



Morphological and thermal properties of mammalian insulation: the evolutionary transition to blubber in pinnipeds

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Carnivora includes three independent evolutionary transitions to the marine environment: pinnipeds (seals, sea lions, and walrus), sea otters, and polar bears. Among these, only the pinnipeds have retained two forms of insulation, an external fur layer and an internal blubber layer for keeping warm in water. In this study we investigated key factors associated with the transition to the use of blubber, by comparing blubber characteristics among the pinnipeds. Characteristics included gross morphology (blubber thickness), fat composition (fatty acid profiles, percentage lipid, and water), and thermal conductivity. Sea lions, phocids, and walrus, which have lower fur densities than fur seals, have thicker blubber layers than fur seals ($P < 0.001$). Comparisons of lipid content, water content, and fatty acid composition indicated significant differences in the composition of the inner and outer regions of the blubber between groups ($P < 0.001$), consistent with the hypothesis that phocids and sea lions utilize the outer layer of their blubber primarily for thermal insulation, and the inner layer for energy storage. Fur seals, by contrast, rely more on their fur for thermal insulation, and utilize their blubber layer primarily for energy storage. Comparing across carnivore species, differences in total insulation (fur and/or blubber) are influenced substantially by body size and habitat, and to a lesser extent by latitudinal climate. Overall, these results indicate consistent evolutionary trends in the transition to blubber and evidence for convergent evolution of thermal traits across lineages. © 2012 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2012, **107**, 774–787.

ADDITIONAL KEYWORDS: aquatic adaptation – blubber thickness – carnivora – fatty acid composition – lipid content – marine mammal – morphology – pinnipedia – thermal conductivity – thermal resistance.

INTRODUCTION

During the course of mammalian evolutionary history, several lineages secondarily invaded the aquatic environment, and this transition was associated with the development of specialized, waterproof

insulation to aid in retaining body heat. Indeed, the development of specialized insulation represents the most efficient long-term solution for maintaining a high, stable core body temperature while living in water (Williams & Worthy, 2002). The transition from terrestrial to aquatic living by mammals was accompanied by numerous modifications in the type of insulation used to retain body heat. Early changes in pelage included flattening of the guard hairs and

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increased hair density to act as a water-resistant barrier and reduce hydrodynamic drag while swimming (Ling, 1970; Liwanag *et al.*, 2012).

Subcutaneous fat in mammals is an energy storage tissue that may also serve other functions (Pond, 1978). As mammals became more specialized for living in water, the reliance on external insulation provided by fur decreased while internal insulation provided by thick blubber layers dominated. Blubber is a continuous, subcutaneous layer of adipose tissue, reinforced by collagen and elastic fibres (Parry, 1949; Ling, 1974; Lockyer, McConnell & Waters, 1984, 1985; Pabst, Rommel & McLellan, 1999). For aquatic mammals with a blubber layer, the fat acts not only as an energy store but also as an insulator (Scholander *et al.*, 1950), and can contribute to body streamlining as well as buoyancy control (Pabst *et al.*, 1999; Fish, 2000). The blubber layer serves as the primary form of insulation for fully aquatic mammals, and evolved independently at least three times among mammals: in cetaceans, sirenians, and pinnipeds.

Across groups, the most recent invaders of the marine environment – the sea otter (1.6 Mya) and the polar bear (0.5 Mya) – utilize a dense, non-wettable fur for insulation; the deep-rooted marine mammal lineages including the sirenians (manatees, dugongs, 50 Mya) and cetaceans (50 Mya) use blubber; and the intermediate group, the pinnipeds (seals, sea lions, and walrus, 29–23 Mya) use some combination of the two (Fish, 2000; Berta, Sumich & Kovacs, 2006). The walrus (*Odobenus rosmarus*, Linnaeus) is nearly hairless (Scheffer, 1964) and thus relies entirely on blubber as an insulator. Although phocids (true seals) have retained a fur covering, these pinnipeds rely primarily on a thick blubber layer when immersed due to the poor insulating quality of the wetted fur (Kvadsheim & Aarseth, 2002). Interestingly, members of the remaining marine mammal line, the otariids (fur seals and sea lions), demonstrate two distinct strategies for keeping warm in the cold waters in which they forage. Fur seals have both dense fur and a moderate blubber layer, while sea lions rely solely on blubber to prevent excessive heat loss (Pabst *et al.*, 1999; Liwanag *et al.*, 2012). The diversity in thermal insulation among the Pinnipedia makes this an ideal group with which to examine the evolutionary pathway involved in the transition to blubber as an insulator.

The aims of this study were to (1) examine the evidence for the evolutionary transition to blubber in aquatic living animals and (2) investigate the relative importance and effectiveness of internal and external forms of insulation in mammals. To accomplish this, we measured and compared morphological traits (blubber thickness), physical properties (thermal conductivity), and biochemical aspects (water and lipid

content, fatty acid composition) of the blubber of pinnipeds. The effectiveness of the total insulation (fur and blubber) was then compared with the pelts of terrestrial and semi-aquatic carnivore species, to place the results in a phylogenetic context and to elucidate the characteristics associated with the transition to a marine existence.

MATERIALS AND METHODS

BLUBBER SAMPLING

Samples were collected from five fur seal species, three sea lion species, and five phocid species (see Supporting Information, Table S1). Because samples were obtained opportunistically from stranded or harvested animals, a range of age classes (pups, juveniles, subadults, and adults) were available. Only adult and subadult animals (from 1 year to sexual maturity) were used for blubber comparisons. Blubber measurements and samples were taken from full sculps (fur, skin, and blubber) of deceased pinnipeds ($n = 90$); detailed methods for each measurement are described below. Fresh tissue samples were collected only from good condition carcasses, as determined by tissue colour (pink or off-white, as opposed to yellow). Fresh sculp samples were 25×25 -cm pieces taken from the dorsum, just caudal to the shoulders. All fresh sculp 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, and values from known emaciated or poor condition animals were omitted.

