Morphological and thermal properties of mammalian insulation: the evolution of fur for aquatic living

HEATHER E. M. LIWANAG[1]*, ANNALISA BERTA[2], DANIEL P. COSTA[1], MASAKO ABNEY[1,3] and TERRIE M. WILLIAMS[1]

[1]*Department of Ecology and Evolutionary Biology, University of California, Santa Cruz, Long Marine Laboratory, 100 Shaffer Road, Santa Cruz, CA 95060, USA*
[2]*Department of Biology, San Diego State University, 5500 Campanile Drive, San Diego, CA 92182, USA*
[3]*Department of Ecology, Evolution and Marine Biology, University of California, Santa Barbara, CA 93106, USA*

Carnivora includes three independent evolutionary transitions to the marine environment: pinnipeds (seals, sea lions, and walruses), sea otters, and polar bears. All three lineages must contend with the thermal challenges of submersion in the marine environment. In the present study, we investigated changes in the fur associated with the transition from a terrestrial to an aquatic lifestyle, comparing fur characteristics among these lineages with those of semi-aquatic and strictly terrestrial carnivores. Characteristics included gross morphology (hair cuticle shape, circularity, length, and density) and thermal conductivity. We found consistent trends in hair morphology associated with aquatic living, such that marine carnivores have significantly flatter ($P < 0.001$), shorter ($P < 0.001$), and denser hairs ($P < 0.001$) than terrestrial carnivores. However, sea lions, phocids, and walrus, which have thicker blubber layers than fur seals, have lower fur densities than fur seals ($P < 0.001$). Species utilizing fur for insulation in water also showed an elongation of hair cuticle scales compared to terrestrial species and marine species relying on blubber for insulation ($P < 0.001$). By testing pelts under hydrostatic pressure, we determined that flattening of the hairs, cuticular scale elongation, and increased fur density are critical characteristics for maintaining an insulating air layer within the fur during submersion. Overall, these results indicate consistent evolutionary modifications to the fur associated with the transition to aquatic living, as well as a decrease in fur function associated with a greater reliance on blubber in some groups. © 2012 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2012, **106**, 926–939.

ADDITIONAL KEYWORDS: adaptation – carnivora – conductivity – cuticle – density – guard hair – hydrostatic pressure – marine mammal – morphology – underhair.

INTRODUCTION

During the course of mammalian evolution, numerous lineages secondarily invaded the aquatic environment. These included the cetaceans (whales and dolphins), sirenians (manatees and dugongs), pinnipeds (seals, sea lions, and walrus), sea otters (*Enhydra lutris*, Linnaeus), and polar bear (*Ursus maritimus*,

Phipps). Key to the successful transition to living in water was modification of the type of insulation used to retain body heat and maintain a stable, relatively high core body temperature during prolonged submergence. Indeed, thermal stability is considered an evolutionary hallmark for fully aquatic mammals (Irving, 1969, 1973).

Rather than a single insulating mechanism, two evolutionary pathways for insulation have been described for these marine living mammalian lineages (Scholander *et al.*, 1950; Ling, 1970; Pabst, Rommel & McLellan, 1999; Berta, Sumich & Kovacs,

*Corresponding author. Department of Biology, Adelphi University, 1 South Avenue, Garden City, NY 11530, USA. E-mail: hliwanag@adelphi.edu

2006). First, mammals modified the ancestral form of insulation, external fur, to serve as the primary thermal barrier in water. Accordingly, the two most recent mammalian lineages to reinvade the marine environment, the sea otter (1.6 Mya) and the polar bear (0.5 Mya), still use fur as the primary insulator (Pabst *et al*., 1999; Berta *et al*., 2006). Other mammalian lineages developed a second type of insulation in the form of an internal blubber layer, which could also serve as an energy store. This is observed for the Cetacea and Sirenia, which invaded the water during the early Eocene (50 Mya), and have secondarily lost the hair covering that characterizes mammals (Hart & Fisher, 1964; Ling, 1970; Berta *et al*., 2006).

In general, specialization for aquatic living resulted in a trend in which there is a decreased reliance on external insulation via dense fur and a replacement by internal insulation provided by thick blubber layers. Interestingly, the Pinnipedia, comprisng the group that evolved during the late Oligocene (29–23 Mya) between the Cetacea and sea otters, is the only mammalian lineage that has retained both types of insulation. In particular, the otariids (fur seals and sea lions) show two distinct mechanisms of retaining body heat: fur seals have dense, waterproof fur and a moderate blubber layer, whereas sea lions rely solely on their blubber for insulation in water. This diversity in thermal insulation among pinnipeds provides a unique opportunity to evaluate the key characteristics of mammalian insulation that facilitate thermal stability in the aquatic environment.

The present study aimed to examine the evolutionary changes in fur insulation for aquatic living animals. To accomplish this, we measured and compared morphological traits (shape of the hair cuticle, hair circularity, hair length, and hair density) and physical properties (thermal conductivity, compression of the insulation under pressure) of the fur of carnivore species, with particular focus on the pinnipeds. These traits were then examined in a phylogenetic context to determine which characters were critical for thermoregulation in water, and to identify how each mammalian lineage utilized these characters to facilitate a marine existence.

