RESEARCH ARTICLE

Lipid class and depth-specific thermal properties in the blubber of the short-finned pilot whale and the pygmy sperm whale

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SUMMARY

Blubber, the specialized hypodermis of cetaceans, provides thermal insulation through the quantity and quality of lipids it contains. Quality refers to percent lipid content; however, not all lipids are the same. Certain deep-diving cetacean groups possess blubber with lipids – wax esters (WE) – that are not typically found in mammals, and the insulative quality of 'waxy' blubber is unknown. Our study explored the influence of lipid storage class – specifically WE in pygmy sperm whales (*Kogia breviceps*; *N*=7) and typical mammalian triacylglycerols in short-finned pilot whales (*Globicephala macrorhynchus*; *N*=7) – on blubber's thermal properties. Although the blubber of both species had similar total lipid contents, the thermal conductivity of *G. macrorhynchus* blubber ($0.20\pm0.01\,\mathrm{W\,m^{-1}\,^{\circ}C^{-1}}$) was significantly higher than that of *K. breviceps* ($0.15\pm0.01\,\mathrm{W\,m^{-1}\,^{\circ}C^{-1}}$; *P*=0.0006). These results suggest that lipid class significantly influences the ability of blubber to resist heat flow. In addition, because the lipid content of blubber is known to be stratified, we measured its depth-specific thermal conductivities. In *K. breviceps* blubber, the depth-specific conductivity values tended to vary inversely with lipid content. In contrast, *G. macrorhynchus* blubber displayed unexpected depth-specific relationships between lipid content and conductivity, which suggests that temperature-dependent effects, such as melting, may be occurring. Differences in heat flux measurements across the depth of the blubber samples provide evidence that both species are capable of storing heat in their blubber. The function of blubber as an insulator is complex and may rely upon its lipid class, stratified composition and dynamic heat storage capabilities.

Key words: odontocete, cetacean, thermal conductivity, wax ester, triacylglycerol.

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INTRODUCTION

Blubber, the specialized hypodermis of the cetacean integument composed of adipocytes and structural connective tissue fibers, functions in buoyancy control and serves as a site of energy storage, while streamlining and insulating the body (e.g. Parry, 1949; Doidge, 1990; Koopman et al., 1996; Pabst et al., 1999; Hamilton et al., 2004; Struntz et al., 2004; Dunkin et al., 2005; Koopman, 2007; Dunkin et al., 2010). The insulative function of blubber is important to marine endotherms as water conducts heat away from the body at a rate ~25 times faster than air of the same temperature (Parry, 1949; Schmidt-Nielsen, 1997).

The insulative ability of blubber depends upon both its quality and quantity (e.g. Worthy and Edwards, 1990; Kvadsheim et al., 1994; Dunkin et al., 2005). Thermal conductivity ($\mathrm{W\,m^{-1}\,^{\circ}C^{-1}}$), a measure of how well heat flows through a given material, has been used to assess the thermal quality of blubber. The quantity, or thickness of blubber, as well as its quality, dictates its thermal conductance ($\mathrm{W\,m^{-2}\,^{\circ}C^{-1}}$), which provides an absolute value of the heat transfer across its depth (reviewed in Dunkin et al., 2005).

In previous studies, the amount (% wet mass) of lipid present has been used as the compositional metric of blubber quality, and has been found to be inversely related to its thermal conductivity (e.g. Worthy and Edwards, 1990; Kvadsheim et al., 1996; Dunkin et al., 2005). For example, the blubber of the harbor porpoise (*Phocoena phocoena*), a species that inhabits cold temperate waters, has high lipid content (81.6±3.6%) and low thermal conductivity ($0.10\pm0.01\,\mathrm{W\,m^{-1}\,^{\circ}C^{-1}}$), whereas that of the similarly sized tropical

spotted dolphin (*Stenella attenuata*) has low lipid content (54.9±2.8%) and high thermal conductivity ($0.20\pm0.02\,\mathrm{W\,m^{-1}\,^{\circ}C^{-1}}$) (Worthy and Edwards, 1990).

No study to date, though, has investigated how the specific class of lipid in blubber influences its thermal quality. Most cetaceans, and all those for which the thermal properties of blubber have been reported (Table 1), store their lipids as triacylglycerols (TAG), three fatty acids attached to a glycerol backbone (reviewed in Koopman, 2007). In contrast, kogiids, physeterids, and ziphiids (sperm and beaked whales) store their blubber lipids primarily as wax esters (WE), a long-chain fatty acid attached to a long-chain fatty alcohol (Lockyer, 1991; Koopman, 2007). These latter odontocetes, which are pelagic deep divers (reviewed in Piscitelli et al., 2010), are apparently unique within mammals for using WE as their primary storage lipid (Koopman, 2007). Although the overall lipid content of the blubber in these species is similar to those that store their lipids as TAG, the thermal consequences of possessing 'waxy' blubber are currently unknown (Koopman, 2007).

In this study, the thermal quality and quantity of blubber from the pygmy sperm whale (*Kogia breviceps* Blainville 1838; Family Kogiidae), which stores its blubber lipids as WE, were compared with that of another deep diver, the short-finned pilot whale (*Globicephala macrorhynchus* Gray 1846; Family Delphinidae) (Aguilar Soto et al., 2008), which stores its blubber lipids as TAG. Both are medium-sized odontocetes with warm temperate to tropical distributions (e.g. Payne and Heinemann, 1993; Bernard and Reilly, 1999; Piscitelli et al., 2010). The two species experience large

Table 1. Thermal conductivity (k) values for blubber from various marine mammals and for other materials

Material	k (W m^{-1} °C^{-1})	Source	Method
Pinniped blubber			
Mirounga leonina	0.07	Doidge, 1990; Bryden, 1964	Unknown
Phoca vitulina	0.18	Worthy, 1985	Heat flux disc
Phoca groenlandica	0.18	Worthy, 1985	Heat flux disc
Phoca groenlandica	0.19	Kvadsheim et al., 1994	Standard material
Phoca hispida	0.2	Scholander et al., 1950	Hot plate
Halichoerus grypus	0.18	Worthy, 1985	Heat flux disc
Cetacean blubber			
Balaenoptera acutorostrata	0.20–0.28	Kvadsheim et al., 1996	Standard material
Balaenoptera acutorostrata	0.18	Folkow and Blix, 1992	Hot plate
Balaenoptera physalus	0.21	Parry, 1949	Hot plate
Delphinapterus leucas (blubber)	0.102	Doidge, 1990	Heat flux plate
Delphinapterus leucas (epidermis)	0.249	Doidge, 1990	Heat flux plate
Phocoena phocoena	0.06	Yasui and Gaskin, 1986	Heat flux disc
Phocoena phocoena	0.1	Worthy and Edwards, 1990	Heat flux disc
Stenella attenuata	0.2	Worthy and Edwards, 1990	Heat flux disc
Tursiops truncatus:	0.11–0.24	Dunkin et al., 2005	Heat flux disc and standard material
Other lipids			
Human fat	0.21	Doidge, 1990; Hensel, 1973	Unknown
Olive oil	0.17	oliveoilsource.com	Unknown
Stearic acid (C18:0)	0.16	Doidge, 1990; Weast, 1989	Unknown
Palmitic acid (C16:0)	0.17	Doidge, 1990; Weast, 1989	Unknown
Oleic acid (C18:1)	0.23	Doidge, 1990; Weast, 1989	Unknown

variations in ambient temperatures during the course of a single dive, which makes them excellent subjects of investigation for comparing the thermal properties of these two different classes of blubber lipid.