BLUBBER THICKNESS AND WATER CONTENT

The thermal properties of blubber change with a variety of parameters, including thickness, water and lipid content, and fatty acid composition. Blubber thickness for each sample was determined by averaging 12 measurements (three on each perimeter side) to the nearest 0.01 mm, using digital calipers (ABSOLUTE Digimatic Caliper Series 500, Mitutoyo, Aurora, IL, USA). With literature values included (see supporting Table S2 for published sources of data), blubber thickness was analysed for four fur seal species [Antarctic fur seal (*Arctocephalus gazella*, Peters), $N = 2$; Cape fur seal (*Arctocephalus pusillus pusillus*, Schreber), $N = 6$; northern fur seal (*Callorhinus ursinus*, Linnaeus), $N = 6$; subantarctic fur seal (*Arctocephalus tropicalis*, Gray), $N = 4$], three sea lion species [California sea lion (*Zalophus californianus*, Lesson), $N = 56$; Steller sea lion (*Eumetopias jubatus*, Schreber), $N = 2$; New Zealand sea lion (*Phocarcos*

hookeri, Peters), $N = 182$], walrus (*Odobenus rosmarus*, $N = 16$), and nine phocid species [Bearded seal (*Erignathus barbatus*, Erxleben), $N = 7$; Caspian seal (*Pusa caspica*, Gmelin), $N = 19$; gray seal (*Halichoerus grypus*, Fabricius), $N = 4$; harbor seal (*Phoca vitulina*, Linnaeus), $N = 28$; harp seal (*Pagophilus groenlandicus*, Erxleben), $N = 18$; hooded seal (*Cystophora cristata*, Erxleben), $N = 5$; northern elephant seal (*Mirounga angustirostris*, Gill), $N = 3$; ribbon seal (*Histiophoca fasciata*, Zimmerman), $N = 2$; ringed seal (*Pusa hispida*, Schreber), $N = 88$].

Water content was determined from a 1×1 -cm subsample of the full vertical blubber depth (see supporting Table S1 for species and sample sizes). This subsample was weighed to the nearest 0.001 g using a digital scale (PR2003 DeltaRange, Mettler-Toledo, Inc., Columbus, OH, USA), placed in a freeze dryer for a minimum of 24 h, and then re-weighed. Samples were re-weighed again the following day to ensure that the mass of each sample was stable (± 0.005 g). Reduction in mass was attributed to water loss and calculated as a percentage of the original mass of the subsample.

LIPID CONTENT AND FATTY ACID ANALYSIS

Lipid content and fatty acid composition were determined for a subset of blubber samples ($N = 22$; see supporting Table S1 for species and sample sizes), beginning with 1×1 -cm subsamples of the full vertical blubber depth. For fatty acid composition, the outer region (top portion) of the blubber, immediately below the epidermis, was analysed separately from the inner region of the blubber, next to the underlying skeletal muscle, in roughly equal halves. Lipids were extracted from the subsamples using a modification of the procedure of Folch, Lees & Sloane-Stanley (1957) (Budge, Iverson & Koopman, 2006). Briefly, lipids were extracted with 2 : 1 chloroform/methanol, washed with salt solution, dried over anhydrous sodium sulphate, and evaporated under nitrogen. The extracted lipid was weighed and used to calculate lipid content as a percentage of the original subsample mass. With literature values included (see supporting Table S3 for published sources of data), lipid content was compared for four fur seal species (Antarctic fur seal, $N = 2$; Cape fur seal, $N = 2$; northern fur seal, $N = 7$; subantarctic fur seal, $N = 2$), one sea lion species (California sea lion, $N = 41$), walrus ($N = 15$), and six phocid species (bearded seal, $N = 6$; Caspian seal, $N = 33$; harbor seal, $N = 23$; harp seal, $N = 19$; northern elephant seal, $N = 12$; ringed seal, $N = 79$).

To analyse fatty acid composition, fatty acid methyl ethers (FAMES) were prepared from the extracted lipid, using H_2SO_4 in methanol (Budge *et al.*, 2006). Duplicate analyses of FAMES were performed using

temperature-programmed gas liquid chromatography according to Budge & Iverson (2003). Samples were analysed on a Perkin Elmer Autosystem II capillary gas chromatograph with a flame ionization detector using a flexible fused silica column ($30 \text{ m} \times 0.25 \text{ mm}$ i.d.) coated with 50% cyanopropyl polysiloxane ($0.25 \mu\text{m}$ film thickness, Agilent Technologies, DB-23; Palo Alto, CA, USA). Helium was used as the carrier gas and the gas line was equipped with an oxygen scrubber. Results were calibrated according to Budge & Iverson (2003). Briefly, individual peaks associated with each FAME were compared with a series of response factors calculated from FAME standards (Nu-Chek Prep, Elysian, MN, USA). Up to 69 FAMES were identified according to Iverson, Frost & Lowry (1997). Of these, 49 were used for analysis based on the following criteria: an average presence among all samples of at least 0.1%, and a maximum value among all samples of at least 0.3%. FAMES were described using the shorthand nomenclature of A:Bn-X, where A represents the number of carbon atoms, B the number of double bonds, and X the position of the double bond closest to the terminal methyl group.

To examine the extent to which the blubber may have been modified by endogenous processes, the $\Delta 9$ desaturation index ($\Delta 9$ -DI) was calculated. $\Delta 9$ -DI is the ratio of potentially endogenous monounsaturated fatty acids (MUFAs) to the corresponding saturated fatty acids (SFAs) from which they could have originated (Käkelä & Hyvärinen, 1996). $\Delta 9$ -DI was calculated according to the formula:

$$\Delta 9\text{-DI} = \frac{(\text{wt}\% 14:1n-5) + (\text{wt}\% 16:1n-7) + (\text{wt}\% 16:1n-9) + (\text{wt}\% 18:1n-9) + (\text{wt}\% 18:1n-7)}{(\text{wt}\% 14:0) + (\text{wt}\% 16:0) + (\text{wt}\% 18:0)} \quad (1)$$

where wt% represents the percentage by weight of the indicated FAME.

THERMAL CONDUCTIVITY

Thermal conductivity was measured for squares (approx. 10×10 cm) trimmed from sculp samples (see supporting Table S1 for species and sample sizes), using the standard material method (Kvadsheim, Folkow & Blix, 1994; Kvadsheim & Aarseth, 2002; Dunkin *et al.*, 2005; Liwanag *et al.*, 2012). The fur was cleaned using cold running water, and the air layer was then restored to the fur using a hairdryer on the cool setting (Williams *et al.*, 1988; Kvadsheim & Aarseth, 2002; Liwanag *et al.*, 2012). Blubber, 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, Katy, TX, USA) with a lower, highly insulated compartment and an upper, chilled compartment modelled after Dunkin *et al.* (2005). The insulated compartment contained the heat source, a sealed aluminium box through which heated water (35 °C) was circulated from a constant-temperature water bath (Lauda RM20, Brinkmann Instruments, Toronto, Ontario, Canada). The upper chamber was cooled with ice packs to create a steady thermal gradient.

