}]</th>
<th>Volume of sphere (m³) [V_{sphere}]</th>
<th>Axillary girth (m)</th>
<th>Axillary core radius (m) [R_{ax}]</th>
<th>Cylinder height (m) [H_{cyl}]</th>
<th>Volume of cylinder (m³) [V_{cyl}]</th>
<th>Tail girth (m)</th>
<th>Tail core radius (m) [R_{tc}]</th>
<th>Cone height (m) [H_{cone}]</th>
<th>Volume of cone (m³) [V_{cone}]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonate</td>
<td>0.56667</td>
<td>0.33554</td>
<td>0.04732</td>
<td>0.26277</td>
<td>0.03371</td>
<td>0.30875</td>
<td>0.00110</td>
<td>0.07133</td>
<td>0.00527</td>
<td>0.15111</td>
<td>0.000005</td>
<td></td>
</tr>
<tr>
<td>Small pup</td>
<td>0.71920</td>
<td>0.34027</td>
<td>0.04732</td>
<td>0.30553</td>
<td>0.03951</td>
<td>0.42809</td>
<td>0.00212</td>
<td>0.08294</td>
<td>0.00636</td>
<td>0.18280</td>
<td>0.000008</td>
<td></td>
</tr>
<tr>
<td>Large pup</td>
<td>0.81200</td>
<td>0.34500</td>
<td>0.04905</td>
<td>0.37307</td>
<td>0.05156</td>
<td>0.49818</td>
<td>0.00417</td>
<td>0.10127</td>
<td>0.01026</td>
<td>0.20400</td>
<td>0.000023</td>
<td></td>
</tr>
<tr>
<td>Juvenile</td>
<td>0.85917</td>
<td>0.34973</td>
<td>0.04950</td>
<td>0.68807</td>
<td>0.10152</td>
<td>0.53451</td>
<td>0.01731</td>
<td>0.18678</td>
<td>0.02373</td>
<td>0.21333</td>
<td>0.000126</td>
<td></td>
</tr>
<tr>
<td>Subadult</td>
<td>1.06333</td>
<td>0.35020</td>
<td>0.04907</td>
<td>0.72760</td>
<td>0.10692</td>
<td>0.68624</td>
<td>0.02466</td>
<td>0.19751</td>
<td>0.02477</td>
<td>0.26563</td>
<td>0.000172</td>
<td></td>
</tr>
<tr>
<td>Adult</td>
<td>1.21333</td>
<td>0.35150</td>
<td>0.04895</td>
<td>0.73809</td>
<td>0.10814</td>
<td>0.81723</td>
<td>0.03001</td>
<td>0.20036</td>
<td>0.02489</td>
<td>0.28422</td>
<td>0.000185</td>
<td></td>
</tr>
</tbody>
</table>

Mean values are provided for each age class.
Table 10. Model parameters used to estimate heat loss that varied across treatments.

<table>
<thead>
<tr>
<th>Age class</th>
<th>Thermal conductivity (W·m⁻¹·°C⁻¹) [(k_i)]</th>
<th>Pelt thickness (m) [(L_i)]</th>
<th>Total head radius (m) [(R_{hi})]</th>
<th>Total axillary radius (m) [(R_{ai})]</th>
<th>Total tail radius (m) [(R_{ti})]</th>
<th>Total heat loss (W/m²) [(Heat_{tot})]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>in air</td>
<td>in water</td>
<td>oiled</td>
<td>dry</td>
<td>wet</td>
<td>oiled</td>
</tr>
<tr>
<td>Neonate</td>
<td>0.1792</td>
<td>0.0466</td>
<td>0.1466</td>
<td>0.0315</td>
<td>0.0061</td>
<td>0.0054</td>
</tr>
<tr>
<td>Small pup</td>
<td>0.1714</td>
<td>0.0660</td>
<td>0.1845</td>
<td>0.0323</td>
<td>0.0068</td>
<td>0.0062</td>
</tr>
<tr>
<td>Large pup</td>
<td>0.1458</td>
<td>0.0620</td>
<td>0.1985</td>
<td>0.0254</td>
<td>0.0059</td>
<td>0.0057</td>
</tr>
<tr>
<td>Juvenile</td>
<td>0.1176</td>
<td>0.0560</td>
<td>0.1665</td>
<td>0.0225</td>
<td>0.0060</td>
<td>0.0055</td>
</tr>
<tr>
<td>Subadult</td>
<td>0.1198</td>
<td>0.0483</td>
<td>0.1444</td>
<td>0.0216</td>
<td>0.0067</td>
<td>0.0064</td>
</tr>
<tr>
<td>Adult</td>
<td>0.1428</td>
<td>0.0676</td>
<td>0.1772</td>
<td>0.0231</td>
<td>0.0070</td>
<td>0.0061</td>
</tr>
</tbody>
</table>