In addition, how thermal conductivity varied across the depth of blubber was investigated because histological and biochemical data have revealed that its composition is stratified (e.g. Parry, 1949; Lockyer, 1991; Lockyer, 1993; Koopman et al., 1996; Koopman et al., 2002; Evans et al., 2003; Struntz et al., 2004; Koopman, 2007; Montie et al., 2008). For example, Struntz et al. (Struntz et al., 2004) and Montie et al. (Montie et al., 2008) described the blubber of the bottlenose dolphin (*Tursiops truncatus*) as having three layers – superficial, middle and deep – across which both adipocyte size and structural fiber density varied significantly. Lipid content and fatty acid composition can also vary across the blubber depth. Harbor porpoises, during periods of nutritional stress, metabolize lipids primarily from their inner blubber layer, which contains both higher lipid content and higher concentrations of dietary fatty acids than their outer blubber, where adipocyte size and shape are constantly maintained (Koopman et al., 2002).

Koopman (Koopman, 2007) examined biochemical stratification in blubber of 30 cetacean species, and demonstrated that both the pygmy sperm whale and the short-finned pilot whale possess a significantly higher percentage of lipid in their inner than in their outer blubber layer. Because adipocytes, lipid content and fatty acid composition can vary across blubber depth (e.g. Koopman et al., 1996; Koopman et al., 2002; Struntz et al., 2004; Koopman, 2007; Montie et al., 2008), and conductivity has been demonstrated to vary with lipid content (e.g. Worthy and Edwards, 1990; Kvadsheim et al., 1996; Dunkin et al., 2005), these layer-specific differences in composition suggest that the thermal properties of blubber should similarly vary across its depth. No study has yet investigated depth-specific thermal properties in blubber.

Thus, the objectives of this study were to utilize the pygmy sperm whale and short-finned pilot whale to: (1) compare the thermal quality and quantity of blubber from a species that stores lipids as WE and one that stores lipids as TAG; (2) examine how the thermal conductivity of blubber varied across its depth; and (3) assess the relationship between conductivity and lipid content across different blubber depths.

MATERIALS AND METHODS
Specimens and samples
Integument samples [epidermis, dermis and hypodermis (blubber)] were obtained from adult female short-finned pilot whales (*G. macrorhynchus*) (*N*=7) that stranded during a multi-species mass stranding event on Bodie Island, NC, on 15 January 2005 (Hohn et al., 2006) and adult male (*N*=4) and female (*N*=3) pygmy sperm whales (*K. breviceps*) that stranded either as part of a mass stranding event on 2 September 2006 or as individuals during the years 2005 through 2010. All specimens were in fresh to moderate carcass condition [Smithsonian Institution Code 1 through 3 (Geraci and Lounsbury, 2005)] and normal to robust body condition (Cox et al., 1998; McLellan et al., 2002). All blubber samples were in excellent condition, and displayed no evidence of decomposition. For each animal, a standard set of external morphometrics (adapted from Norris, 1961) was collected. *Globicephala macrorhynchus* were between 297 and 386 cm in length, and *K. breviceps* were between 250 and 301 cm in length. Full-depth samples of the integument (~20×20 cm), which were utilized for both lipid and thermal studies, were taken from a dorsal, mid-thoracic site on each whale (Fig. 1). The blubber samples were vacuum-sealed (Koch 1700, Kansas City, MO, USA) and stored at –20°C until analysis.

The thicknesses of the full integument and blubber layer were measured at each side of the sample using precision digital calipers (Absolute Digimatic calipers, Mitutoyo, Tylertown, MS, USA), and the mean values were used in all calculations. To investigate depth-specific lipid composition and thermal properties, the blubber layer had to be divided. The blubber of *G. macrorhynchus* did not appear stratified at the gross level (Fig. 2A). Because previous studies (e.g. Koopman et al., 1996; Struntz et al., 2004; Montie et al., 2008) had identified delphinid blubber to be stratified into superficial, middle and deep layers, the thickness of *G. macrorhynchus* blubber (excluding the epidermis) was measured, and that thickness was divided into
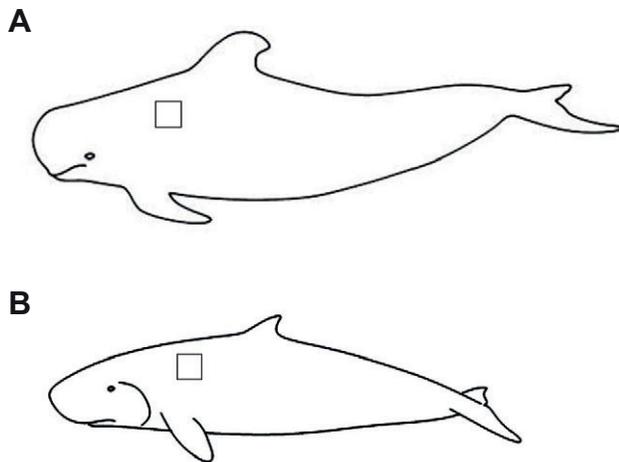
A

B

Fig. 1. Blubber sample sites (squares) from (A) short-finned pilot whales (*Globicephala macrorhynchus*) and (B) pygmy sperm whales (*Kogia breviceps*) used in thermal experiments and lipid composition analyses. Images not drawn to scale.
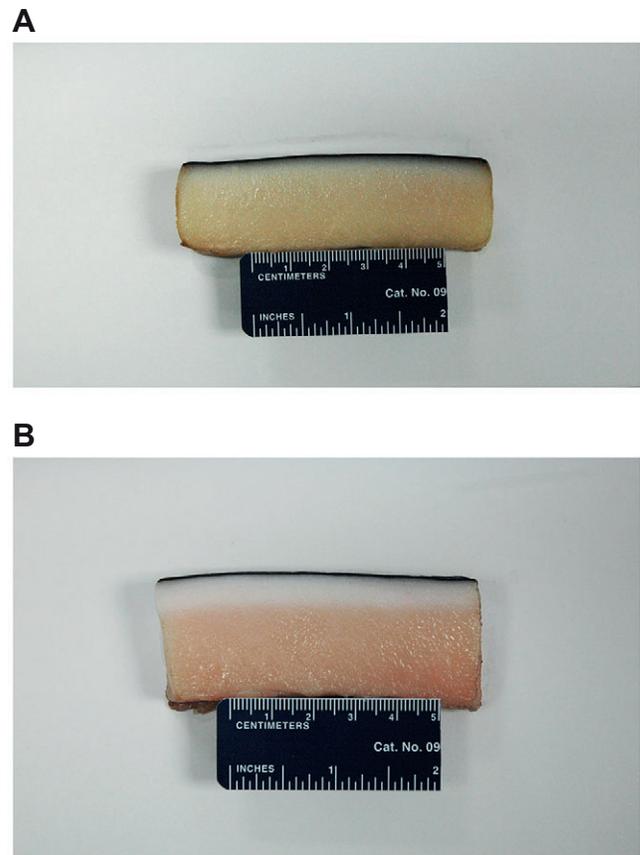
A

B

Fig. 2. (A) Photograph of a typical *G. macrorhynchus* integument sample with no grossly observable stratification pattern; therefore, the thickness was divided into equal thirds for experiments. (B) Photograph of a typical *K. breviceps* blubber sample that was grossly stratified into two clearly distinct layers. The superficial layer was more fibrous and white in color than the deep layer. Therefore, this blubber was divided at the interface between the fibrous superficial layer and the deep layer.

equal thirds. This method may not fully represent the morphological or biochemical stratification of the blubber layer, but it permitted a standardized method with which to investigate depth-specific properties. In contrast, *K. breviceps* blubber was visually stratified into two clearly distinct layers – a feature that has not previously been reported for this species, nor, to the best of our knowledge, for any other odontocete cetacean (Fig. 2B). The superficial layer, which represented ~38% of the blubber layer's thickness, was more densely fibrous, rubbery and white in color than the deep layer. Therefore, the blubber of *K. breviceps* was divided at the interface between the fibrous superficial layer and the deep layer.

