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Ontogenetic changes in southern sea otter (*Enhydra lutris nereis*) fur morphology

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Abstract

Many animals exhibit morphological changes across ontogeny associated with adaptations to their environment. Sea otters (*Enhydra lutris*) have the densest fur of any animal, which is composed of guard hairs, intermediate hairs, and underhairs. Sea otters live in cold water environments, and their fur traps a layer of air to remain properly insulated, due to morphological adaptations that allow the hairs to trap air when submerged. When a sea otter is born, it has a natal pelage which it will eventually molt and replace with a pelt resembling the adult pelage. 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 (*E. 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. Natal pelage guard hairs were similarly shaped but smaller in diameter 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 pelage had a lower hair density than the pelage of older age classes, 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 more vulnerable to heat loss.

KEYWORDS
development, fur density, insulation, lanugo, natal pelage

1 | INTRODUCTION

One of the most defining characteristics in Class Mammalia is the presence of fur (Cleveland, 2011). Mammalian fur functions to aid in camouflage, behavioral signaling, waterproofing, and most importantly insulation (Cleveland, 2011). Fur acts as an insulator by trapping a layer of still air between an animal's skin and the surrounding environment (Frisch et al., 1974; Reynolds, 1993; Scholander et al., 1950; Sokolov, 1962). 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; Zagrebelny, 1998), 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 (Liwanag, Berta, Costa, Abney, et al., 2012; Scheffer, 1964). Each hair is composed of a cortex, an outer cuticle, and a central medulla (Williams & Davis, 1995).

Over evolutionary time, some mammals have evolved adaptations in the fur to facilitate an aquatic or semiaquatic lifestyle, including increased hair density, elongated cuticular scales on guard hairs and underhairs, and the flattening and lengthening of guard hairs (Liwanag, Berta, Costa, Abney, et al., 2012). 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 (Liwanag, Berta, Costa, Abney, et al., 2012; Swift, 1977). 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 (Kuhn et al., 2010; Weisel et al., 2005). 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, Berta, Costa, Abney, et al., 2012). 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 (Castellini & Mellish, 2015; Kenyon, 1969; Morrison et al., 1974; Tarasoff, 1974; Young, 1976). Sea otters also have the densest fur of any animal (Tarasoff, 1974), and that fur exhibits adaptations to aquatic living such as longer, flatter guard hairs, and elongated cuticular scales (Liwanag, Berta, Costa, Abney, et al., 2012). It is presumed that sea otters, like other mammals, have two different types of pelage, that is, 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 (Fish et al., 2002; Kuhn et al., 2010; Tarasoff et al., 1972; Williams et al., 1992), 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-week-old (Payne & Jameson, 1984). We also hypothesized that the adult pelage would have a higher hair density compared to age classes bearing the natal pelage.

2 | MATERIALS AND METHODS

2.1 | Sample collection

In collaboration with California Department of Fish and Wildlife (CDFW), southern sea otter (*E. lutris nereis* Merriam, 1904) 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 US Marine Mammal Protection Act (MMPA), the US Fish and Wildlife Service's regulations implementing the MMPA at 50 CFR 18.22(a), and the US Fish and Wildlife Service's regulations implementing the US 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. The original pelt samples were from the back (dorsal) region and were 24 × 20 cm. 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 before analysis for 1 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.

2.2 | Guard hair morphology

Following the methods described in Liwanag, Berta, Costa, Abney, et al. (2012), 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 (ABSO-LUTE Digimatic Caliper Series 500; Mitutoyo©).

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, Berta, Costa, Abney, et al. (2012). 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 ×40 objection (×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:

$$Hair\ circularity = \frac{minor\ diameter}{major\ diameter}$$

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.

2.3 | Shape of the hair cuticle

To mount the hairs, we placed one individual hair on a conductive carbon tab (PELCO Tabs™; Ted Pella, Inc) 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.