An elastomer (Plastisol vinyl, Carolina Biological Supply, Burlington, NC, USA) was used as the standard material ($k = 0.124 \pm 0.008 \text{ W m}^{-1} \text{ °C}^{-1}$). The standard material was placed flush against the heat source, and the blubber of 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., Clifton, NJ, USA) placed between the surface of the heat source and the standard material, three thermocouples between the standard material and the blubber, three thermocouples between the blubber and the skin (just beneath the dermis), and three thermocouples on top of the fur. All thermocouples were wired to a Fluke Hydra data logger (model 2625A, Fluke Inc., Everett, WA, USA), 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 analysed for the final 30 min of each trial.

Thermal conductivity was calculated across each layer (blubber and pelt) and across the entire sample, using the Fourier equation (Kreith, 1958):

$$H = k \cdot A \cdot \Delta T \cdot L^{-1} \quad (2)$$

where H is heat transfer (J s^{-1}), k is thermal conductivity ($\text{W m}^{-1} \text{ °C}^{-1}$), A is the area (m^2) through which the heat is moving, ΔT is the temperature differential ($^{\circ}\text{C}$) across the material, and L is the thickness of the material (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. With literature values included (Scholander, Irving & Grinnell, 1942; Scholander *et al.*, 1950; Hart & Irving, 1959; Bryden, 1964; Worthy, 1991; Kvadsheim *et al.*, 1994), blubber conductivity was compared for five fur seal species (Antarctic fur seal, $N = 2$; Cape fur seal, $N = 6$; Guadalupe fur seal, $N = 1$; subantarctic fur seal, $N = 4$; northern fur seal, $N = 1$), three sea lion species

(California sea lion, $N = 31$; Galápagos sea lion, $N = 1$; Steller sea lion, $N = 2$), and six phocid species [gray seal, $N = 1$; harbor seal, $N = 5$; harp seal, $N = 3$; northern elephant seal, $N = 6$; ringed seal, $N = 3$; southern elephant seal (*Mirounga leonina*, Linnaeus), $N = 1$].

STATISTICAL ANALYSES

Numerical values for all data are presented as means \pm 1SD. The relationship between each characteristic and body mass was examined on a log–log scale, and any covariance was taken into account prior to further statistical analysis. As suggested by Harvey & Pagel, (1991), a nested ANOVA was used to incorporate phylogenetic influences into statistical comparisons. Statistical significances among means were determined for families (Otariidae, Odobenidae, Phocidae) and species nested in families, using nested ANOVA and the Tukey Honestly Significant Difference test (JMP Software, SAS Institute, Cary, NC, USA). ANCOVA was used to incorporate the relative influences of body mass, habitat, and latitude on total insulation (Systat Software, Inc., Chicago, IL, USA). Where unspecified, statistical significance corresponds to $P < 0.05$.

To examine differences in fatty acid (FA) composition between the inner and outer blubber layers, Principal Components Analysis (PCA) was performed on proportional FA data that were transformed using the following logarithmic function (Aitchison, 1983): $x_i = \log(x_i g^{-1}(x))$, where x_i is a given FA expressed as a percentage of the total for that sample, $g(x)$ is the geometric mean of the FA data for all samples, and x_i represents the transformed FA data. The resulting principal component factors were analysed with Discriminant Function Analysis (DFA), using a backward stepwise function; FA composition was compared using MANOVA (Systat Software). PCA and DFA were performed separately for fur seals, sea lions, and phocids. To examine the relationship between fatty acid composition and thermal conductivity, linear regression was performed for individual FA proportions that were transformed as described above (Systat Software).

RESULTS

BLUBBER THICKNESS

Blubber thickness ranged from 6.41 to 63.50 mm among pinniped species (see supporting Table S2 for comparative data). On average, phocid seals and walrus (*Odobenus rosmarus*) maintained the thickest blubber layers, which were 3–5 times thicker than fur seal blubber and 1.5–2 times thicker than sea lion blubber (Fig. 1). Overall, fur seals maintained the thinnest blubber layers. Blubber thickness varied inversely with fur density among pinniped species (Fig. 1).

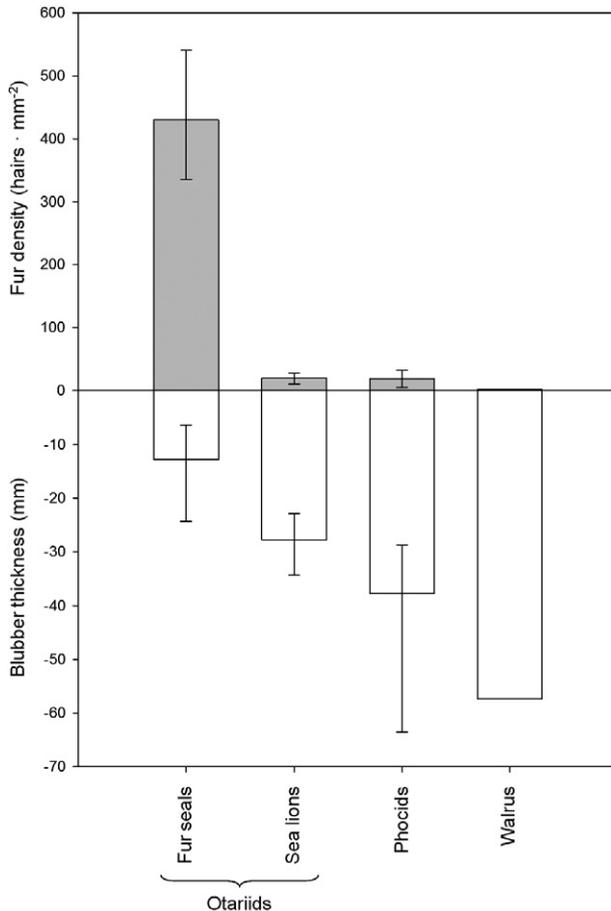


Figure 1. Blubber thickness relative to fur density among pinnipeds. Heights of the bars indicate means for the indicated species or groups. Whiskers indicate range of species means (no whiskers for single species). Fur seals: $N=6$ species for fur density; $N=4$ species for blubber depth. Sea lions: $N=4$ species for fur density; $N=3$ species for blubber depth. Phocids: $N=13$ species for fur density; $N=9$ species for blubber depth. Data for fur density are from Scheffer (1964), Frisch *et al.* (1974), and Liwanag *et al.* (2012). Data for blubber thickness are from the present study, Addison & Smith (1974), West, Burns & Modafferi (1979a,b), Stewart & Lavigne (1984), Pitcher (1986), Folkow & Blix (1987), Wiig (1989), Savelle & Friesen (1996), Cameron *et al.* (1997), Kleivane *et al.* (1997), Krahn *et al.* (1997), Nilssen *et al.* (1997), Rosen & Renouf (1997), Addison & Smith (1998), Hall *et al.* (1999), Muir *et al.* (2000), Severinsen, Skaare & Lydersen (2000), Kucklick *et al.* (2002), Lydersen *et al.* (2002), Addison, Ikonoumou & Smith (2005), Willis *et al.* (2005), Ylitalo *et al.* (2005), Del Toro *et al.* (2006), Kucklick *et al.* (2006), Stapleton *et al.* (2006), the Central Puget Sound Marine Mammal Stranding Network, Massey University, the New Zealand Ministry of Fisheries and the New Zealand Department of Conservation.