Specimens used in this study were collected under the authority of a NOAA Stranding Agreement to the University of North Carolina Wilmington (UNCW IACUC no. 2003-013).

Lipid extraction, classification and analysis

Lipids were extracted and analyzed from full-depth blubber samples and from layer-specific blubber samples (deep, middle and superficial layers for *G. macrorhynchus* and deep and superficial layers for *K. breviceps*). Approximately 0.5 g full-depth sections of blubber, in addition to 0.5 g sections of each layer, were excised, weighed and extracted *via* a modified Folch procedure. Total lipid content was determined as percent wet mass (Folch et al., 1957; Iverson, 1988; Koopman et al., 1996). The lipids were then re-suspended in hexane, flushed with nitrogen to avoid oxidation and stored at −20°C until further analysis.

Lipid classes were separated and quantified using thin layer chromatography with flame ionization detection (Iatroscan Mark VI Mitsubishi Kagaku Iatron, Tokyo, Japan). Samples were analyzed in duplicate by spotting 1 μl on chromarods that were developed in hexane:ethyl acetate:formic acid (94:6:1) and quantified by the Iatroscan. Peaks were identified and integrated using PeakSimple 329 Iatroscan software (SRI Instruments, Torrance, CA, USA), based on lipid class standards (Nu Chek Prep, Elysian, MN, USA). Levels of lipid classes were calculated by applying standard curves generated from known concentrations.

Integument thermal experiments

Approximately 24 h prior to measuring the integument's thermal properties, the large vacuum-sealed sample was taken from the freezer and an 8×8 cm full-depth frozen sample was excised using a Stryker saw (Stryker Corporate, Kalamazoo, MI, USA). This sub-sample was thawed on ice and any remaining cutaneus trunci muscle was removed prior to the thermal experiment. These methods permitted a standardized sample treatment and the ability to compare results with those of other published studies where the blubber was frozen prior to the time of experimentation (see Table 1).

Two thermal properties of blubber were investigated. Thermal conductivity, k, $(\mathrm{W\,m^{-1}\,^{\circ}C^{-1}})$ can be calculated with the Fourier equation:

$$k = dQ / A(T_2 - T_1), \qquad (1)$$

where d is the blubber thickness (m), Q is the rate of heat transfer (W), A is the surface area of the material $(\mathrm{m^2})$ and T_2-T_1 is the temperature differential across the depth of the blubber sample (°C) (Parry, 1949; Kvadsheim et al., 1994; Schmidt-Nielsen, 1997).

Thermal conductance, C, $(\mathrm{W\,m^{-2}\,^{\circ}C^{-1}})$ can be calculated as:

$$C = H / (T_2 - T_1), \qquad (2)$$

where the H denotes heat flux, a rate of energy transfer per unit area $(\mathrm{W\,m^{-2}})$. The inverse of conductance is insulation, R $(\mathrm{m^2\,^{\circ}C\,W^{-1}})$, which measures the absolute resistance to heat flow (Schmidt-Nielsen, 1997).

Thermal conductivity of the whole integument was calculated using both the heat flux disc and standard material methods. In the first method, a heat flux disc is placed in series with the heat source and blubber sample; when steady state is achieved, the Fourier equation (Eqn 1) is used to calculate conductivity (e.g. Doidge, 1990; Worthy and Edwards, 1990). In the second method, a standard material of known thermal conductivity is placed in series with a heat source and the blubber sample (Kvadsheim et al., 1994). When steady state is achieved, the Fourier equation can again be used to calculate the blubber's thermal conductivity by setting equal the heat flow through the standard material and blubber samples.

Both methods were utilized in this study, because each yields a unique opportunity to measure specific thermal properties of blubber. Only the heat flux disc method yields data that permit the calculation of both thermal conductivity and conductance. Dunkin et al. (Dunkin et al., 2005) demonstrated, though, that heat flux differed across the deep and superficial surfaces of bottlenose dolphin (*T. truncatus*) blubber under steady-state conditions and therefore that placement of the heat flux disc strongly influences the resulting calculated conductivity value. In the present study, two heat flux discs were used, one positioned on the deep surface of the blubber, and one on the epidermal surface, to investigate whether this phenomenon was observed for *G. macrorhynchus* and *K. breviceps*. Only the standard material method can be used to measure thermal conductivity of blubber throughout its depth, as this method relies solely upon temperature measurements taken at different positions across a material's depth. Small thermocouples, which can easily be inserted into the blubber, make such depth-specific measurements possible (see detailed methods below).

Using standardized methods established in the literature (e.g. Worthy and Edwards, 1990; Kvadsheim et al., 1994; Kvadsheim et al., 1996) and an experimental setup similar to that of Dunkin et al. (Dunkin et al., 2005), a dual compartment heat flux chamber (69 quart Coleman Cooler, Albany, NY, USA) was used; the lower compartment consisted of an insulated heat source of a constant temperature (37°C), and the upper compartment consisted of a cooled (15–19°C) air space (Fig. 3). This setup created a controlled temperature differential that simulated the conditions of the temperature difference between the warm inner body of a whale (e.g. Rommel et al., 1994) and the colder environment.

The constant temperature heat source was an aluminum box in the lower compartment through which heated water (37°C) was continuously circulated from a water bath (RE-120 Lauda Ecoline, Brinkmann Instruments, Toronto, ON, Canada). The upper part of the aluminum box was an open platform upon which the standard material and blubber were placed. Insulating foam surrounded the standard material and the blubber, and ensured unidirectional heat flow through the blubber sample (Fig. 3). The upper compartment was filled with ice packs, which cooled the air temperature between 15 and 19°C for all experiments. Air temperature was measured using a thermocouple suspended ~10 cm above the blubber sample. All temperatures were measured using copper-constantan (Type T) thermocouples (Omega Engineering, Stamford, CT, USA).

The standard material was an elastomer (Plastisol vinyl, Carolina Biological Supply, Burlington, NC, USA; k=0.11±0.01 W m^{-1} °C^{-1}) that was 8×8×0.67 cm. This elastomer was placed flush against the surface of the heated aluminum box. Two thermocouples (probes 1 and 2; Fig. 3) monitored temperature at the interface between the aluminum and elastomer standard material. Three more thermocouples (probes 3, 4 and 5; Fig. 3) monitored temperature between the standard material and the deep hypodermal surface of the sample. For *G. macrorhynchus*, one side of the blubber sample was arbitrarily chosen,

and deep, middle and superficial thermocouples were inserted into the blubber at those three equidistant positions (Fig. 3A). The tip of each thermocouple was inserted ~5 mm into the lateral face of the blubber sample (Fig. 3A). The superficial thermocouple was placed ~1 mm below the pigmented epidermis (Fig. 3A). For *K. breviceps*, one thermocouple was inserted at the interface between the fibrous superficial layer and the deep layer and another thermocouple was inserted ~1 mm below the pigmented epidermis (Fig. 3B). The sample, with thermocouples already inserted across the depth, was then positioned on the standard material. A final four thermocouples (probes 6–9; Fig. 3) were positioned on the epidermis and held in place with thin pieces of medical tape (Johnson and Johnson All Purpose Cloth Tape).

Two heat flux discs (Model HA-13-18-PC, Thermonetics Corp., San Diego, CA, USA) were used to directly measure heat flow (Fig. 3). The deep heat flux disc was placed at the interface between the standard material and the deep hypodermis. The superficial heat flux disc was positioned on the epidermis and also held in place with thin strips of the same medical tape. The tape was carefully placed on the outer silicone edges of the disc so that it did not contact the active thermal surface (diameter of which was 2.54 cm). All thermocouples and heat flux discs were connected to a Fluke Hydra Data Logger (Model 2625A, Fluke, Everett, WA, USA).