2.4 | Hair density

We estimated sea otter hair density following the methods of Liwanag, Berta, Costa, Abney, et al. (2012) 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 × 2.5 cm squares from the original samples. Next, we shaved the subsamples before fixing them in formalin (FisherSci; Buffered 10%) for 4 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). Tissue samples were cut into 2.5 × 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 ×15 magnification (Figure 1).

To calculate the number of hairs per unit area, we used ImageJ Software (National Institute of Health) to draw a rectangular area (1800 μm by 1000 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.

2.5 | Statistical analysis

All statistical analyses were performed in R Studio Software (Version 1.2.5019). 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 package). Using the Tukey outputs, we made visualizations of paired comparisons for different age classes (multcompView package). The number of underhairs, intermediate hairs, and 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).

3 | RESULTS

Guard hair length differed significantly across age classes ($F_{5,32} = 17.05$, $p < .001$; Figure 2). Neonate and small pup pelts had significantly longer guard hairs than adult ($p < .001$), subadult ($p < .001$), juvenile ($p < .001$), and large pup pelts ($p = .004$, $p < .001$). There was no significant difference in guard hair length between neonate and small pup pelts ($p = .849$). Additionally, there was no significant difference in guard hair length among large pup, juvenile, subadult, and adult pelts (p range: .514–.999 for all comparisons).

There was no significant difference in guard hair circularity across ontogeny ($F_{5,32} = 0.371$, $p = .864$; Table 1). However, there was a significant difference in guard hair minor diameter across age classes ($F_{5,32} = 6.172$, $p < .001$; Table 1). The minor diameter of neonate guard hairs was significantly smaller than the minor diameter of subadult ($p = .038$) and adult ($p = .001$) guard hairs. Similarly, the