Table 1. Percentage lipid content (by weight) of pinniped blubber

Group	Lipid content (%)
Otariids	52.12 ± 18.767
Walrus	71.75 ± 12.004
Phocids	85.60 ± 11.156

Values are presented as means \pm 1SD. All three groups are significantly different from one another. Otariids include four fur seals and one sea lion. Phocids include six species. Data are from the present study, Addison & Smith (1974), Beck, Smith & Hammill (1993), Kleivane *et al.* (1997), Krahn *et al.* (1997), Addison & Smith (1998), Hall *et al.* (1999), Muir *et al.* (2000), Severinsen *et al.* (2000), Kajiwara *et al.* (2001), Kajiwara *et al.* (2002), Kucklick *et al.* (2002), Lydersen *et al.* (2002), Kannan *et al.* (2004), Addison *et al.* (2005), Del Toro *et al.* (2006), Kucklick *et al.* (2006), and Stapleton *et al.* (2006).

Blubber thickness generally increased with body mass among pinnipeds, but the response differed among groups. To account for the differential responses among groups, mass-specific blubber thickness was calculated for each individual. Comparing blubber thickness on a mass-specific basis revealed a different pattern from that observed for absolute blubber thickness. Phocid seals ($N=9$ species) had significantly higher mass-specific blubber thicknesses ($0.54 \pm 0.31 \text{ mm kg}^{-1}$) compared with otariids ($0.22 \pm 0.11 \text{ mm kg}^{-1}$; $N=7$), which in turn had significantly higher mass-specific blubber thicknesses compared with walrus ($0.06 \pm 0.04 \text{ mm kg}^{-1}$; $F_{15,425} = 40.875$, $P < 0.001$). Within the otariids, fur seals ($N=4$ species) had greater mass-specific blubber thicknesses ($0.32 \pm 0.22 \text{ mm kg}^{-1}$) than sea lions ($0.22 \pm 0.10 \text{ mm kg}^{-1}$; $N=3$ species, $P < 0.001$). Within the phocids, phocines ($N=7$ species) had significantly greater mass-specific blubber thicknesses ($0.55 \pm 0.31 \text{ mm kg}^{-1}$) than the northern elephant seal (*Mirounga angustirostris*), a monachine ($0.09 \pm 0.04 \text{ mm kg}^{-1}$; $P = 0.011$).

LIPID AND WATER CONTENT

Lipid content ranged from 50 to 97% among pinnipeds (see supporting Table S3 for comparative data). Phocid blubber had the highest lipid content compared with the other pinniped groups ($F_{11,229} = 29.7469$, $P < 0.001$; Table 1). Among the phocids, harp (*Pagophilus groenlandicus*) and ringed seal (*Pusa hispida*) blubber had significantly greater lipid content than northern elephant seal (*Mirounga angustirostris*) blubber, while the other species did not differ significantly. Walrus blubber had a

significantly higher lipid content than otariid blubber, and the otariid species did not differ from each other.

Percentage water content varied linearly with lipid content ($r^2 = 0.72$, $P < 0.001$), according to the equation:

$$\%H_2O = -0.92 (\%Lipid) + 76.84\% \quad (3)$$

where $\%H_2O$ is the percentage water content by mass, and $\%Lipid$ is the percentage lipid content by mass.

THERMAL CONDUCTIVITY

Thermal conductivity of the blubber was significantly higher for otariids ($0.276 \pm 0.068 \text{ W m}^{-1} \text{ }^\circ\text{C}^{-1}$) than for phocids ($0.192 \pm 0.040 \text{ W m}^{-1} \text{ }^\circ\text{C}^{-1}$; $F_{1,65} = 24.997$, $P < 0.001$). Blubber conductivity did not differ significantly among species within either family, but fur seal conductivity ($0.280 \pm 0.087 \text{ W m}^{-1} \text{ }^\circ\text{C}^{-1}$) had twice the variance of sea lions ($0.274 \pm 0.061 \text{ W m}^{-1} \text{ }^\circ\text{C}^{-1}$). Thermal conductivity varied linearly with lipid content ($r^2 = 0.426$, $P = 0.001$), according to the equation:

$$k = -0.004 (\%Lipid) + 0.510 \quad (4)$$

where k is thermal conductivity ($\text{W m}^{-1} \text{ }^\circ\text{C}^{-1}$) and $\%Lipid$ is the percentage lipid content by mass (Fig. 2). For sea lions and phocids only, thermal conductivity varied with lipid content ($r^2 = 0.612$, $P = 0.001$) according to the equation:

$$k = -0.002 (\%Lipid) + 0.343. \quad (5)$$

This relationship is similar to that reported for cetacean blubber (Worthy & Edwards, 1990).