The outputs of the thermocouples (°C) and the heat flux discs (mV) were recorded continuously. When the heat flux values at both the deep and superficial heat flux discs became stable (a standard deviation of less than 5 W m^{-2} around the mean for a period of at least 30 min) and the temperature values of all thermocouples became stable (a standard deviation of less than 0.1°C around the mean for a period of at least 30 min), the experiment was concluded (Fig. 4). On average, equilibrium was reached within 2 h for all experiments, and values remained stable for up to 6 h of continuous recording. The data were then downloaded to a laptop computer for analysis and heat flux readings were converted from mV to W m^{-2} using the calibration coefficients provided by the manufacturer. Conductivity and conductance values were calculated according to Eqns 1 and 2 as described previously.

A series of calibration experiments [similar to those of Dunkin et al. (Dunkin et al., 2005)] were also performed on standard materials of known thermal conductivities: polystyrene foam (Dow Chemical Company, Midland, MI, USA; k=0.03 W m^{-1} °C^{-1}) and white pine wood [reported in Liley (Liley, 1996); k=0.1040 W m^{-1} °C^{-1}]. For foam and wood, thermal conductivity values calculated using both the superficial and deep heat flux disc outputs, as well as the standard material method, were similar and consistent with those reported in the literature (largest range across all methods ±0.0002 W m^{-1} °C^{-1}).

Statistical analyses

Data met assumptions of normality and equal variances (*via* Levene's test) required for parametric tests. *t*-tests were used to determine significant differences between *G. macrorhynchus* and *K. breviceps* with regard to blubber thickness, epidermis thickness, integument thickness, total and depth-specific lipid content, thermal conductivities (for the entire integument; blubber only; and deep, middle and superficial layers), insulation, conductance and heat flux disc outputs. Repeated-measures ANOVA, with pairwise tests using a Bonferroni-type correction [to reduce the chances of making a Type I error (Tabachnick and Fidell, 1996)], were performed to determine significant differences in lipid content or thermal conductivities across the superficial, middle and deep blubber layers in *G. macrorhynchus*. Paired *t*-tests were used to compare the lipid
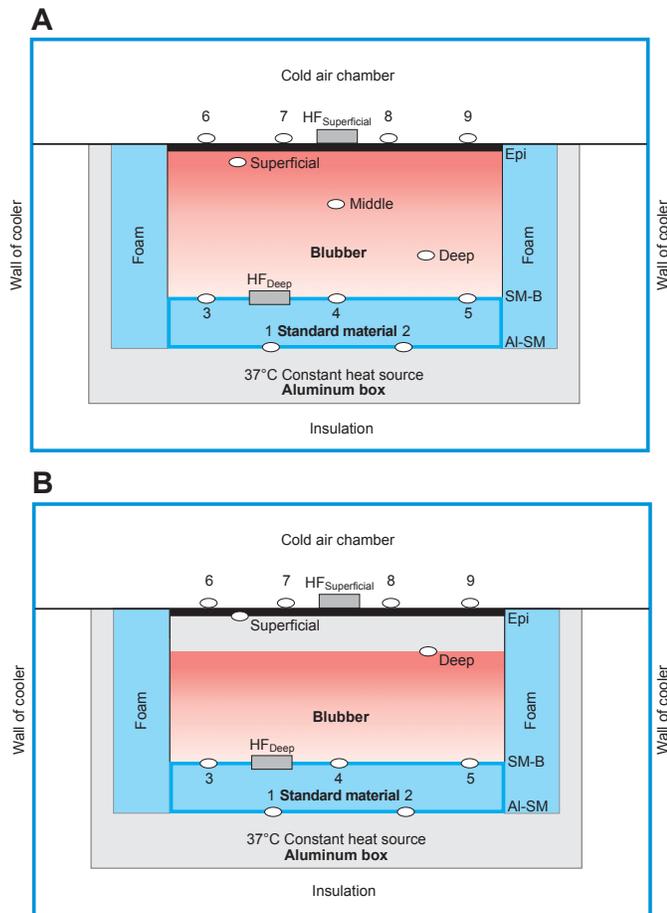
Fig. 3. (A) Thermal chamber showing the placement of thermocouples (ovals) and heat flux discs (HF$_{Superficial}$ and HF$_{Deep}$; rectangles) for *G. macrorhynchus* and (B) for *K. breviceps*. Al-SM is the interface between the heat source and the standard material; SM-B is the interface between the standard material and the deep blubber surface; and Epi refers to the epidermis. Because *K. breviceps* blubber was grossly stratified into two layers, the superficial layer is colored gray to represent the difference between the two layers. The figure is not drawn to scale and certain aspects have been exaggerated for clarity.
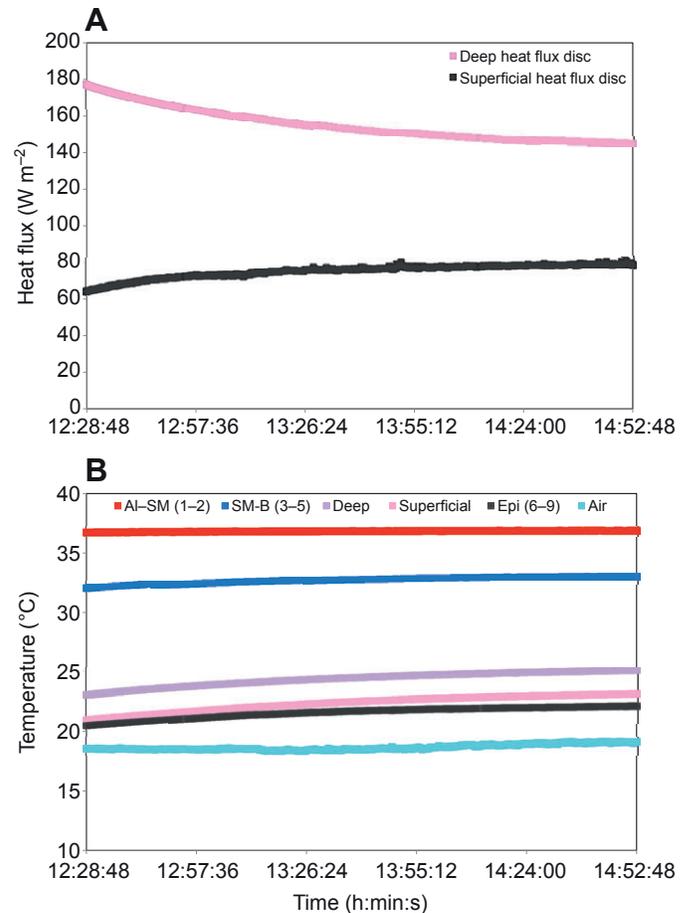

Fig. 4. (A) Example outputs from the deep and superficial heat flux discs using blubber from BRF 092, an adult female pygmy sperm whale. (B) Example of temperature values recorded from the same animal. Al-SM (1–2) refers to the average temperature of the two probes placed at the interface between the heat source and the standard material; SM-B (3–5) is the average of the three probes placed between the standard material and the deep blubber surface; Epi (6–9) is the average of the four probes that were taped onto the surface of the epidermis and were in contact with the air. The blubber depth was divided into the two grossly observable layers, and deep and superficial probes were placed at these positions. For both traces, data from the last 30 min of the experiment, during which time temperature and heat flux values were stable, were used in thermal calculations.

content and thermal conductivities of the superficial and deep blubber layers in *K. breviceps*. For each species, thermal conductivities obtained using the three methods (standard material, superficial heat flux disc and deep heat flux disc) were also compared using repeated-measures ANOVA. Significance was assessed using α=0.05.