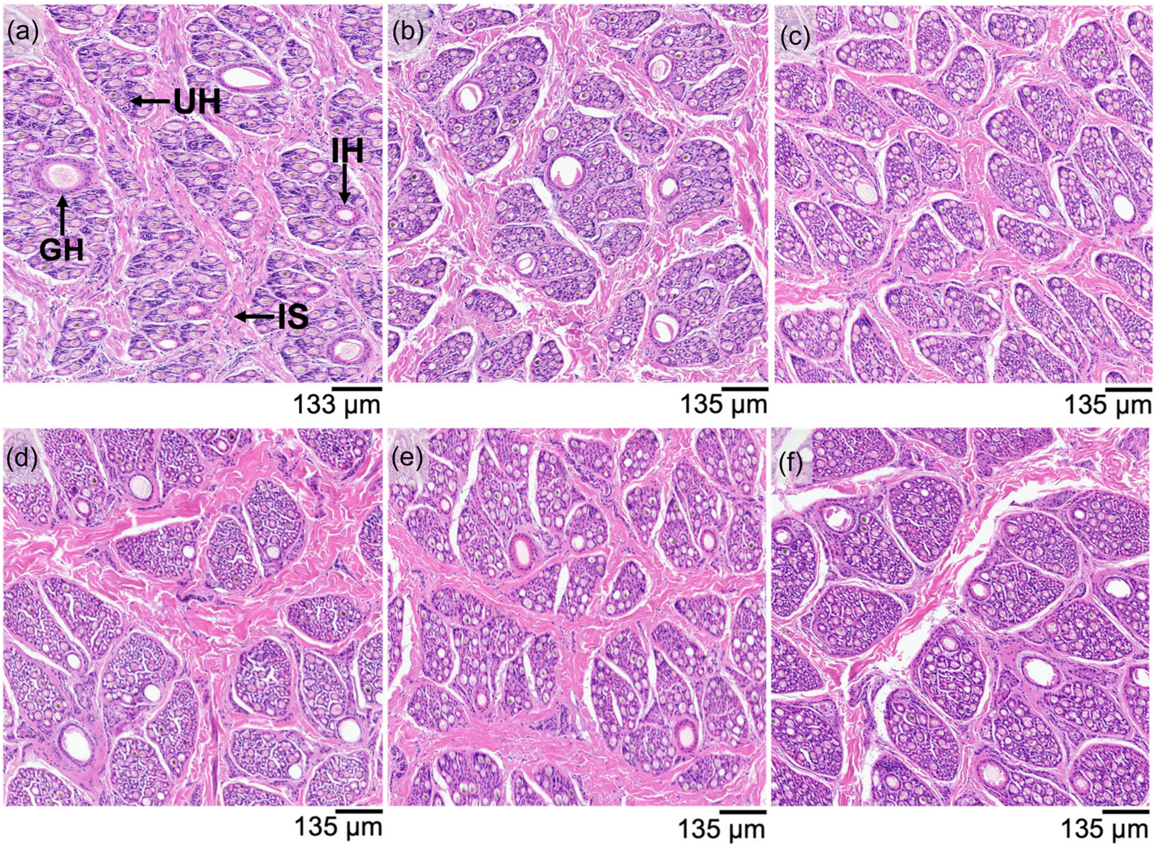

FIGURE 1 Neonate (a), small pup (b), large pup (c), juvenile (d), subadult (e), and adult (f) southern sea otter (*Enhydra lutris nereis*) longitudinal sections from the skin of the dorsal region of the body, visualized on hematoxylin and eosin-stained slides. Arrows and labels in (a) designate a specific type of hair follicle: GH, guard hair follicle; IH, intermediate hair follicle; IS, interstitial space; UH, underhair follicle. All images were taken at ×15 magnification.

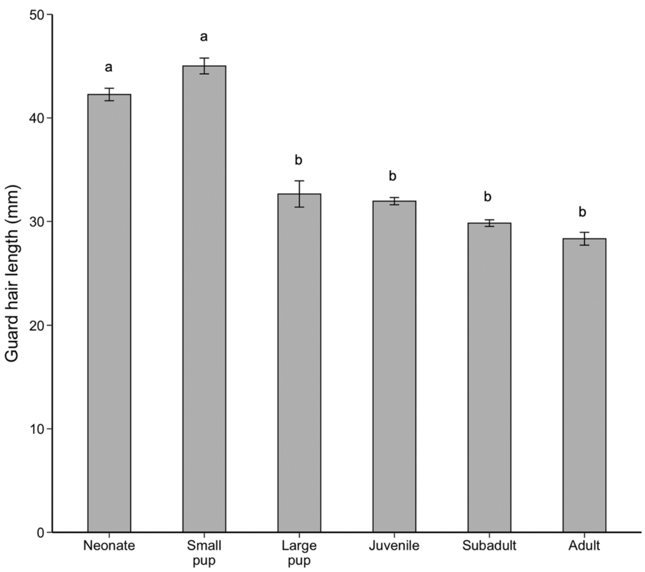

FIGURE 2 Sea otter guard hair length (mm) across ontogeny. Bar heights indicate means and error bars indicate standard error for the associated age class: neonate (*N* = 9), small pup (*N* = 5), large pup (*N* = 5), juvenile (*N* = 6), subadult (*N* = 6), and adult (*N* = 7). Different letters above the bars indicate statistically significant differences among means.

TABLE 1 Minor and major diameter (μm) and hair circularity (minor diameter/major diameter) for sea otter guard hairs across ontogeny.

Age class	*N*	Minor diameter (μm)	Major diameter (μm)	Hair circularity
Neonate	9	24.96 ± 2.49[a]	86.27 ± 8.01[ab]	0.2919 ± 0.038
Small pup	5	24.13 ± 3.53[a]	81.22 ± 9.02[a]	0.2969 ± 0.038
Large pup	5	26.50 ± 4.13[ab]	89.23 ± 14.07[ab]	0.2978 ± 0.036
Juvenile	6	26.83 ± 3.04[ab]	89.81 ± 6.96[ab]	0.3012 ± 0.050
Subadult	6	29.61 ± 8.11[bc]	98.11 ± 24.20[bc]	0.2947 ± 0.037
Adult	7	35.27 ± 5.81[c]	113.29 ± 12.93[c]	0.3144 ± 0.049