FATTY ACID COMPOSITION

The inner and outer layers of the blubber of each sample were analysed separately for fatty acid composition. For fur seals ($N = 4$ species), seven principal component factors were defined, with the first two factors explaining 59.7% of the variance among samples. However, DFA revealed that none of these factors was significantly different between the inner and outer layers of fur seal blubber (Wilks's lambda = 0.952, $F_{7,14} = 0.0964$, $P = 0.998$). For sea lions ($N = 1$ species), five principal component factors were defined, with the first factor alone explaining 57.6% of the variance. Fatty acid composition did differ between the inner and outer layers of sea lion blubber (Wilks's lambda = 0.829, $F_{1,20} = 5.076$, $P = 0.0356$), with one principal component factor contributing to the difference. This factor revealed that sea lions tended to have more 14:1n-5 and less 18:0 in the outer layer of the blubber compared with the inner layer. For phocids ($N = 3$ species), five principal component factors were defined, with the first factor alone explaining 51.7%

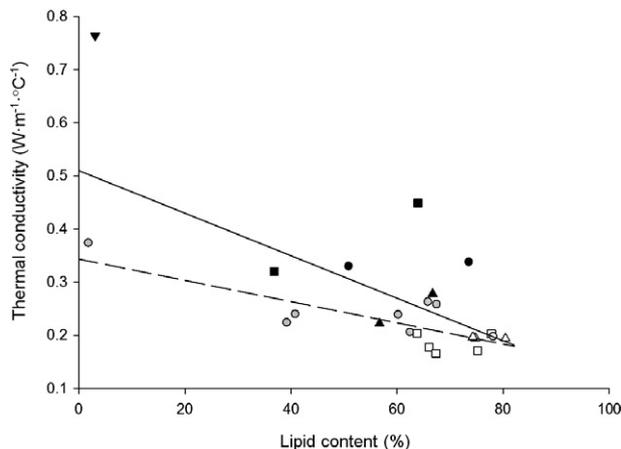


Figure 2. Change in thermal conductivity with lipid content of blubber for phocids (white), sea lions (grey), and fur seals (black). Each point represents an individual sample, with different symbols representing different species within each group (black circles: Antarctic fur seal, black squares: Cape fur seal, black triangles: subantarctic fur seal, black inverted triangle: northern fur seal; grey circles: California sea lion; white circle: ringed seal, white squares: northern elephant seal, white triangles: harbour seal). The solid line represents the best-fit linear regression for all the data, and is described in the text. The dashed line represents the best-fit linear regression for sea lions and phocids, and is also described in the text. The two points with low lipid contents are from poor-condition animals, and serve to demonstrate the increase in thermal conductivity that accompanies a loss of lipid content. Data are from the present study only.

of the variance. Fatty acid composition differed significantly between the inner and outer layers of phocid blubber (Wilks's lambda = 0.456, $F_{2,21} = 3.627$, $P = 0.0444$), with two principal component factors contributing to the difference. This revealed that phocids tended to have more 17:1 and less 20:0 fatty acids in the outer layer of the blubber compared with the inner layer, and to a lesser extent more 18:1n-7 and less 24:1 in the outer layer.

$\Delta 9$ -DI was significantly higher for the outer blubber layer than for the inner layer in phocids (paired t -test, $t = -6.292$, $P < 0.001$) and sea lions ($t = -3.876$, $P = 0.003$). However, there was no significant difference in $\Delta 9$ -DI between the blubber layers for fur seals ($t = -2.076$, $P = 0.065$). Overall, fur seal blubber had a significantly lower $\Delta 9$ -DI (1.6 ± 0.5) compared with sea lions ($F_{2,32} = 12.031$, $P = 0.045$) and phocids ($P < 0.001$). Sea lions had a lower $\Delta 9$ -DI (2.2 ± 0.7) than phocids (2.7 ± 0.4), but the difference was not statistically significant ($P = 0.069$).

When fatty acids were grouped by structural type and examined across the full blubber depth, there were significant differences in composition among

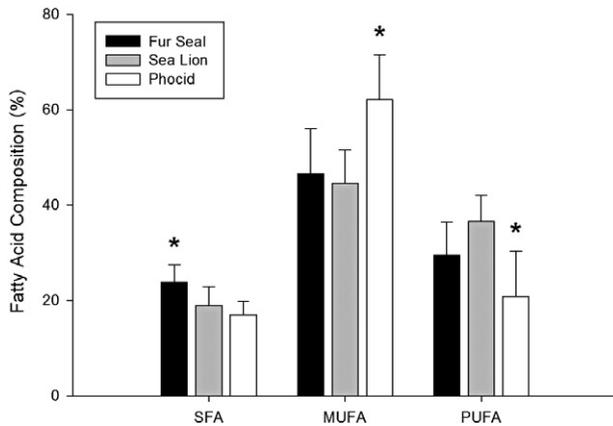


Figure 3. Percentage composition of fatty acid types in pinniped blubber. Heights of the bars and lines indicate means and standard deviations for the indicated pinniped groups. Asterisks indicate significant differences among groups for each fatty acid type. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Fur seals had significantly higher levels of SFAs compared with sea lions and phocids, whereas phocids had significantly higher levels of MUFAs and significantly lower levels of PUFAs compared with fur seals and sea lions. $N=4$ species for fur seals, $N=1$ species for sea lions, and $N=3$ species for phocids. Data are from the present study only.

pinniped groups (Fig. 3). Fur seal blubber had a higher percentage of saturated fatty acids than sea lion or phocid blubber ($F_{2,32} = 11.814$, $P < 0.001$). Phocid blubber had a higher percentage of monounsaturated fatty acids ($F_{2,32} = 14.974$, $P < 0.001$) and a lower percentage of polyunsaturated fatty acids ($F_{2,32} = 13.318$, $P < 0.001$) compared with fur seal or sea lion blubber (Fig. 3).

Some individual fatty acids exhibited significant correlations with thermal conductivity of the blubber (Table 2). Four of these FAs (17:1, 18:0, 20:0, and 24:1) were among those found to be significantly different between the inner and outer layers of sea lion or phocid blubber. Because of the increased Type I error rate associated with multiple comparisons (Rice, 1989), we took special note of correlations with P values ≤ 0.005 . However, we also report the data for correlations with P values at and slightly above 0.05 to avoid an increased Type II error rate (Cabin & Mitchell, 2000).

TOTAL INSULATION

To examine the overall insulation available to each animal, thermal resistance was calculated according to the equation $R = L/k$, where R is the thermal resistance ($\text{m}^2 \text{ } ^\circ\text{C W}^{-1}$), L is the thickness of the insulation (m), and k is the thermal conductivity ($\text{W m}^{-1} \text{ } ^\circ\text{C}^{-1}$). To

Table 2. Correlative relationships between individual fatty acids (FAs) and thermal conductivity of blubber

Fatty acid	Relationship to conductivity	P
16:1n-11	Negative	< 0.001*
16:1n-9	Negative	0.018
16:1n-7	Negative	0.033
17:1	Negative	0.001*
18:0	Positive	0.082
18:3n-3	Negative	0.003*
20:0	Positive	0.003*
20:3n-6	Positive	0.012
22:4n-6	Positive	0.033
22:5n-6	Positive	0.024
22:6n-3	Positive	0.022
24:1	Positive	0.005*