MATERIAL AND METHODS

FUR SAMPLING

Samples were collected from 25 species of terrestrial and marine carnivores (See Supporting information, Table S1). Because samples were obtained opportunistically, a range of age classes (pups, juveniles, subadults, and adults) were available. Because young mammals typically exhibit similar fur characteristics to adult conspecifics (Meyer, Schnapper & Hülmann,

2002), all age classes were utilized for comparisons of fur characteristics. Fur samples were collected from full sculps (fur, skin, and blubber; $N = 96$) of deceased marine carnivores, fresh pelts (fur and skin; $N = 19$), tanned pelts ($N = 10$), or hairs removed from live animals ($N = 5$). Fresh tissue samples were collected only from good condition carcasses, as determined by tissue colour. Fresh sculp samples were 25×25 cm pieces taken from the dorsum, just caudal to the shoulders. Fresh pelt samples were also taken from the same location, although the size of each sample varied (range = 5×5 cm to 25×25 cm). Hairs removed from tanned pelts or live animals were taken from the mid-dorsum. The tanning process could stretch the skin, resulting in an underestimation of hair density; however, other hair characteristics are not affected by tanning. Thus, the only tanned pelt used to determine hair density was that of the ermine (*Mustela erminea*, Linnaeus), for which no other samples were available. All fresh sculp and pelt samples were wrapped in plastic wrap and stored in heavy-duty freezer bags to prevent desiccation. All samples were kept frozen at –20 °C until used for analyses. Additional data were taken from the literature, where available. Measurements incorporated from other studies were from adult or subadult animals.

SHAPE OF THE HAIR CUTICLE

Using tweezers, three guard hairs and three underhairs were carefully removed from each sample under a dissecting microscope (SMZ645; Nikon Instruments Inc.). Hairs were washed with alcohol and prepared for scanning electron microscopy by mounting them on aluminum stubs with cyanoacrylic adhesive (Krazy Glue®). The stub and hairs were then sputter-coated with gold-palladium. The coated specimens were viewed and digitally photographed with an ISI WB-6 scanning electron microscope to visualize cuticular scale patterns. Magnifications were matched as closely as possible, although they were ultimately determined by the focal window of the microscope. Scale patterns were visualized near the base of each hair. Using ImageJ software (National Institute of Health), scale shape was quantified with the ratio of scale length to scale width, for which elongated scales have larger values.

HAIR LENGTH AND DIAMETER

Three guard hairs and three underhairs were removed from each sample, as described above. Each hair was washed with alcohol, laid flat in a coat of clear acrylic polish on a plastic sheet, and covered again with polish to keep the hair flat. Hair length was measured to the nearest 0.01 mm with digital

calipers (ABSOLUTE Digimatic Caliper Series 500; Mitutoyo). The hair embedded in dried polish was then peeled from the sheet cover and placed under a dissecting microscope. A cross section perpendicular to the length of the hair was taken at the widest point on the hair, and mounted vertically on a 1.3-cm Styrofoam block. This mount was viewed under a compound microscope (CH-3; Olympus America Inc.), under the ×20 objective for guard hairs and ×40 or ×100 objective for underhairs. Using an ocular micrometer, the maximum and minimum diameter of each hair was determined. Note that hairs were easily distinguished from the polish mount under the microscope, so that only the hair diameter was measured. The ratio of the minimum diameter to the maximum diameter gave an index of circularity of the hair shaft, with perfect circularity represented by a ratio of 1.0. For hair lengths and diameters, values for three guard hairs and three underhairs were averaged to yield a single value per individual for each hair type.

FUR DENSITIES

Fur densities were determined *sensu* Scheffer (1964a). A small (5 × 5 cm) square of skin with fur was isolated from the original sample, and any subcutaneous fat or blubber removed. The pelt sample was fixed in 10% buffered formalin for a minimum of 5 days, and then flattened in a press. Because skin tends to contract slightly when removed from the animal and even further when dried, the pressing process helped to restore the sample more closely to its living dimensions (Scheffer, 1964a; Fish *et al.*, 2002). After the sample was flattened, the hair was cut with scissors and then shaved nearly flush with the skin using a safety razor. Six circular discs were cut with a trephine from each shaved sample. The trephine was a cylindrical metal tube sharpened at one end, with a 0.88-cm inner diameter. Because the diameter of the trephine could change with repeated use, the inner diameter was measured with digital calipers (Mitutoyo) before use on each sample, and this value was used to calculate the area of each disc cut. Each disc was stored between two glass microscope slides until needed. Although the area of the disc might become distorted after cutting, the number of hair bundles could not change from further treatment of the disc; thus, the original area cut by the trephine was used to calculate disc area and ultimately hair density for each sample disc.

Mammalian fur is organized into bundles, each of which consists of a single guard hair accompanied by a number of underhairs (if present). To count the number of bundles present per unit area, we took digital photographs of three discs from each pelt sample, under a dissecting microscope (SZX7; Olympus America Inc.) with a ×1 objective. Using ImageJ software, every bundle on each disc was marked digitally, and the marks were summed. Mean bundle density was determined from the average of three discs per pelt sample. To count the number of underhairs per bundle, underhairs were manually counted for 20 representative bundles under a compound microscope with the ×10 or ×20 objective (CH-3; Olympus America Inc.). The mean number of underhairs per bundle was determined from the average of 3 discs per pelt sample. This value was combined with the mean number of bundles per unit area to determine the average fur density for each pelt sample.