Mean values are provided for each age class.
C. HEAT LOSS EQUATIONS

**Head (sphere)**

Head diameter = \( \frac{\text{head girth}}{\pi} \)

Head radius = \( \frac{\text{head girth}}{2\pi} \)

\( R_{hc} = \text{head radius} - \frac{3}{4}L_{water} \)

**Axillary (cylinder)**

Axillary radius = \( \frac{\text{axillary girth}}{2\pi} \)

\( R_{ac} = \text{axillary radius} - L_{water} \)

\( H_{cyl} = L_{body} - H_{cone} - \text{head diameter} \)

**Tail (cone)**

Tail radius = \( \text{axillary radius} \cdot 0.2714504 \)

\( R_{tc} = \text{tail radius} - \frac{3}{4}L_{water} \)

**Total head radius**

\( R_{ht, \text{air}} = R_{hc} + \frac{3}{4}L_{air} \)

\( R_{ht, \text{water}} = R_{hc} + \frac{3}{4}L_{water} \)

\( R_{ht, \text{oiled}} = R_{hc} + \frac{3}{4}L_{oiled} \)

**Total axillary radius**

\( R_{at, \text{air}} = R_{ac} + L_{air} \)

\( R_{at, \text{water}} = R_{ac} + L_{water} \)

\( R_{at, \text{oiled}} = R_{ac} + L_{oiled} \)

**Total tail radius**

\( R_{tt, \text{air}} = R_{tc} + \frac{3}{4}L_{air} \)

\( R_{tt, \text{water}} = R_{tc} + \frac{3}{4}L_{water} \)

\( R_{tt, \text{oiled}} = R_{tc} + \frac{3}{4}L_{oiled} \)
Heat flux: sphere

\[ Q_{\text{sphere}} = \frac{T_{MB} - T_A}{\frac{1}{4} \pi k_i \left[ \frac{1}{R_{hc}} - \frac{1}{R_{ht}} \right]} \]

Heat flux: cylinder

\[ Q_{\text{cyl}} = 2\pi \cdot H_{\text{cyl}} \cdot k_i \left[ \frac{T_{MB} - T_A}{\ln \left( \frac{R_{ac}}{R_{at}} \right)} \right] \]

Heat flux: cone

\[ Q_{\text{cone}} = \left[ \frac{\pi k_i (T_{MB} - T_A)}{H_{\text{cone}}} \right] (R_{tc} \cdot R_{tt}) \]

Volumes

\[ V_{\text{sphere}} = \frac{4}{3} \pi (R_{hc})^3 \]

\[ V_{\text{cyl}} = \pi (R_{ac})^2 \cdot H_{\text{cyl}} \]

\[ V_{\text{cone}} = \frac{1}{3} \pi (R_{tc})^2 \cdot H_{\text{cone}} \]

Heat loss totals

\[ Heat_{\text{tot}} = \frac{Q_{\text{sphere}} + Q_{\text{cyl}} + Q_{\text{cone}}}{V_{\text{sphere}} + V_{\text{cyl}} + V_{\text{cone}}} \]
**Abbreviation Key**

- $R_{hc}$: head core radius (m)
- $R_{ac}$: axillary core radius (m)
- $R_{tc}$: tail core radius (m)
- $R_{ht}$: total head radius (m)
- $R_{at}$: total axillary radius (m)
- $R_{tt}$: total tail radius (m)
- $k$: thermal conductivity of the pelt for the specific treatment (W·m$^{-1}$·°C$^{-1}$)
- $L_{i}$: pelt thickness for the specific treatment (m)
- $L_{body}$: total body length (m)
- $H_{cone}$: cone height (m) [tail length]
- $H_{cyl}$: cylinder height (m) [trunk length]
- $Q_{sphere}$: heat flux of a sphere (W)
- $Q_{cyl}$: heat flux of a cylinder (W)
- $Q_{cone}$: heat flux of a cone (W)
- $V_{sphere}$: volume of sphere (m$^3$)
- $V_{cyl}$: volume of cylinder (m$^3$)
- $V_{cone}$: volume of cone (m$^3$)
- $T_{MB}$: temperature at the muscle-skin interface (set to 36.5°C)
- $T_{A}$: ambient temperature (set to 16°C for in air and 13°C for in water and oiled)
- $Heat_{tot}$: total conductive heat transfer for the specific treatment (W/m$^3$)