Data are shown only for FAs that exhibited significant (or nearly significant) relationships with conductivity. P values marked with an asterisk met a more conservative significance cut-off of 0.005, to account for multiple comparisons. FAs in bold were also found to be significantly different between the inner and outer layers of blubber in sea lions or phocids.

evaluate the insulation in a phylogenetic context, thermal resistance values were compared across carnivore species. Thermal resistance was calculated for the pelts of terrestrial carnivores, polar bear, and river otter, and for full sculps of pinniped species. Thermal resistance varied ($F_{7,110} = 67.002$, $P < 0.001$) according to habitat (terrestrial, semi-aquatic, marine) and latitudinal climate (tropical, temperate, polar; Fig. 4). The temperate and polar terrestrial species, along with the polar bear (*Ursus maritimus*, Phipps), had significantly greater thermal resistances compared with all other groups ($P \leq 0.007$). The pinnipeds, river otter (*Lontra canadensis*, Schreber), and tropical terrestrial species had comparatively lower thermal resistances, which were not significantly different from each other ($P = 1.000$). PCA revealed that body size and habitat strongly covaried, with larger animals in the aquatic habitat. In contrast, latitudinal climate was independent of body size and habitat among the species sampled.

DISCUSSION

THE EVOLUTIONARY TRANSITION TO BLUBBER

The use of fur or blubber by aquatic mammals involves several trade-offs. Blubber maintains a large thermal gradient between the animal's core and body surface, but requires a relatively large thickness (compared with fur) to achieve that gradient

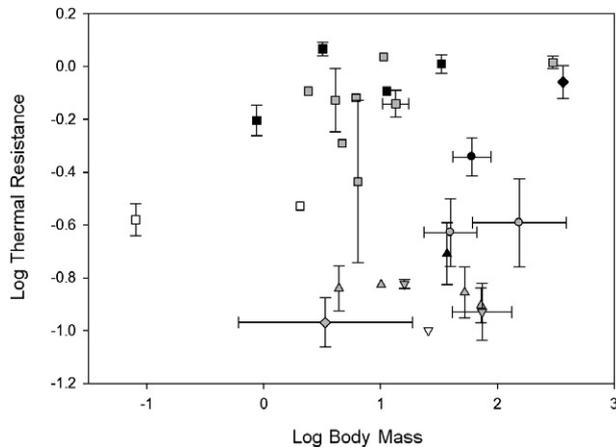


Figure 4. Thermal resistance of the total insulation ($\text{m}^2 \text{ } ^\circ\text{C W}^{-1}$) in relation to body mass (kg) for carnivores. Symbols and lines indicate species means and standard deviations. Symbol shapes indicate groups as follows: terrestrial species are represented by squares, phocids by circles, otariids by triangles (upward triangles for fur seals, downward triangles for sea lions), the river otter by the grey diamond, and the polar bear by the black diamond. Symbol colours indicate latitude: polar (black), temperate (grey), and tropical (white). Polar terrestrial species include the Arctic fox, lynx, marten, and wolf. Temperate terrestrial species include the bobcat, domestic cat, coyote, domestic dog, grizzly bear, raccoon, red fox, and skunk. Tropical terrestrial species include the kinkajou and least weasel. The polar bear represents a polar semi-aquatic species. The river otter represents a temperate semi-aquatic species. The polar phocid is the ringed seal, and the polar otariid is the Antarctic fur seal. Temperate phocids include the northern elephant seal and harbour seal. Temperate otariids include the Cape fur seal, California sea lion, Guadalupe fur seal, northern fur seal, Steller sea lion, and subantarctic fur seal. The tropical otariid is the Galápagos sea lion. Data are from the present study, Scholander *et al.* (1950), Hammel (1955), and Liwanag *et al.* (2012). Note that terrestrial species (squares) generally show higher insulating values compared with pinnipeds (circles and triangles). The polar bear groups with the terrestrial carnivores, whereas the river otter groups with the aquatic species. Polar (black) and temperate species (grey) generally have higher insulating values compared with tropical species (white). In addition, phocid species (circles) demonstrate higher insulating values compared with otariids (triangles).

(Scholander *et al.*, 1950; Costa & Kooyman, 1982; Worthy, 1991). As an insulator, fur is comparatively lighter than blubber and a better insulator in air, but its effectiveness is reduced in water (Scholander *et al.*, 1950; Hammel, 1955; Johansen, 1962; Ling, 1970; Frisch, Ørtisland & Krog, 1974; Morrison, Rosenmann & Estes, 1974; Doncaster *et al.*, 1990; Fish,

2000; Kvadsheim & Aarseth, 2002). The insulating air layer of fur allows the skin to be maintained at body temperature; however, this air layer requires the energetically expensive process of grooming to maintain (Williams, 1989; Yeates, Williams & Fink, 2007). The effectiveness of fur as a thermal barrier is compromised at depth due to compression of the air layer during a dive, which will also affect buoyancy control (Repenning, 1976; Lovvorn & Jones, 1991; Pabst *et al.*, 1999; Liwanag *et al.*, 2012). In contrast, blubber may not provide the same level of buoyancy relative to fur, and it is relatively incompressible with depth (Repenning, 1976; Lovvorn & Jones, 1991). Furthermore, because blubber is living tissue, it can be bypassed by perfusion when the animal needs to dissipate excess heat (Schmidt-Nielsen, 1990; Mauck *et al.*, 2003; Meagher *et al.*, 2008).

Quality and quantity of blubber can change seasonally (Ryg, Smith & Ørtisland, 1988; Aguilar & Borrell, 1990; Worthy & Edwards, 1990; Samuel & Worthy, 2004; Budge *et al.*, 2008) as well as with animal condition (Aguilar & Borrell, 1990; Dunkin *et al.*, 2005). In addition, life-history strategy, phase of reproduction, and diet can alter blubber thickness, lipid content, and composition (Pabst *et al.*, 1999; Struntz *et al.*, 2004; Montie *et al.*, 2008). Despite this variation, we were able to observe consistent patterns in blubber characteristics among pinniped groups. Whereas fur seals had thinner blubber than sea lions on average (Fig. 1), the quantity of blubber was greater for fur seals on a mass-specific basis, consistent with previous reports of the relationship between lipid mass and total body mass in otariids (Costa, 1991). The comparatively large quantity of walrus blubber (Fig. 1) can be attributed primarily to the extremely large body size (over 1000 kg, on average) exhibited by this species (Fay, 1981), such that the differences between walrus and otariid blubber thickness disappeared or rather reversed when corrected for mass. Phocid seals, however, had a larger quantity of blubber compared with otariids on both an absolute and a mass-specific basis. Yet thickness alone does not determine thermal characteristics, as changes in quality can produce differences in thermal conductivity of up to 400% (Worthy & Edwards, 1990; Pabst *et al.*, 1999; Dunkin *et al.*, 2005).