EFFECTS OF HYDROSTATIC PRESSURE

To examine the effects of hydrostatic pressure on the integrity of the insulation, a sample was isolated from the original sculp for a subset of pinniped species ($N = 8$ species; see Supporting information, Table S1). Each sample was cut to exactly 4.0 cm laterally and 5.2 cm dorso-ventrally to fit the dimensions of the experimental chamber. Because we determined no detectable changes in blubber under pressure up to 70 m simulated depth, the underlying blubber was removed from sculp samples to facilitate adhesion to the container insert. The fur was cleaned using cold running water, and the air layer restored to the fur using a hairdryer *sensu* Williams *et al.* (1988) and Kvadsheim & Aarseth (2002). The pelt sample was then fit into a clear plastic container insert (length 4.0 cm, width 5.2 cm, height 10.0 cm) and the skin adhered to the bottom with silicon adhesive. Dry fur thickness was measured for both guard hairs and underhairs to the nearest 0.01 mm with digital calipers (Mitutoyo) three times on each side, and the mean values were used for calculations. Water was then carefully poured onto the sample, and wet fur thickness was measured for guard hairs and underhairs using the same method.

The plastic container with the immersed sample was placed into a hyperbaric chamber (TS3; Trident Systems Inc.) with a viewing window, such that the side of the container was flush against the viewing window. Dives to 70 m (i.e. the maximum capability of the chamber) were simulated once on each lateral side of the sample, with the fur dried and air layer restored between trials. During each simulated dive, height of the guard hairs, underhairs, and water level were marked every 10 m for both descent and ascent, on transparent tape adhered to the viewing window. These marks were subsequently measured to the nearest 0.01 mm with the digital calipers (Mitutoyo). Reductions in water level were attributed to loss of air from the pelt during the dive. To detect any air

remaining in the fur after a dive, entrapped air was forcibly removed from the fur by vigorously rubbing a dissecting probe through the pelt, and the resulting change in water level (if any) was measured.

THERMAL CONDUCTIVITY

Thermal conductivity was measured for squares (approximately 10×10 cm) trimmed from sculp or pelt samples, using the standard material method (Kvadsheim, Folkow & Blix, 1994; Kvadsheim & Aarseth, 2002; Dunkin *et al*., 2005). The fur was cleaned using cold running water, and then the air layer was restored to the fur using a hairdryer on the cool setting (Williams *et al*., 1988; Kvadsheim & Aarseth, 2002). Skin and dry fur thickness were measured to the nearest 0.01 mm with digital calipers (Mitutoyo) three times on each side, and the mean values were used for calculations.

Measurements were conducted in a heat flux chamber (162 quart Igloo Marine ice chest; Igloo Commercial) with a lower, highly insulated compartment and an upper, chilled compartment modeled after Dunkin *et al*. (2005). The insulated compartment contained the heat source, a sealed aluminum box through which heated water (35 °C) was circulated from a constant-temperature water bath (Lauda RM20; Brinkmann Instruments). The upper chamber was cooled with ice packs to create a steady thermal gradient.

An elastomer (Plastisol vinyl; Carolina Biological Supply) was used as the standard material ($k = 0.124 \pm 0.008$ W m^{-1} °C^{-1}). The standard material was placed flush against the heat source, and the sample was placed in series with the standard so that the fur was exposed to the cold air. The standard material and sample were surrounded by insulation to ensure unidirectional heat flow through the materials.

Temperatures were measured using three copper-constantin (Type T) thermocouples (Physitemp Instruments, Inc.) placed between the surface of the heat source and the standard material, three thermocouples between the standard material and the sample, and three thermocouples on top of the fur. In addition, two thermocouples were placed at the base of the fur. All thermocouples were wired to a Fluke Hydra data logger (model 2625A; Fluke Inc.), which recorded the outputs every 6 s onto a laptop computer. Trials lasted a minimum of 2 h to ensure that the apparatus reached steady state, and data were analyzed for the final 30 min of each trial.

Thermal conductivity was calculated across the full pelt and across the fur layer only, using the Fourier equation (Kreith, 1958):

$$H = k \times A \times \Delta T \times L^{-1} \tag{1}$$

where H is heat transfer in J s^{-1}, k is thermal conductivity in W m^{-1} °C^{-1}, A is the area (in m^2) through which the heat is moving, ΔT is the temperature differential (in °C) across the material, and L is the thickness of the material in m. Assuming that heat transfer is equal across both the standard material and the sample, the equations for both materials can be set equal and solved for the thermal conductivity of the sample.

STATISTICAL ANALYSIS

Numerical values for all data are presented as the mean ± SD. The relationship between each characteristic and body mass was examined on a log-log scale, and any covariance taken into account before further statistical analysis. As suggested by Harvey & Pagel, (1991), a nested analysis of variance (ANOVA) was used to incorporate phylogenetic influences into statistical comparisons. Statistical significances among means were determined for families using a composite phylogeny (Flynn *et al*., 2005; Higdon *et al*., 2007), and species nested in families, using nested ANOVA and the Tukey's honestly significant difference test (JMP Software; SAS Institute). Where unspecified, $P < 0.05$ was considered statistically significant.
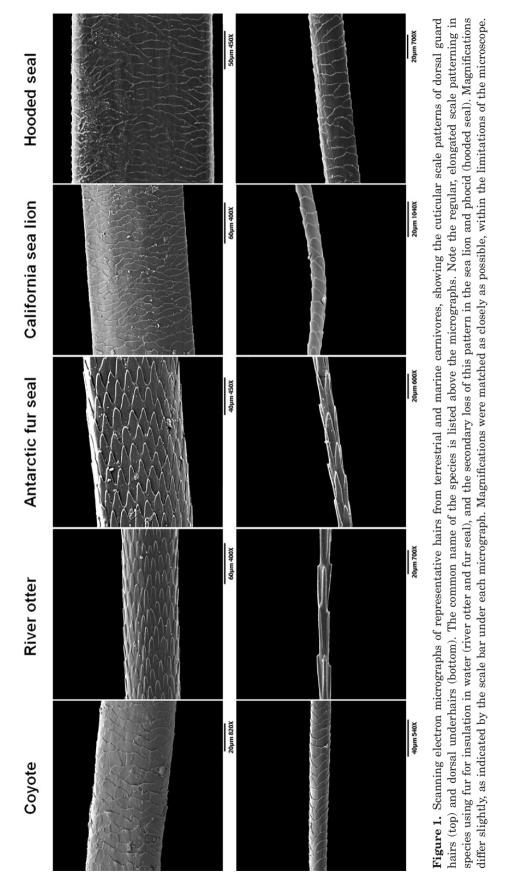
RESULTS