Note: Values are presented as mean ± 1 SD. *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.

minor diameter of small pup guard hairs was significantly smaller than the minor diameter of subadult ($p = .046$) and adult ($p = .003$) guard hairs. The minor diameter of large pup and juvenile guard hairs was significantly smaller than the minor diameter of adult guard hairs

($p = .03$ for both comparisons). Guard hair major diameter was significantly different across age classes ($F_{5,32} = 6.057$, $p < .001$; Table 1). The major diameters of neonate ($p = .0028$), large pup ($p = .034$), and juvenile ($p = .028$) guard hairs were significantly smaller than the major diameter of adult guard hairs but did not significantly differ from subadult guard hairs. The major diameter of small pup guard hairs was significantly smaller than the major diameter of subadult ($p = .038$) and adult ($p = .0022$) guard hairs. Across sea otter ontogeny, the minor and major diameters increased in tandem to generate a similar ellipsoid shape (Figure 3).

There was no visual difference in cuticular scales between the neonate and adult underhairs (Figure 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 (Figure 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 (Figure 1).

Hair density differed significantly across age classes ($F_{5,12} = 18.237$, $p < .001$; Figure 5; Table 2). Neonate pelts had a significantly lower hair density than juvenile ($p = .0023$), subadult ($p = .0013$), and adult ($p < .0001$) pelts. Small pup, large pup, and juvenile pelts had a significantly lower hair density than adult pelts (p range: .0003–.0474). Small pups also had a significantly lower hair density than subadult pelts ($p = .0427$). There were no significant differences in hair density among neonates, small pups, and large pups (p range: .0745–.9116), and there were no significant differences among any other age classes (p range: .0784–.9989; Figure 5).

There was no significant difference in the number of guard hairs present across ontogeny ($F_{5,12} = 1.13$, $p = .397$). Similarly, there was no significant difference in the number of intermediate hairs present across all age classes ($F_{5,12} = 2.54$, $p = .085$). However, there was a significant difference in the number of underhairs present across ontogeny ($F_{5,12} = 20.33$, $p < .001$; Table 2). Adult sea otter pelts had more underhairs compared to all other age classes (p range: <.0001–.0200). Neonate pelts had significantly fewer underhairs than juvenile ($p = .0015$), subadult ($p = .0027$), and adult ($p < .0001$) pelts. There was no significant difference in the number of underhairs across all other age classes (p range: .0594–.9987).

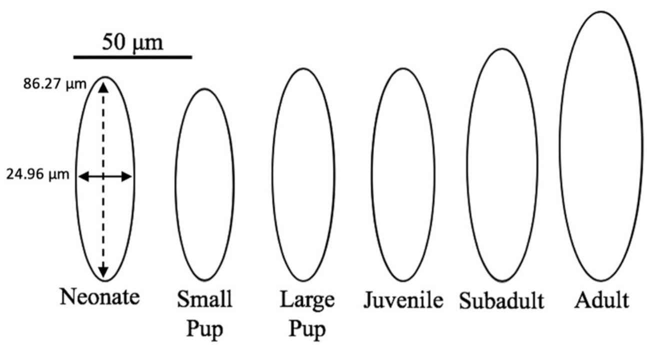

FIGURE 3 Sea otter guard hair cross section across age classes: neonate ($N = 9$), small pup ($N = 5$), large pup ($N = 5$), juvenile ($N = 6$), subadult ($N = 6$), and adult ($N = 7$). Ellipses represent the shape formed by the mean minor diameter (solid arrow) and major diameter (dashed arrow) for 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.