In terms of quality, measurements for the conductivity of animal fat have generally fallen into the range $0.1\text{--}0.21 \text{ W m}^{-1} \text{ } ^\circ\text{C}^{-1}$, with reported values up to $0.28 \text{ W m}^{-1} \text{ } ^\circ\text{C}^{-1}$ (reviewed by Dunkin *et al.*, 2005). Interestingly, the average thermal conductivity of otariid blubber ($0.276 \pm 0.068 \text{ W m}^{-1} \text{ } ^\circ\text{C}^{-1}$, range = $0.161\text{--}0.449$) fell at the high end of the range of known values, although the ranges do overlap considerably. The similar quality of blubber among the otariid species, as measured by both lipid content and

thermal conductivity, is consistent with the occurrence of rapid evolution within this group (Higdon *et al.*, 2007; Yonezawa, Kohno & Hasegawa, 2009).

Phocid blubber appears to be greater in both quantity and quality. Not only was phocid blubber of a greater quantity beyond that associated with large body size, but phocid blubber also had a significantly lower thermal conductivity and higher lipid content compared with otariid and walrus blubber (Table 1). As the insulating quality of blubber appears to be directly correlated to its lipid content (Fig. 2; Worthy & Edwards, 1990), this indicates better quality blubber in the later diverging pinniped group. The otariids represent extant examples of the evolutionary transition from fur to blubber among aquatic mammals, and the lower quality of otariid blubber may be a constraint related to the transitional state of this group. Within the otariids, fur seals generally showed a greater variation in lipid content and thus thermal conductivity of the blubber (Fig. 2); this may reflect the use of the blubber for energy storage rather than for insulation. Indeed, the linear relationship between lipid content and blubber conductivity more closely matched that of cetacean blubber (Worthy & Edwards, 1990) when examined for sea lions and phocids only (Fig. 2). Sea lions appear to compensate for a lack of waterproof fur with a larger body size and associated thicker blubber layer (Fig. 1). Within the phocids, the monachine seals appeared to utilize a similar strategy, with larger body sizes, thicker blubber, and shorter and less dense fur compared to phocines (Liwana *et al.*, 2012).

THE USE OF BLUBBER FOR ENERGY OR INSULATION

Vertical stratification of blubber has been well documented in cetaceans (Ackman, Eaton & Jangaard, 1965; Ackman, Epstein & Eaton, 1971; Ackman *et al.*, 1975; Aguilar & Borrell, 1990; Koopman, Iverson & Gaskin, 1996; Hooker *et al.*, 2001; Samuel & Worthy, 2004; Reynolds, Wetzel & O'Hara, 2006; Budge *et al.*, 2008), and it has been suggested that the outer and inner layers of the blubber serve different functions (Aguilar & Borrell, 1990; Montie *et al.*, 2008). We hypothesized that pinniped species relying more on blubber than fur for insulation would demonstrate stratification of the blubber associated with two different uses: the outer layer of the blubber would function more for insulation, while the inner layer would serve as an energy store. The differing fatty acid compositions we observed between the blubber layers in phocids and sea lions, but not in fur seals, support this hypothesis. While the minimal blubber layer in fur seals may limit the extent to which stratification occurs, the homogeneous nature of the blubber layer combined with significantly greater

fur densities (Fig. 1) suggest that fur seals rely on blubber as an energy store and rely more on their fur for thermoregulation in water.

This hypothesis was further supported by the differences in structural groups among the blubber of pinnipeds. In general, unsaturated FAs tend to have lower melting points than SFAs, and thus tend to remain more fluid at low temperatures (Hilditch & Williams, 1964; Hochachka & Somero, 2002). Because of this, unsaturated FAs are generally better suited for thermoregulation, as the outer blubber layers in particular would be expected to sustain lower temperatures resulting from the thermal gradient across the insulating layer. Phocids and sea lions had significantly higher levels of unsaturated FAs compared with fur seals (Fig. 3), suggesting that fur seal blubber may be less suited for lower temperatures and thus may not be as effective for the maintenance of a thermal gradient. As homeoviscous theory indicates that changes associated with lipid fluidity may be induced by exposure to certain temperatures (Hochachka & Somero, 2002), these results are also consistent with fur seal blubber being maintained at higher temperatures because the fur provides the primary thermal gradient between the subcutaneous tissues and the surrounding environment.

Further evidence was provided by the differences in $\Delta 9$ -DI among the groups. $\Delta 9$ -DI is an indicator of the extent to which the animal has modified SFAs to endogenously form MUFAs. In sea lions, $\Delta 9$ -DI increased from 2.0 in the inner blubber to 2.4 in the outer layer; for phocids, $\Delta 9$ -DI increased from 2.3 in the inner blubber to 3.1 in the outer layer. The higher $\Delta 9$ -DI in the outer blubber layer is consistent with the use of the outer layer for thermoregulation, as this layer will experience lower temperatures when generating a thermal gradient across the tissue (Käkelä & Hyvärinen, 1996). While many unsaturated FAs, and polyunsaturated fatty acids (PUFAs) in particular, are derived largely from the diet of pinnipeds, $\Delta 9$ -DI is calculated from MUFAs that mammals can form endogenously (Käkelä & Hyvärinen, 1996; Budge *et al.*, 2006). Thus, higher levels of these FAs in the outer blubber suggest that in sea lions and phocids endogenously derived MUFAs may be preferentially deposited into the outer blubber layer to maintain its fluidity while it serves as an insulator. In contrast, fur seals, as with terrestrial carnivores (Käkelä & Hyvärinen, 1996), rely on their fur to create a thermal gradient; this allows fur seals to maintain the blubber layer at higher temperatures and does not require the endogenous addition of MUFAs to maintain blubber fluidity.