SHAPE OF THE HAIR CUTICLE

Shape of the hair cuticle was visualized for eight terrestrial, two semi-aquatic, and 14 marine carnivore species (Fig. 1; see also Supporting information, Table S1). Terrestrial species showed an irregular cuticular scale pattern on both guard hairs and underhairs, with few exceptions. Unlike the other terrestrial species examined, two felid species showed distinctive cuticular scaling. The domestic cat (*Felis catus*, Linnaeus) had elongated cuticular scales on both guard hairs and underhairs, while the bobcat (*Lynx rufus*, Schreber) had elongated and pointed scales on the underhairs. However, the strictly terrestrial families including the felids had significantly shorter cuticular scales ($F_{7,16} = 425.4173$, $P < 0.001$) compared to the mustelids and otariids (Fig. 2).

Regardless of aquatic or terrestrial lifestyles, all of the mustelids examined maintained regular cuticular scaling patterns with elongated scales (Fig. 2). Thus, the primarily terrestrial ermine (*Mustela erminea*) showed extremely regular, elongated scale patterning on both guard hairs and underhairs. This was also evident for the river otter (*Lontra canadensis*, Schreber) and sea otter (*Enhydra lutris*).

Similar to the otters, all five fur seal species examined showed regular, elongated scalar patterning on both guard hairs and underhairs (Figs 1, 2). By

Figure 1. Scanning electron micrographs of representative hairs from terrestrial and marine carnivores, showing the cuticular scale patterns of dorsal guard hairs (top) and dorsal underhairs (bottom). The common name of the species is listed above the micrographs. Note the regular, elongated scale patterning in species using fur for insulation in water (river otter and fur seal), and the secondary loss of this pattern in the sea lion and phocid (hooded seal). Magnifications differ slightly, as indicated by the scale bar under each micrograph. Magnifications were matched as closely as possible, within the limitations of the microscope.

Figure 2. Elongation (maximum length/maximum width) of the cuticular scales of carnivore guard hairs (black bars) and underhairs (white bars). Heights of the bars and lines indicate the mean ± SD for the indicated families or groups. Letters above the bars indicate statistically significant differences among means, with uppercase letters for guard hairs and lowercase letters for underhairs. Felidae includes the domestic cat, bobcat, and mountain lion; Canidae includes the red fox, coyote, and domestic dog; Ursidae includes the polar bear; Mephitidae includes the striped skunk; Procyonidae includes the raccoon; Mustelidae includes the ermine, river otter, and sea otter; Otariidae includes four fur seals and three sea lions; and Phocidae includes five phocid seals. Data are reported from the present study only.

contrast, all three sea lion species showed irregular and shortened cuticular scale patterns on both guard hairs and underhairs, which were more characteristic of terrestrial carnivores. The five phocid seal species also demonstrated irregular and shortened cuticular scale patterning on guard hairs and underhairs, as well as a reduction in the prominence of the scales. In addition, the polar bear (*Ursus maritimus*) demonstrated a marked reduction in the prominence of cuticular scales on both guard hairs and underhairs.

HAIR LENGTHS AND DIAMETERS

Circularity of the hair was calculated as the ratio of the minimum diameter to the maximum diameter, and did not correlate consistently with body mass. Guard hairs were significantly flatter ($F_{24,84} = 84.697$, $P < 0.001$) in the aquatic groups compared to the terrestrial species, with some exceptions (Fig. 3; see also Supporting information, Table S2). Otariids and

phocids ($N = 12$ species) had significantly shorter guard hairs compared to felids ($N = 3$), canids ($N = 3$), and polar bear ($N = 1$). The raccoon (*Procyon lotor*, Linnaeus, $N = 1$), skunk (*Mephitis mephitis*, Schreber, $N = 1$), and mustelids ($N = 3$) demonstrated intermediate circularities. There were no significant differences in guard hair circularity within families, except for the mustelids. Both the river otter and sea otter had significantly flatter guard hairs compared to the primarily terrestrial ermine ($P < 0.050$).

Underhair circularity showed significant differences among families ($F_{23,73} = 6.464$, $P < 0.001$), although the pattern was not as marked as that for guard hairs (Fig. 3; see also Supporting information, Table S2). Underhairs were significantly flatter in the otariids ($N = 7$ species) compared to felids ($N = 3$), canids ($N = 3$), mustelids ($N = 3$), and phocids ($N = 5$). The circularity of raccoon ($N = 1$), skunk ($N = 1$), and polar bear ($N = 1$) underhairs did not differ significantly from that of any other group. Significant differences in underhair circularity were not detected among species within families.