RESULTS
Lipid content, thermal conductivity and conductance of integument

There were no significant differences between male and female *K. breviceps* for any parameter measured; therefore, all individuals of this species were pooled for subsequent analyses. The blubber of *G. macrorhynchus* and *K. breviceps* was each dominated by different lipid classes. *Globicephala macrorhynchus* blubber was composed of 99.5±0.07% TAG, and *K. breviceps* blubber was composed of 82.1±3.80% WE. The total percentage of lipid present in the entire blubber layer tended to be higher in *G. macrorhynchus* than in *K. breviceps*, but this difference was not significant (*P*=0.07; Table 2). The blubber was significantly thinner in *G. macrorhynchus* than in

K. breviceps (*P*=0.006; Table 2), but the epidermal thickness was similar across species (Table 2).

The thermal conductivity (k; W m^{-1}°C^{-1}) of the integument was compared across species using values obtained from both the heat flux disc and the standard material methods (Table 2). Each method yielded the same pattern – the thermal conductivity of the entire integument of *G. macrorhynchus* was higher than that of *K. breviceps* (Fig. 5). The standard material method and the results from the superficial heat flux disc showed that the conductivity of *G. macrorhynchus* integument was significantly higher than that of *K. breviceps* (*P*=0.0006 and 0.03, respectively). When results from the deep heat flux disc were used to calculate conductivity, this difference across species was not significant (*P*=0.17). Although the deep heat flux disc yielded the same absolute difference in value across species as did the superficial disc (Table 2, Fig. 5), the variation around the mean for was higher at this deep position. The

Table 2. Morphological, compositional and thermal data for integument from *Globicephala macrorhynchus* and *Kogia breviceps*

	G. macrorhynchus (N=7)	K. breviceps (N=7)
Blubber thickness (mm)	19.61±0.96*	27.98±1.35
Epidermis thickness (mm)	1.04±0.02	0.98±0.11
Total lipid content (% wet mass)	62.17±2.02	56.64±1.73
Deep lipid content (% wet mass)	67.60±3.40	75.29±2.61
Middle lipid content (% wet mass)	75.67±2.20	–
Superficial lipid content (% wet mass)	43.26±2.85	26.22±3.02
Conductance, C ($W\,m^{-2}\,°C^{-1}$)		
Using superficial heat flux disc	10.79±0.71*	6.91±0.44
Using deep heat flux disc	17.37±0.93*	11.76±0.93
Insulation, R ($m^2\,°C^{-1}\,W^{-1}$)		
Using superficial heat flux disc	0.10±0.01*	0.15±0.01
Using deep heat flux disc	0.06±0.01*	0.09±0.01
Conductivity, k, using standard material method ($W\,m^{-1}\,°C^{-1}$)		
k integument	0.19±0.01*	0.14±0.01
k blubber	0.20±0.01*	0.15±0.01
k epidermis	0.19±0.04	0.10±0.03
k deep blubber	0.23±0.02	0.14±0.01
k middle blubber	0.21±0.01	–
k superficial blubber	0.16±0.01	0.20±0.003
Conductivity, k, using heat flux disc method ($W\,m^{-1}\,°C^{-1}$)		
k integument using deep heat flux disc	0.35±0.01	0.32±0.02
k integument using superficial heat flux disc	0.22±0.01*	0.19±0.01
Heat flux ($W\,m^{-2}$)		
Deep heat flux disc ($W\,m^{-2}$)	156.66±7.48	148.93±5.05
Superficial heat flux disc ($W\,m^{-2}$)	96.73±3.54	87.67±3.26
Δ Heat flux discs ($W\,m^{-2}$)	59.93±5.85	61.26±4.53

Values are means ± s.e.m. For all measurements across rows, significant differences between species are marked with an asterisk (*$P<0.05$).
Depth-specific lipid contents and thermal conductivities were not compared across species, as these were not homologous positions.

dramatically different conductivity values resulting from use of the deep heat flux disc, relative to both the superficial disc and the standard material method, will be discussed in more detail below.

Conductance and insulation values could only be calculated using the heat flux disc method. Conductance of the entire integument of *G. macrorhynchus* was significantly higher than that of *K. breviceps* (superficial heat flux disc $P=0.0009$; deep heat flux disc $P=0.0011$; Table 2). Insulation, the inverse of conductance, was significantly higher in *K. breviceps* than in *G. macrorhynchus* (superficial heat flux disc $P=0.0019$; deep heat flux disc $P=0.0022$; Table 2). As was observed with conductivity, there were large differences in the conductance and insulation values obtained from the deep and superficial heat flux discs for each species.

Lipid content and thermal conductivity across blubber depth
Within each species, the percentages of TAG or WE present did not differ significantly across the blubber depth; however, total lipid content did differ. In *G. macrorhynchus*, the deep and middle blubber layers had higher lipid contents than did the superficial layer ($P<0.0001$; Table 2, Fig. 6). In *K. breviceps*, the deep layer contained more lipid than did the superficial layer ($P<0.0001$; Table 2, Fig. 7).

The standard material method permitted depth-specific conductivity values to be calculated (Table 2). *Globicephala macrorhynchus* blubber did not display the expected inverse relationship between thermal conductivity and lipid content. The deep and middle blubber layers, with higher lipid contents, also had significantly higher conductivity values than the superficial layer ($P=0.021$; Fig. 6). In contrast, the superficial layer in *K. breviceps* blubber, with a lower lipid content, tended to have a higher conductivity than the deep layer, although these values were not significantly different ($P=0.08$; Fig. 7).

Comparison of standard material and heat flux disc methods
In all blubber samples, regardless of species, the deep heat flux disc recorded values that were higher than those recorded by the superficial disc (both species $P<0.0001$; Table 2). The mean difference in heat flux across the blubber of *G. macrorhynchus* (58.93 $W\,m^{-2}$, range=31.4–80 $W\,m^{-2}$) was similar to that across *K. breviceps* blubber (61.26 $W\,m^{-2}$, range=46–81 $W\,m^{-2}$, $P=0.86$; Table 2). In contrast, the mean differences in heat flux across the standard calibration materials were ±4.2 $W\,m^{-2}$ for polystyrene foam (range=2.7–8.4 $W\,m^{-2}$) and ±9.3 $W\,m^{-2}$ for white pine wood (range=4.8–15.0 $W\,m^{-2}$).

Because the deep disc recorded higher heat flux values for both species, calculations using the deep heat flux disc data resulted in higher integument conductivity values (~1.5–1.8 times higher) than those calculated using either the superficial disc or the standard material method ($P<0.001$; Table 2, Fig. 5). In addition, for both species, the integument conductivity values calculated using the superficial heat flux disc outputs were higher than those calculated using the standard material method ($P<0.001$).