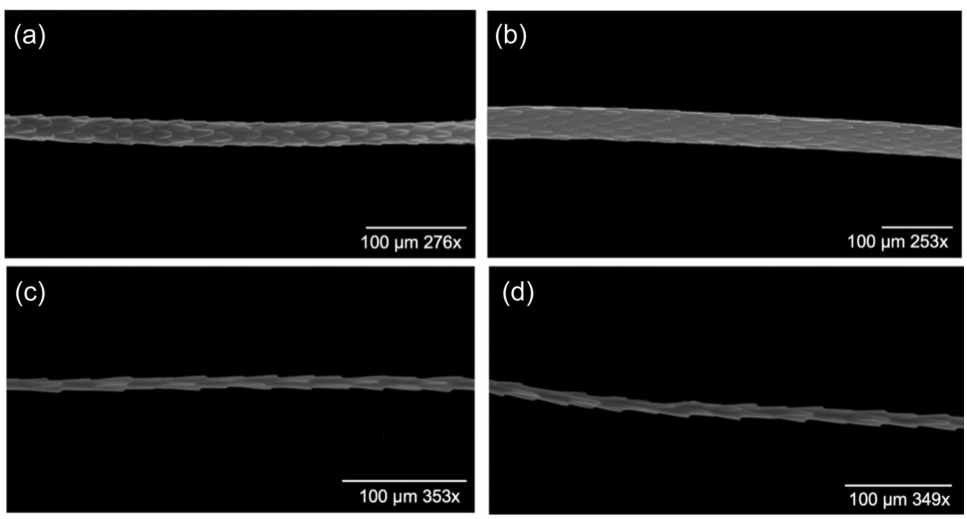

FIGURE 4 Scanning electron micrographs of sea otter hairs: (a) guard hair from the pelt of a neonate, (b) guard hair from the pelt of an adult, (c) underhair from the pelt of a neonate, and (d) underhair from the pelt of an adult. These images show the cuticular scale patterns on individual hairs. Magnifications differ slightly, as indicated on each micrograph. Magnifications were matched as closely as possible, within the limitations of the microscope.

4 | 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, Berta, Costa, Abney, et al., 2012).

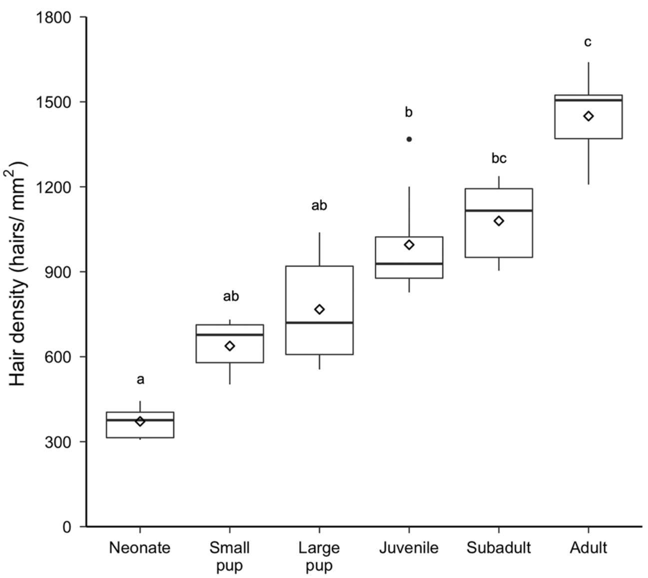

FIGURE 5 Sea otter hair density (hairs/mm^2) across age classes: neonate (N = 3), small pup (N = 3), large pup (N = 3), juvenile (N = 3), subadult (N = 3), and adult (N = 3). The horizontal line within each box indicates the median value of the interquartile range, and the box boundaries indicate the 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.

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, Berta, Costa, Abney, et al., 2012; Liwanag, Berta, Costa, Budge, et al., 2012). 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, little was previously known about the natal pelage and the pelage of intermediate age classes. Previous studies have analyzed sea otter guard hair length for adult sea otters (Fish et al., 2002; Kuhn & Meyer, 2010; Liwanag, Berta, Costa, Budge, et al., 2012; Tarasoff et al., 1972; Williams et al., 1992), 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 (Figure 2). Our results suggest that the natal pelage is shed for the adult pelage between the small pup (1- to 2-month-old) and large pup (3- to 5-month-old) age classes (Figure 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 (Figure 2). The contrast between the natal pelage and adult pelage in sea otters is visually apparent (Figure 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 (Figure 6). In contrast, the wet adult pelage is more streamlined (Figure 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 (Figure 6).