Hair length did not correlate consistently with body mass. Hair length differed significantly among families ($F_{47,106} = 8.918$, $P < 0.001$), with longer hairs in terrestrial species (Fig. 4; see also Supporting information Table S3). Guard hairs were significantly shorter in mustelids ($N = 16$ species), otariids ($N = 10$), phocids ($N = 13$), and walrus ($N = 1$) compared to felids ($N = 3$), canids ($N = 3$), polar bear ($N = 1$), raccoon ($N = 1$), and skunk ($N = 1$). Phocids also had significantly shorter guard hairs compared to mustelids. Within the otariids, fur seals ($N = 6$ species) had significantly longer guard hairs than sea lions ($N = 6$ species; $P < 0.001$). Within the phocid family, members of the Phocinae ($N = 7$ species) had significantly longer guard hairs compared to members of the Monachinae ($N = 6$ species; $P = 0.011$).

Underhair length also demonstrated a significant trend among families, with shorter hairs in aquatic groups ($F_{34,82} = 7.016$, $P < 0.001$; Fig. 4; see also Supporting information Table S3). Underhairs were significantly shorter in mustelids ($N = 16$ species), otariids ($N = 8$), and phocids ($N = 4$) compared to felids ($N = 3$), canids ($N = 3$), polar bear ($N = 1$), and skunk ($N = 1$). Underhairs were significantly longer in the raccoon ($N = 1$) compared to mustelids and otariids but did not differ significantly from phocids. Underhairs are completely absent in the walrus. Within otariids, fur seals ($N = 5$ species) had significantly longer underhairs than sea lions ($N = 3$ species; $P < 0.001$). Differences among phocine and monachine underhair length could not be tested because northern elephant seals lack underhairs, and underhair length was not reported for the species examined in Scheffer (1964b).
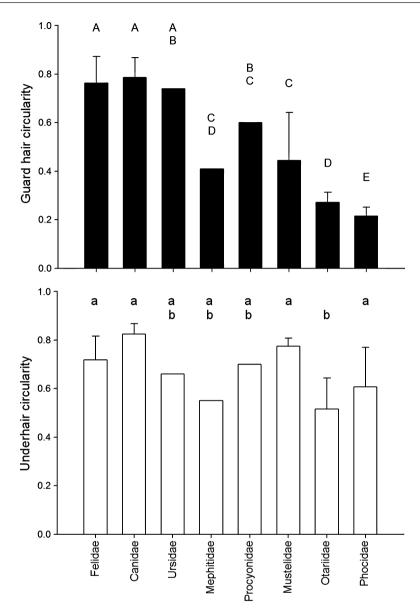
Figure 3. Circularity (minimum diameter/maximum diameter) of carnivore guard hairs (black bars, top panel) and underhairs (white bars, bottom panel). Heights of the bars and lines indicate the mean ± SD for the indicated families (no error bars for single species). Letters above the bars indicate statistically significant differences among means, with uppercase letters for guard hairs and lowercase letters for underhairs. Felidae includes the domestic cat, bobcat, and mountain lion; Canidae includes the red fox, coyote, and domestic dog; Ursidae includes the polar bear; Mephitidae includes the striped skunk; Procyonidae includes the raccoon; Mustelidae includes the ermine, river otter, and sea otter; Otariidae includes four fur seals and three sea lions; and Phocidae includes five phocid seals. Values for fur seals and sea lions were not significantly different for this character, and are thus presented together in Otariidae. Data are reported from the present study only.

FUR DENSITIES

As with the other fur characteristics, fur density showed no consistent relationship with body mass. Fur densities varied significantly among families ($F_{30,72} = 21.364$, $P < 0.001$), with greater fur densities in families with species relying primarily on fur for insulation in water (Fig. 5; see also Supporting information Table S4). Accordingly, the mustelids ($N = 5$ species) had significantly greater fur densities than all other groups. The otariids ($N = 10$) had significantly greater fur densities than the canid (red fox, *Vulpes vulpes*, Linnaeus) and phocids ($N = 13$) but did not differ significantly from the other groups. There
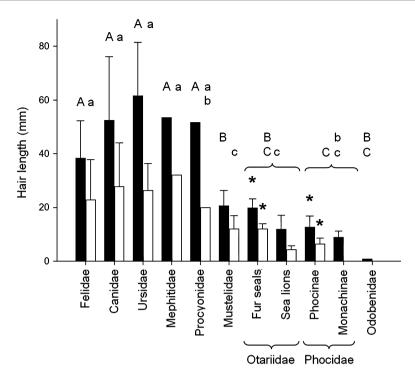
Figure 4. Lengths of carnivore guard hairs (black bars) and underhairs (white bars). Heights of the bars and lines indicate the mean ± SD for the indicated families (no error bars for single species). Letters above the bars indicate statistically significant differences among family means, with uppercase letters for guard hairs and lowercase letters for underhairs. Asterisks indicate significant differences between groups within families. Felidae includes the domestic cat, bobcat, and mountain lion; Canidae includes the red fox, coyote, and domestic dog; Ursidae includes the polar bear; Mephitidae includes the striped skunk; Procyonidae includes the raccoon; Mustelidae includes the ermine, mink, and 14 otter species; Otariidae includes six fur seals and four sea lions for guard hairs, five fur seals and three sea lions for underhairs; Phocidae includes 13 species (seven phocines, six monachines) for guard hairs and four species (three phocines, one monachine) for underhairs; Odobenidae includes the walrus. Data are reported from the present study, Scheffer (1964b), Frisch *et al*. (1974), Hilton & Kutscha (1978), Williams *et al*. (1992), Fish *et al*. (2002), and Kuhn & Meyer (2010a).