DISCUSSION
Thermal properties of the integument in two deep-diving cetacean species, the short-finned pilot whale (*G. macrorhynchus*) and the pygmy sperm whale (*K. breviceps*), were investigated *via* the simultaneous use of the standard material and heat flux disc methods. Both methods yielded consistent results, and all species comparisons were significant except that of thermal conductivity calculated using the output of the deep heat flux disc. Results suggest that blubber composed predominantly of WE may provide enhanced insulation relative to that composed predominantly of TAG. Blubber's thermal conductivity did vary across its depth, but the relationship between lipid content and conductivity was complex.
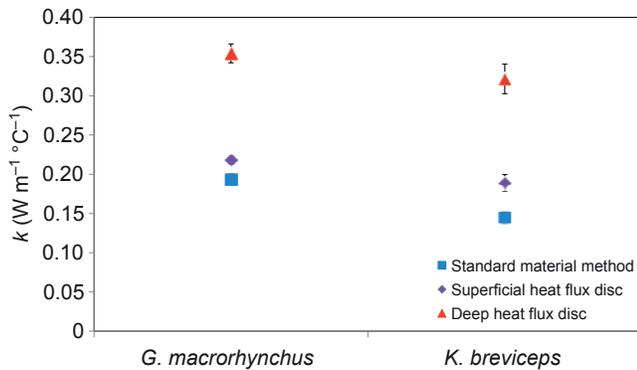
Fig. 5. Comparison of the standard material method, superficial heat flux disc and deep heat flux disc used to calculate the integument conductivity (k) values for *G. macrorhynchus* and *K. breviceps*. Values represent means ± s.e.m.

Fig. 6. Percentage of lipid and thermal conductivities (k) in the superficial, middle and deep blubber layers of *G. macrorhynchus*. Values represent means ± s.e.m.

Results of this study support those of Dunkin et al. (Dunkin et al., 2005) and suggest that blubber, regardless of lipid class, behaves as a phase change material whose thermal properties are temperature dependent.

Blubber's quality and quantity

Blubber's insulative ability depends upon both its quality, or thermal conductivity, and its quantity, or thickness (e.g. Parry, 1949; Worthy and Edwards, 1990; Koopman et al., 1996; Pabst et al., 1999; Hamilton et al., 2004; Struntz et al., 2004; Dunkin et al., 2005; Montie et al., 2008). *Kogia breviceps* blubber had a lower thermal conductivity and was thicker than that of *G. macrorhynchus* (Table 2). Thus, *K. breviceps* blubber provided significantly better insulation, ~1.5 times that of *G. macrorhynchus* blubber. Insulation is important to these animals because both species may experience as much as a 20°C drop in ambient temperatures over the course of a dive (Goold and Clarke, 2000). *Globicephala macrorhynchus* has been recorded to dive to a maximum depth of 1018 m for 21 min (Aguilar Soto et al., 2008). Less is known about *K. breviceps* dive behavior, but dietary analyses suggest that they feed on deep-water squid typically occurring between 500 and 1500 m (reviewed in Piscitelli et al., 2010) and one visual tracking study observed a maximum dive duration of 52 min (Barlow et al., 1997). Perhaps the enhanced insulation provided by the WE blubber of *K. breviceps* facilitates deeper, longer dives in this species.

However, other functional consequences of possessing WE blubber must be considered because not all marine mammals that perform prolonged deep dives possess WE-enriched blubber. For example, the blubber of elephant seals (*Mirounga* spp.) is composed entirely of TAG, and these animals undergo continuous, deep dives for months at a time while at sea (Le Boeuf et al., 1989). Elephant seals, though, experience periodic fasts, during which they rely upon lipids, stored as TAG, within their blubber (Fedak et al., 1994). Although TAG are easily metabolized, most mammals cannot metabolize WE (reviewed in Swaim et al., 2009), and because kogiids are not known to undergo fasts, they may not be reliant upon lipid resources stored within their blubber.

Koopman (Koopman, 2007) speculated that kogiids and ziphiids may gain some functional advantage – whether related to thermoregulation or buoyancy control – by possessing WE-enriched blubber. The results of our study suggest that lipid class may significantly influence blubber's ability to resist heat flow. The thermal conductivities of the blubber of *G. macrorhynchus* and *K.*
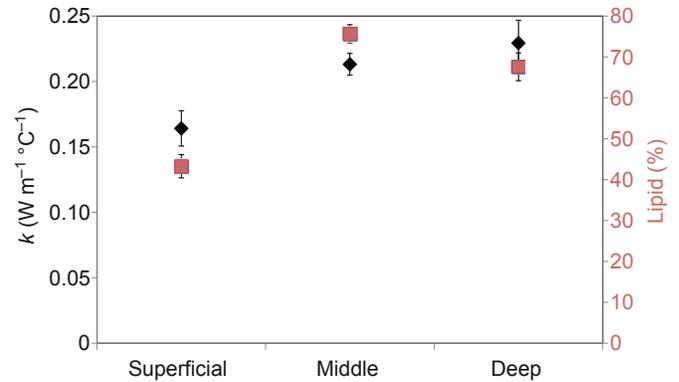
breviceps differed, despite their similar lipid contents (Table 2): *G. macrorhynchus* blubber typically had higher conductivity values, even when its blubber had higher lipid contents than that of *K. breviceps* blubber (Fig. 8).

Differences in the chemical composition of the TAG and WE lipids may affect the way in which these lipids are arranged within an adipocyte, and thus may affect the lipid's thermal properties. In blubber, TAG and WE storage lipids can be any combination of saturated, monounsaturated or polyunsaturated fatty acids. However, the fatty alcohol portion of a WE is almost always saturated or monounsaturated (Sargent et al., 1976; reviewed in Budge et al., 2006). The number of 'kinks' in the carbon chain, due to the addition of double bonds, appears to affect an individual fatty acid's conductivity. For example, stearic (18:0) and palmitic (16:0) acids are saturated fatty acids that have lower thermal conductivity values (0.16 and $0.17\,\mathrm{W\,m^{-1}\,^\circ C^{-1}}$, respectively) than oleic acid ($18{:}1_{n-9}$), which is a monounsaturated fatty acid with a thermal conductivity of $0.23\,\mathrm{W\,m^{-1}\,^\circ C^{-1}}$ (Weast, 1989). This result suggests that the efficiency of heat transfer correlates with the degree of lipid unsaturation.

In this study, the WE blubber of *K. breviceps* had a lower thermal conductivity than the TAG blubber of *G. macrorhynchus*. Other cetaceans with blubber composed of TAG have thermal conductivity values that are reported to be equal to or lower than that of *K. breviceps*, though the geographic ranges, as well as blubber's percent wet mass lipid and thickness, may not be comparable across species (Table 1). For example, the beluga whale (*Delphinapterus leucas*) and the harbor porpoise (*P. phocoena*) are two cold temperate to Arctic species that possess TAG blubber with lower thermal conductivity values than the WE blubber of *K. breviceps* (Table 1). Worthy and Edwards (Worthy and Edwards, 1990) found that the thermal conductivity of *P. phocoena* blubber, composed of TAG (Koopman, 2007), was $0.10\pm0.01\,\mathrm{W\,m^{-1}\,^\circ C^{-1}}$; however, *P. phocoena* blubber also possessed a much higher lipid content [81.6±3.6% (Worthy and Edwards, 1990)] than the *K. breviceps* blubber used in this study. The overall insulation value, which takes both quantity and quality into account, of *P. phocoena* blubber was $0.15\,\mathrm{W\,m^{-2}\,^\circ C^{-1}}$ (Worthy and Edwards, 1990), the same insulation as that of *K. breviceps* blubber (Table 2). Because influences on insulation might vary by family, a broader phylogenetic analysis of blubber's composition and thermal properties would be valuable. In future studies, the TAG blubber of *P. phocoena* could be compared with
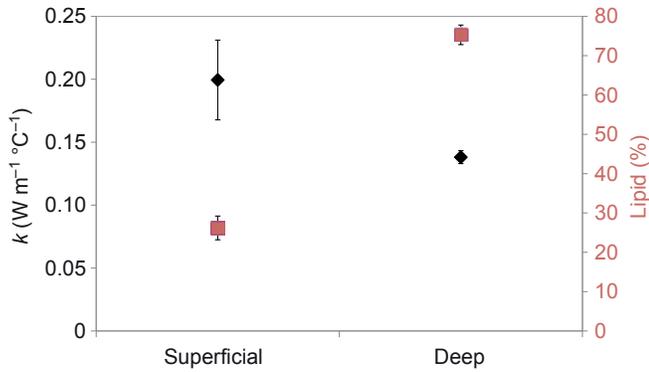
Fig. 7. Percentage of lipid and thermal conductivities (k) in the superficial and deep blubber layers of *K. breviceps*. Values represent means ± s.e.m.
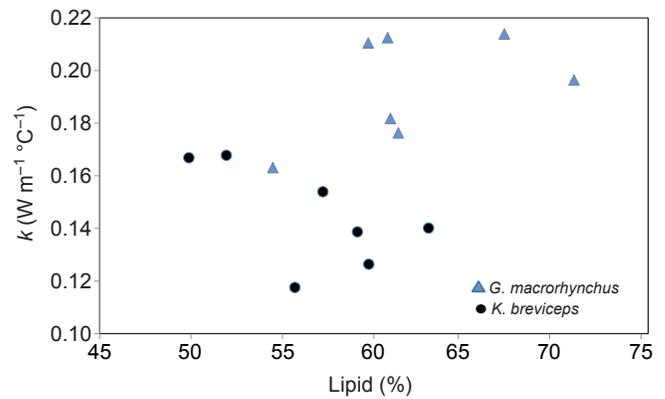