Across ontogeny, the conserved flatness or almond-like shape of sea otter guard hairs in cross section is important for the protection

TABLE 2 Number of underhairs, intermediate hairs, and guard hairs, and overall hair density (hairs/mm^2) for sea otter pelts across ontogeny.

Age class	N	Number of underhairs	Number of intermediate hairs	Number of guard hairs	Hair density (hairs/mm^2)
Neonate	3	665.33 ± 123.14[a]	9.78 ± 2.73	4.11 ± 1.36	371. 65 ± 53.00[a]
Small pup	3	1274.67 ± 203.61[ab]	16.00 ± 5.15	7.56 ± 5.15	637.99 ± 87.45[ab]
Large pup	3	1430.38 ± 344.00[ab]	16.50 ± 3.51	3.50 ± 1.77	767.25 ± 191.94[ab]
Juvenile	3	1904.88 ± 340.53[b]	8.38 ± 2.07	5.75 ± 2.19	995.31 ± 188.84[bc]
Subadult	3	1890.13 ± 272.14[b]	19.13 ± 8.90	6.00 ± 1.69	1079.59 ± 137.05[bc]
Adult	3	2863.33 ± 296.87[c]	20.56 ± 5.79	6.78 ± 0.97	1449.69 ± 152.03[c]

Note: Values are presented as mean ± 1 SD. 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 and guard hairs.

FIGURE 6 Photo of small pup (left) and mother sea otter (right). Note the differences in wet pelage appearance. The natal pelage of the pup is generally 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.

of the air layer (Figure 3). Our findings are consistent with previously reported morphology values for adult sea otter guard hairs (Kuhn & Meyer, 2010; Williams et al., 1992; Supporting Information: 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, Berta, Costa, Abney, et al., 2012), 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 (Figure 4). The underhairs themselves and the cuticular scales were similarly sized in both the natal pelage and adult pelage (Figure 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 nonaquatic adult mustelids exhibit elongation of the cuticular scales on their hairs (Liwanag, Berta, Costa, Abney, et al., 2012); 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 and size characteristics of intermediate hairs in other mammalian species (Erdsack et al., 2015), but no study has estimated the number of intermediate hairs. Our samples had intermediate hairs present across all age classes (Figure 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 (Figure 1), which is in contrast to previous descriptions of the organization of mammalian hair bundles (Kuhn et al., 2010; Liwanag, Berta, Costa, Abney, et al., 2012; Scheffer, 1964).

The hair density for nonadult 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 (Fish et al., 2002; Kenyon, 1969; Kuhn et al., 2010; Sokolov, 1962; Tarasoff, 1972, 1974; Williams et al., 1992; Supporting Information: 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 (Figure 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 (Figure 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 (Figure 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 (Gmuca et al., 2015; Pearson et al., 2014). 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 before what we consider the "molt" from the natal pelage to a more mature pelage type. Past observational studies of sea otters have noted a change from the natal pelage to an adult-like pelage between 10 and 14 weeks (between small pup and large pup age classes; 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 (Castellini, 2009; Williams et al., 1992). 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.

5 | CONCLUSIONS

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.

AUTHOR CONTRIBUTIONS

Kate Riordan: Conceptualization; methodology; funding acquisition; investigation; visualization; data curation; formal analysis; project administration; writing—original draft. **Annika E. Dean**: Methodology; investigation; writing—review & editing. **Payton Adema**: Methodology, investigation; writing—review & editing. **Nicole M. Thometz**: Conceptualization; writing—review & editing. **Francesca I. Batac**: Conceptualization; methodology; resources; writing—review & editing. **Heather E. M. Liwanag**: Conceptualization; methodology; funding acquisition; resources; investigation; visualization; data curation; project administration; supervision; writing—review & editing.

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DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the Supporting Information of this article.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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