was no significant difference in fur density among the felids ($N = 2$), polar bear or walrus (*Odobenus rosmarus*, Linnaeus). There were significant differences in fur density among species within mustelids, otariids, and phocids. Among mustelids, the sea otter had significantly denser fur than the river otter, mink (*Neovison vison*, Schreber), and ermine (Fig. 5). The semi-aquatic river otter had a fur density significantly greater than that for the primarily terrestrial ermine, whereas the semi-aquatic mink had a comparably intermediate fur density. There was no statistical difference in fur density for the Eurasian otter (*Lutra lutra*, Linnaeus), likely as a result of reduced statistical power. Among the otariids, fur seals ($N = 6$ species) had significantly denser fur (431.5 ± 112.9 hairs·mm^{-1}) than sea lions (23.2 ± 11.2 hairs·mm^{-1}; $N = 4$ species), except for the New Zealand fur seal (*Arctocephalus forsteri*, Lesson) and New Zealand sea lion (*Phocarctos hookeri*, Peters), which were not significantly different from any other otariid species (Fig. 6). Note that the lack of significance here is likely a result of the reduced statistical power asso-

ciated with single samples. Among the phocids, phocines ($N = 7$ species) had significantly greater fur densities (25.8 ± 11.9 hairs·mm^{-1}) than monachines (15.0 ± 7.2 hairs·mm^{-1}; $N = 6$ species; $P = 0.018$).

EFFECTS OF HYDROSTATIC PRESSURE

Of the species examined, only fur seal pelts were able to maintain an air layer in the fur upon immersion; the sea lion and phocid pelts were saturated with water when immersed (Fig. 7). During the simulated 70-m dives, 78.3–99.6% of the trapped air in the fur seal pelts bubbled out at 20 m during ascent. For fur seals, guard hairs were compressed 5.6–28.9% upon immersion, and compressed another 4.3–15.1% during the dive; underhairs were compressed 2.9–18.9% upon immersion, and compressed another 5.3–25.1% during the dive. Sea lion and phocid guard hairs were compressed 29.1–52.3% upon immersion, and compressed another 0.0–2.1% during the dive. Underhairs were either not present or not visible for measurement in the sea lion and phocids.
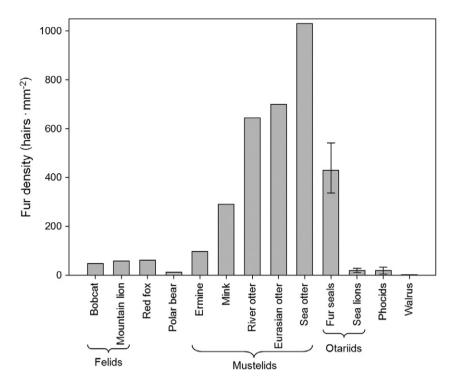
Figure 5. Fur density among carnivores. Heights of the bars indicate means for the indicated species or groups. Whiskers indicate range of species means (no whiskers for single species). Fur seals include six species; sea lions include four species; and phocids include 13 species. Data are reported from the present study, Sokolov (1962), Scheffer (1964b), Kenyon (1969), Kaszowski, Rust & Shackleford (1970), Tarasoff (1972), Frisch *et al.* (1974), Tarasoff (1974), Williams *et al.* (1992), Fish *et al.* (2002), and Kuhn *et al.* (2010).

THERMAL CONDUCTIVITY

Thermal conductivity of the fur layer alone was significantly lower than thermal conductivity of the full pelt (fur and skin) for all samples measured in this study (paired *t*-test, t = 2.730, $P = 0.009$). Because previous studies used full pelts for similar measurements (Scholander *et al.*, 1950; Hammel, 1955), thermal conductivity of the full pelt was used for comparisons among species. Note that all conductivity values for terrestrial species are taken from Scholander *et al.* (1950) and Hammel (1955). Thermal conductivity of the pelt in air varied among families ($F_{21,82} = 11.715$, $P < 0.001$), with higher conductivities in fully aquatic species (Table 1). The thermal conductivities of phocid ($N = 3$ species) and otariid ($N = 8$) pelts were significantly greater than mustelid ($N = 3$) and canid ($N = 4$) pelts. The thermal conductivities of procyonid [raccoon and kinkajou (*Potos flavus*, Schreber)] pelts were significantly lower than phocid pelts, although not significantly different from any other group.

DISCUSSION

Fur functions as an insulator by maintaining a relatively still air layer between the animal's skin and the surrounding environment, primarily within the dense underfur (Ling, 1970). However, the insulating value of fur is diminished by the presence of water vapor, and wet fur is further compromised as an insulator (Scholander *et al.*, 1950; Hammel, 1955; Johansen, 1962; Ling, 1970; Frisch, Ørtisland & Krog, 1974; Morrison, Rosenmann & Estes, 1974; Costa & Kooyman, 1982; Doncaster *et al.*, 1990). In addition, the physical forces of drag during swimming and hydrodynamic pressure during diving alter the position of the fur relative to the body (Fish, 2000). Under water, the trapped air layer is key because penetration of the fur by water results in a three-fold increase in heat loss (McEwan, Aitchison & Whitehead, 1974; Williams, 1986; Williams *et al.*, 1988; Kuhn & Meyer, 2009). Because the insulative effectiveness of fur requires the presence of trapped air, the transition to an aquatic lifestyle required several modifications of fur to maintain its insulative function in water.