Fig. 8. Thermal conductivities of the integument of *G. macrorhynchus* and *K. breviceps* shown in relation to percentage of lipid in the blubber of each of these individuals.

that of another cetacean group, the ziphiids, which possess WE blubber with similarly high lipid contents (e.g. *Mesoplodon bidens*, range 71.1–91.4%) (Koopman, 2007).

Factors other than lipid content, lipid class and fatty acid composition may also influence the overall thermal conductivity value of blubber. Future studies might consider whether structural features of blubber not measured in this study (such as vascularization, collagen content or water content) could be contributing to the difference in thermal conductivity between these two species. For instance, *K. breviceps* blubber was grossly stratified, with a fibrous outer layer that differed in both color and texture from the deep layer (see Fig. 2). Investigating the thermal properties of another species, such as the closely related dwarf sperm whale, *K. sima*, which possesses blubber composed of WE but appears to lack the grossly stratified blubber that is seen in *K. breviceps*, would help identify the thermal consequences of possessing this unusually stratified blubber.

Depth-specific thermal conductivities

Lipid content did vary across blubber's depth in both species (Table 2, Figs 6, 7). The deep layer possessed significantly more lipid than the superficial layer in *K. breviceps*, and the deep and middle layers possessed more lipid than the superficial layer in *G. macrorhynchus*.

Because an inverse relationship between blubber's lipid content and thermal conductivity had been demonstrated in other species (e.g. Worthy and Edwards, 1990; Dunkin et al., 2005), it was expected that in both species, the deep blubber layer would have a lower thermal conductivity value than the superficial layer. In *K. breviceps* blubber, the deep layer did tend to have a lower thermal conductivity, although it was not significantly different than that of the superficial layer (Fig. 7). *Globicephala macrorhynchus* blubber displayed a pattern that was opposite of that which was expected; the lipid-rich deep layer had a higher thermal conductivity than the superficial layer, which contained significantly less lipid (Fig. 6).

There are two potential hypotheses regarding this unexpected relationship between conductivity and lipid content in the blubber of *G. macrorhynchus*. First, it is possible that the fatty acid compositions of the TAG molecules differed between the deep and superficial layers. Perhaps the deep layer is composed of fatty acids with higher conductivity values than those in the more superficial layers. Although Koopman (Koopman, 2007) reported that fatty acids in the blubber of *G. macrorhynchus* are not highly stratified, the index used in her study was not designed to explore this specific functional question. Testing this hypothesis will require careful characterization of the individual fatty acid profiles across the blubber depth.

Alternatively, the unexpected relationship between conductivity and lipid content may reflect temperature-dependent thermal properties of lipids. Blubber's depth-specific conductivities were calculated using the standard material method, which relies on measures of depth (thickness) of the sample, and of the difference in temperature (ΔT), across that depth. A typical homogenous, insulating material, such as polystyrene foam, divided into three layers of equal thickness, would have the same ΔT, generating the same calculated conductivity value for each layer. Alternatively, if three different foam layers of equal thickness were stacked together, the temperature differential across each would be dependent upon the individual foam's thermal conductivity. The foam with the lowest thermal conductivity would have the largest ΔT across its depth.

In *G. macrorhynchus*, the blubber was divided equally into thirds, so ΔT was the determining factor for the calculated differences in conductivity across these layers. If blubber were acting as a typical insulator and simply resisting heat flow, then a lipid-rich deep layer would be expected to slow the rate of heat flow more than the superficial layer, and result in a larger ΔT across that deep layer. However, the deep layer of *G. macrorhynchus* had an unexpectedly low ΔT relative to the middle and superficial blubber layers, which suggests that heat entering the deep surface was not directly transmitted through the blubber, but was instead trapped within that layer.

Blubber is a biological material composed of lipids, and lipids change phase at their melting temperature (reviewed in Tan and Che Man, 2000). In this thermal experiment, the deep blubber layer experienced near-core body temperatures, and the outer layer was exposed to cooler environmental temperatures. Differential melting could have occurred throughout the blubber depth. As an analogy, when a stick of butter is placed on a warm pan, the butter directly touching the pan begins to melt, although melting does not take place all at once throughout its depth. When a lipid changes from solid to liquid, it absorbs energy, or stores heat (reviewed in Suppes et al., 2003). If the deep blubber layer possessed lipids that melted at temperatures experienced during these experiments, heat would be absorbed, which could account for the small ΔT we observed across that layer.

Blubber as a heat-storing material

A material that can temporarily store latent heat due to changes in molecular structure at certain temperatures is defined as a phase

change material (reviewed in Suppes et al., 2003). Dunkin et al. (Dunkin et al., 2005) reviewed the evidence that supports classifying the blubber of cetaceans as a phase change material, and other studies (e.g. Sari and Kaygusuz, 2001; Sari, 2003; Sari et al., 2004; Suppes et al., 2003) have identified mixtures of fatty acids, which occur in cetacean blubber, that yield phase change materials with melting points within the range of mammalian body temperatures. Future studies that directly measure the phase behavior of blubber lipids are required to test this hypothesis.

However, the two heat flux discs, which directly measured the rate of energy entering and exiting the blubber, provided further indirect evidence that blubber is capable of temporarily storing heat. The deep disc yielded much higher heat flux (Wm^{-2}) values than the superficial disc (Fig. 4A), a phenomenon also observed in the blubber of bottlenose dolphins by Dunkin et al. (Dunkin et al., 2005). Recall that there were only small differences in heat flux values across the standard materials of polystyrene foam and wood (see also Dunkin et al., 2005), suggesting that there was little heat loss to the sides of any sample in this experimental setup. These results suggest that some of the heat that was entering the deep surface of the blubber was not being directly transmitted through it, but rather temporarily stored within the tissue.

As was also observed by Dunkin et al. (Dunkin et al., 2005), blubber conductivity values calculated using the outputs of the deep heat flux disc were more than 50% higher than conductivity values calculated using either the output of the superficial heat flux disc or the standard material method. (Blubber conductance values, calculated in this study using the output of the deep heat flux disc, were also over 50% higher than those using the superficial disc.) Unlike Dunkin et al. (Dunkin et al., 2005), this study found that conductivity values calculated using the superficial heat flux disc were similar to, but higher than, those calculated using the standard material method. Why these two approaches yielded statistically significant differences in conductivity values for blubber of G. macrorhynchus and K. breviceps but not for T. truncatus is not known at this time. Investigating depth-specific patterns of lipid content and thermal conductivity values across all three species may provide insights into these species-specific differences. These different values, though, are not likely the result of experimental error, but rather reflect the complex thermal behavior of this lipid-rich, stratified biological material.