Overall proportions of SFAs, MUFAs, or PUFAs did not correlate significantly with thermal conductivity. However, a small subset of individual FAs did

correlate significantly with the thermal conductivity of the blubber (Table 2). Highly unsaturated FAs (e.g. 18:3n-3, 20:3n-6, 22:4n-6, 22:5n-6, 22:6n-3) are probably entirely derived from the diet. While their relationship to conductivity is interesting, their levels are unlikely to be subject to physiological modification by the animal. In contrast, levels of SFAs and MUFAs may be modified by the animal via physiological processes, and several of the correlations corresponded to differences observed between the inner and outer layers of blubber. In particular, 20:0 and 24:1 FAs had significant positive correlations with thermal conductivity, suggesting a negative impact on the insulating quality of the blubber; both of these FAs were reduced in the outer (thermal) layer of phocid blubber. In addition, 17:1 had a significant negative correlation with thermal conductivity, indicating a positive impact on insulation, and it was increased in the outer (thermal) layer of phocid blubber. Although the relationship was not statistically significant, 18:0 showed a positive correlation with thermal conductivity, and was reduced in the outer (thermal) layer of sea lion blubber. This may indicate that the endogenous modification of FAs can influence conductivity as well as fluidity of the blubber. Note, however, that the overall amount of fat present (Fig. 2) and individual FAs derived from the diet (Table 2) still have an effect on thermal conductivity; thus, more research is needed to develop a complete understanding of the relationship between FA composition and thermal properties of the blubber.

ECOLOGICAL AND EVOLUTIONARY IMPLICATIONS

The extent to which fur or blubber is utilized as insulation in aquatic mammals appears to reflect the level of evolutionary adaptation for the aquatic environment. The most recently diverged groups, polar bears (0.5 Mya) and sea otters (1.6 Mya), have retained fur as the primary insulator; the earliest diverging groups, cetaceans and sirenians (50 Mya), have secondarily lost the fur and now use blubber; and the intermediate groups, pinnipeds (29–23 Mya), have both retained fur and developed blubber (Pabst *et al.*, 1999; Berta *et al.*, 2006). The evolutionary transition to an aquatic lifestyle was accompanied first by a modification of the ancestral fur state to maintain thermal resistance in water (Liwanag *et al.*, 2012), followed by a gradual replacement of the fur with an internal blubber layer (Fig. 1). Certain morphological changes to the fur associated with aquatic living, including flattening of the hair shaft and shortening of the hairs, seem to reduce the insulating value of the fur in air (Liwanag *et al.*, 2012; Fig. 4). For example, the polar bear has fur with more ancestral (terrestrial-like) characteristics (Liwanag *et al.*,

2012), and this was reflected in the high insulating value of its pelt (Fig. 4). In contrast, the river otter's fur possesses more derived morphological characteristics, resulting in a reduction in the insulating value of the pelt (Fig. 4).

The selective pressures of the aquatic environment on the evolution of mammalian insulation were probably quite strong, as the total insulation among carnivores varied strongly according to habitat (Fig. 4). Terrestrial carnivore pelts generally showed greater insulating values compared with full pinniped sculps, demonstrating that blubber is a poorer insulator than fur (Scholander *et al.*, 1950). Within groups, the influence of climate was also apparent, as polar and temperate species generally had higher insulating values compared with tropical species (Fig. 4). In addition, phocid species demonstrated higher insulating values than otariids, reflecting the greater quantity and quality of blubber in the later divergent pinniped species (Fig. 1, Table 1). The more developed insulation may also help explain the greater diversity of phocids in polar climates (Berta *et al.*, 2006).

Body size also plays an important role among pinnipeds, as walrus and sea lions appear to rely on the increased blubber thickness associated with large body size to increase their levels of insulation. Large body size alone confers a thermal advantage in cold environments, by reducing surface area to volume ratio and thus relative heat loss to the surroundings (Kreith, 1958). However, it appears that large body size also facilitates the evolutionary switch from fur to blubber as the primary insulator. This changeover is evident within the otariid family, as the generally smaller-bodied fur seals rely on their fur for insulation, while the larger-bodied sea lions have made the transition to the use of blubber as the primary insulator. Because a substantial blubber thickness is required to achieve a similar thermal gradient to that provided by fur, smaller-bodied marine mammals may be constrained to the retention of fur as the primary insulator. A disproportionately large blubber layer would severely limit the flexibility and manoeuvrability of the animal, both on land and in the water. Thus, the use of blubber as the primary insulating layer requires a larger body size to allow for greater blubber thicknesses without impeding the animal's movement.

As blubber is a relatively poor insulator compared with fur (Scholander *et al.*, 1950), the selective pressures driving the evolutionary transition to blubber may not be due to blubber's thermal properties. Instead, the hydrodynamic pressures associated with aquatic living and the ability to use blubber as an energy store may have selected for the convergent evolution of blubber across mammalian lineages. Blubber allows for body streamlining, hence reducing

pressure drag in the viscous aquatic medium; its insulative properties are not changed with submergence in water; and it is not compressed appreciably by hydrostatic pressure during diving, thus providing a stable source of both insulation and buoyancy to a diving mammal (Pabst *et al.*, 1999; Fish, 2000). In addition, blubber acts as energy storage that can be utilized during fasting periods and has enabled the separation of breeding and feeding in many species (e.g. Costa, 1991). Fur does not confer these advantages to an aquatic mammal, and in fact is more likely to act as a liability when it comes to hydrodynamics and grooming costs. In particular, the compression and loss of the trapped air layer within the fur during a dive will reduce both the insulation and the buoyancy of the animal (Fish *et al.*, 2002; Liwanag *et al.*, 2012).

Overall, despite the disadvantages, fur is the superior insulator as long as the air layer is maintained among the hairs (Fig. 4). For small-bodied endotherms in the marine environment, fur may be the only option, as the amount of blubber needed to achieve a similar level of insulation would comprise a disproportionate fraction of the animal's body size and thus inhibit manoeuvrability. However, both the use of fur as the primary insulator and small body size limit the diving capabilities for these animals. Larger body sizes facilitate the development of a blubber layer, which is advantageous for swimming and diving. Thus, the most effective insulatory mechanism depends on body size as well as habitat, with larger body size and a blubber layer comprising the best configuration for a fully aquatic lifestyle.

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SUPPORTING INFORMATION

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

Table S1. Sculp samples analysed in the present study. Total number of samples (N) is indicated for each species. Number of samples measured for each characteristic is indicated in the appropriate column. H_2O = % water content; Lipid = % lipid content; FA = fatty acid analysis; k = thermal conductivity.

Table S2. Species means \pm 1 SD (by age class) for blubber thickness, from the present study and published sources. For $N = 1$ from the present study, means and SD are from four perimeter sides of a single sample. Only good condition animals were used to calculate means. Note that only adult and subadult values were used for comparisons in the present study.

Table S3. Species means \pm 1 SD for blubber and subcutaneous fat lipid content, from the present study and published sources. Note that only good condition animals were used to calculate means (where possible). *Value from Addison & Smith (1974) is a median value.