Guard hairs became flattened and maintained the air layer trapped by the fine underhairs in aquatic species, including the semi-aquatic river otter (Fig. 3). Unlike most terrestrial mammals, sea otters and pinnipeds lack arrector pili muscles in their hair follicles, and therefore have little physiological control over the
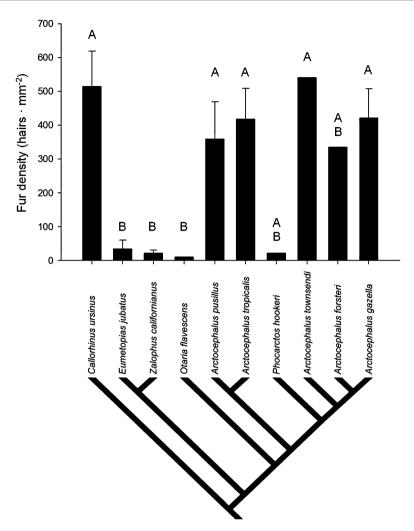
Figure 6. Fur densities among otariid species, with a cladogram illustrating the evolutionary relationships among the species (based on Higdon *et al*., 2007; Yonezawa *et al*., 2009). Heights of the bars and lines indicate the mean ± SD for the indicated species (no error bars for single samples). Uppercase letters above the bars indicate statistically significant differences among means. Note that fur seals have significantly greater fur densities compared to sea lions, and that the paraphyly of these groups indicates convergent evolution for this trait. Data are reported from the present study and Scheffer (1964b).

Table 1. Thermal conductivities of pelts for carnivore groups

Group	Pelt conductivity (W m^{-1} °C^{-1})	Source
Canids	0.048 ± 0.006	Scholander *et al*. (1950)
Ursids	0.069 ± 0.015	Scholander *et al*. (1950)
Procyonids	0.034 ± 0.004	Scholander *et al*. (1950)
Mustelids	0.045 ± 0.030	Present study; Scholander *et al*. (1950)
Otariids	0.111 ± 0.034	Present study
Phocids	0.158 ± 0.104	Present study

Values are presented as the mean ± SD. Canids include the Arctic fox, red fox, wolf, and domestic dog. Ursids include the grizzly bear and polar bear. Procyonids include the raccoon and kinkajou. Mustelids include the marten, least weasel, and river otter. Otariids are represented by five fur seals and three sea lions, and phocids are represented by three species.
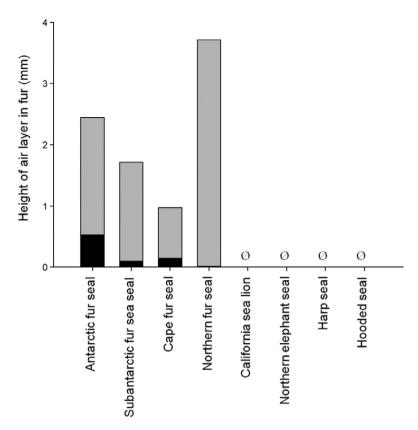
Figure 7. Amount of air trapped in the fur and lost during diving for the pelts of eight pinniped species. Full height of the bars indicates the total height of the air layer trapped in the fur. Grey bars represent the amount of air lost at 20-m depth during the ascent of a 70-m simulated dive. Black bars represent the amount of air remaining in the fur after a 70-m simulated dive. Pelts of the sea lion and phocid seals were saturated upon immersion.

positioning of the hairs (Montagna & Harrison, 1957; Scheffer, 1962; Ling, 1965; Ling 1970). When the animal is submerged, this feature increases the extent to which the hairs lie flat against the body, and the overlapping arrangement of guard hairs protects the underlying air layer by preventing penetration by water (Kuhn & Meyer, 2009). The flattened hairs lay parallel to the body surface, such that forward movement of the animal through the water should maximize the flattening of the hairs against the body (Kuhn & Meyer, 2009). The flat shape of the guard hair facilitates both laying flush when the animal is submerged and natural lifting as the fur dries (Ling, 1970; H. Liwanag, pers. observ.), enhancing its function in both air and water without the need for piloerector muscles.

The flattening of underhairs was not as marked as that observed for guard hairs (Fig. 3). The underhairs are comparatively fine and malleable, and are not typically exposed to the surrounding water. However, the underhairs of pinnipeds were significantly flatter than those of most terrestrial species, demonstrating a consistent pattern for both hair types. The shortening

of both guard hairs and underhairs (Fig. 4) probably facilitates streamlining, thus providing an indirect thermal benefit by moving water past the animal. Hence, the flattening and shortening of the hairs in aquatic species not only provides a waterproof barrier to protect the insulating air layer, but also facilitates movement through the water by reducing pressure drag (Noback, 1951; Ling, 1970; Fish, 2000).

A key characteristic associated with preventing the penetration of water into the fur is the ability of underhairs to interlock with each other. As previously described for otter species (Williams *et al.*, 1992; Weisel, Nagaswami & Peterson, 2005; Kuhn & Meyer, 2010b), both the river otter and sea otter demonstrated characteristic geometric scale patterning on the underhairs (Fig. 1), which enables the flexible underhairs to interlock and more efficiently maintain an air layer even when submerged (Kuhn & Meyer, 2010b). Similar to other otter species (Kuhn & Meyer, 2010a), both the river otter and sea otter also showed regular, elongated scale patterning on the guard hairs (Fig. 1), which may increase the tendency of the hairs to overlap. Interestingly, these same patterns were

observed for guard hairs and underhairs in the ermine, a primarily terrestrial mustelid, and were previously described for the guard hairs of the western polecat (*Mustela putorius*, Linnaeus) (Meyer *et al.*, 2002). The ermine often burrows in snow and does have the ability to swim long distances (Taylor & Tilley, 1984), which could have provided selective pressure for the development of interlocking scales; however, the existence of such patterning on the hairs of the strictly terrestrial polecat suggests a potential exaptation of hairs for water colonization among the mustelids.

Fur seals share the same elongated patterning on both guard hairs and underhairs (Figs 1, 2), consistent with their use of fur to trap air and insulate against the aquatic environment (Fig. 7). There is still debate as to whether mustelids or ursids are the closest outgroup to the pinnipeds (Berta *et al.*, 2006), and so it is unclear whether this shared characteristic represents convergent evolution or retention of a shared common ancestry. However, both sea lions and phocids have clearly lost this patterning secondarily (Figs 1, 2), which provides morphological evidence that both groups no longer utilize the fur for insulation in water. This was further confirmed by the lack of trapped air in immersed sea lion and phocid pelts compared to fur seal pelts (Fig. 7).

Patterns of fur density also indicate initial changes associated with aquatic living that are reduced or lost in the later diverging species (Fig. 5). Significant differences in fur density were observed within the mustelid family alone (ermine, mink, river otter, Eurasian otter, and sea otter), such that more aquatic species showed concomitant increases in fur density (Fig. 5). Such increases in fur density facilitate the interlocking of underhairs and thus the trapping of air when the animal is submerged (Romanenko & Sokolov, 1987; Fish *et al.*, 2002). Because pelage units, or bundles, are typically spaced evenly across the body surface (Scheffer, 1964b), increases in fur density can be attributed to increases in the number of underhairs per bundle. Accordingly, the secondary reduction in fur density observed in sea lions, phocids, and walrus is associated primarily with a reduction or complete loss of underhairs (Scheffer, 1964b; present study). Interestingly, the spacing of pelage units is no longer uniform in the walrus and most phocid species (Scheffer, 1964b), which may contribute further to a decline in fur density in those species. Both decreases in underfur and non-uniform spacing of pelage units are consistent with the loss of thermal function for the fur in water.

The loss of thermal function in water for sea lion fur represents a clear case of convergent evolution with phocids. Although the exact evolutionary relationship among otariid species (fur seals and sea lions) is subject to continued debate (Berta *et al.*, 2006), both morphological and molecular studies agree that the northern fur seal (*Callorhinus ursinus*, Linnaeus) is the earliest diverging species of the extant otariids (Berta & Deméré, 1986; Berta & Wyss, 1994; Lento *et al.*, 1995, 1997; Wynen *et al.*, 2001; Deméré, Berta & Adam, 2003; Higdon *et al.*, 2007; Yonezawa, Kohno & Hasegawa, 2009). The placement of a fur seal at the basal position suggests that dense fur was an ancestral characteristic for otariids, and that sea lions must have secondarily lost this characteristic (Fig. 6). This may be a case of multiple evolutionary transitions, as suggested by the paraphyletic relationships of sea lions and fur seals in recent molecular phylogenies (Wynen *et al.*, 2001; Higdon *et al.*, 2007; Yonezawa *et al.*, 2009). Total evidence analysis, which combines both morphology and molecular data, also indicates that fur seals and sea lions are paraphyletic groupings (Flynn *et al.*, 2005). The morphological changes observed for sea lion and phocid fur (i.e. irregularity of cuticular scales and a reduction in fur density) provide further evidence of convergent evolution associated with aquatic specialization (Figs 2, 5).

The higher thermal conductivity of the pelt in pinnipeds compared to terrestrial carnivores suggests some loss of functionality in air associated with the morphological adaptations of the fur to the aquatic environment (Table 1). Although these values were measured in air, the thermal conductivity of the immersed pelt would only increase relative to its conductivity value in air (Scholander *et al.*, 1950; Williams, 1986; Williams *et al.*, 1988). Thus, pinnipeds with fur that no longer functions in water must rely instead on an alternative form of insulation. Overall, there appears to be a trade-off in terms of the function of fur in different media, such that mammals utilizing both terrestrial and aquatic environments will experience reductions in the effectiveness of their insulation compared to mammals living exclusively in a single medium.

ACKNOWLEDGEMENTS

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Hair, pelt, and full sculp samples analyzed in the present study. Total number of samples (N) is indicated for each species, with sample types indicated by superscripts: A, full sculp; B, fresh pelt; C, tanned pelt; D, hairs removed from live animal. Analyses performed on at least one sample are indicated by an 'X' in the appropriate column. SEM, scanning electron microscopy; k, thermal conductivity; Press., hydrostatic pressure experiments.

Table S2. Species mean ± SD for hair circularity from the present study. N = number of individuals examined for that species. For $N = 1$, the mean ± SD is from three representative hairs. Note that underhairs are absent in the northern elephant seal (*Mirounga angustirostris*).

Table S3. Species means for hair length from the present study and published sources. Dashes indicate missing values. For $N = 1$ from the present study, the mean ± SD is from three representative hairs. *Guard hair data from Hilton & Kutscha (1978) are weighted averages; no SDs were reported.

Table S4. Species mean ± SD for fur density from the present study and published sources. *Values from Frisch *et al*. (1974) are median values.

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