The large differences in the amount of heat entering the deep blubber layer *versus* exiting the epidermis per unit time, along with the unexpected depth-specific conductivity values and low ΔT across the deep blubber in G. macrorhynchus, support Dunkin et al.'s (Dunkin et al., 2005) observation that blubber has the capacity to store heat. The functional consequences of blubber behaving as a phase change material are not yet known. However, if the lipids in blubber change phase within a physiologically relevant temperature range, then blubber may not only be resisting heat flow to and from the cetacean body, it may also be acting as a dynamic thermal buffer, with the capacity to store and release heat.

Conclusions

This study was the first to address how lipid class affects the thermal properties of cetacean blubber by investigating two deep divers, G. macrorhynchus and K. breviceps. The WE blubber of K. breviceps was less conductive than the TAG blubber of G. macrorhynchus, and was thus a material of higher thermal quality. Kogia breviceps blubber was also thicker and provided significantly higher overall insulation than that of G. macrorhynchus. This result suggests that possessing blubber composed of WE may provide a thermal

advantage to K. breviceps, potentially providing an enhanced ability to conserve body heat during deep, long dives.

This study also provided the first data on the thermal properties of blubber across its depth. In K. breviceps, the depth-specific conductivity values tended to vary inversely with lipid content. Unexpectedly, however, the lipid-rich deep blubber layer in G. macrorhynchus was significantly more conductive than superficial layers. In addition, heat flux measurements across blubber's depth provided evidence that heat was being stored in blubber, an observation first noted in bottlenose dolphin blubber by Dunkin et al. (Dunkin et al., 2005). These results suggest that there are temperature-dependent effects on blubber's lipids that influenced its thermal properties. The function of blubber as an insulator is complex and may rely upon its stratified composition and dynamic heat-storage capabilities. The mechanism behind this observed heat storage, including the hypothesis that blubber's lipids are functioning as a phase change material, requires further investigation.

REFERENCES

Aguilar Soto, N., Johnson, M. P., Madsen, P. T., Díaz, F., Domínguez, I., Brito, A. and Tyack, P. (2008). Cheetahs of the deep sea: deep foraging sprints in short-finned pilot whales off Tenerife (Canary Islands). *J. Anim. Ecol.* **77**, 936-947.

Barlow, J., Forney, K., Von Saunder, A. and Urban-Ramirez, J. (1997). A report of cetacean acoustic detection and dive interval studies (CADDIS) conducted in the Southern Gulf of California, 1995. La Jolla, CA: National Marine Fisheries Service SFSC.

Bernard, H. J. and Reilly, S. B. (1999). Pilot whales *Globicephala* Lesson, 1828. In *Handbook of Marine Mammals* (ed. S. H. Ridgway and R. Harrison), pp. 245-279. San Diego, CA: Academic Press.

Bryden, M. M. (1964). Insulating capacity of the subcutaneous fat of the southern elephant seal. *Nature* **203**, 1299-1300.

Budge, S. M., Iverson, S. J. and Koopman, H. N. (2006). Studying trophic ecology in marine ecosystems using fatty acids: a primer on analysis and interpretation. *Mar. Mammal Sci.* **22**, 759-801.

Cox, T. M., Read, A. J., Barco, S., Evans, J., Gannon, D. P., Koopman, H. N., McLellan, W. A., Murray, K., Nicolas, J., Pabst, D. A. et al. (1998). Documenting the bycatch of harbor porpoises, *Phocoena phocoena*, in coastal gillnet fisheries from stranded carcasses. *Fish Bull.* **96**, 727-734.

Doidge, D. W. (1990). Integumentary heat loss and blubber distribution in the beluga (*Delphinapterus leucas*), with comparisons to the narwhal (*Monodon monoceros*). *Can. B. Fish. Aquat. Sci.* **224**, 129-140.

Dunkin, R. C., McLellan, W. A., Blum, J. E. and Pabst, D. A. (2005). The ontogenetic changes in the thermal properties of blubber from Atlantic bottlenose dolphin *Tursiops truncatus*. *J. Exp. Biol.* **208**, 1469-1480.

Dunkin, R. C., McLellan, W. A., Blum, J. E. and Pabst, D. A. (2010). The buoyancy of the integument of Atlantic bottlenose dolphins (*Tursiops truncatus*): effects of growth, reproduction, and nutritional state. *Mar. Mammal Sci.* **26**, 573-587.

Evans, K., Hindell, M. A. and Thiele, D. (2003). Body fat and condition in sperm whales, *Physeter macrocephalus*, from southern Australian waters. *Comp. Biochem. Physiol.* **134A**, 847-862.

Fedak, M. A., Arnbom, T. A., McConnell, B. J., Chambers, C., Boyd, I. L., Harwood, J. and McCann, T. S. (1994). Expenditure, investment, and acquisition of energy in Southern elephant seals. In *Elephant Seals: Population Ecology, Behavior, and Physiology* (ed. B. J. Le Boeuf and R. M. Laws), pp. 354-373. Berkeley, CA: University of California Press.

Folch, J., Lees, M. and Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226**, 497-509.

Folkow, L. P. and Blix, A. S. (1992). Metabolic rates of minke whales (*Balaenoptera acutorostrata*) in cold water. *Acta Physiol. Scand.* **146**, 141-150.

Geraci, J. R. and Lounsbury, V. J. (2005). *Marine Mammals Ashore: A Field Guide for Strandings*. Baltimore, MD: National Aquarium in Baltimore.

Goold, J. C. and Clarke, M. R. (2000). Sound velocity in the head of the dwarf sperm whale, Kogia sima, with anatomical and functional discussion. *J. Mar. Biol. Assoc. U. K.* **80**, 535-542.

Hamilton, J. L., Dillaman, R. M., McLellan, W. A., and Pabst, D. A. (2004). Structural fiber reinforcement of keel blubber in harbor porpoise (*Phocoena phocoena*). *J. Morphol.* **261**, 105-117.

Hensel, H. (1973). Cutaneous thermoreceptors. In *Somatosensory System* (ed. A. Iggo), pp. 79-110. Berlin: Springer-Verlag.

Hohn, A. A., Rotstein, D. S., Harms, C. A. and Southall, B. L. (2006). Report on marine mammal unusual mortality event UMESE0501Sp: multispecies mass stranding of pilot whales (*Globicephala macrorhynchus*), minke whales (*Balaenoptera acutorostrata*), and dwarf sperm whales (*Kogia sima*) in North Carolina on 15-16 January 2005. NOAA Technical Memorandum NMFS-SEFSC-537.

Iverson, S. J. (1988). Composition, intake, and gastric digestion of milk lipids in pinnipeds. PhD thesis, University of Maryland, College Park, MD, USA.

Koopman, H. N. (2007). Phylogenetic, ecological, and ontogenetic factors influencing the biochemical structure of the blubber of odontocetes. *Mar. Biol.* **151**, 277-291.

Koopman, H. N., Iverson, S. J. and Gaskin, D. E. (1996). Stratification and age-related differences in blubber fatty acids of the male harbor porpoise (*Phocoena phocoena*). *J. Comp. Physiol. B* **165**, 628-639.

Koopman, H. N., Pabst, D. A., McLellan, W. A., Dillaman, R. M. and Read, A. J. (2002). Changes in blubber distribution and morphology associated with starvation in the harbor porpoise (*Phocoena phocoena*): evidence for regional differences in blubber structure and function. *Physiol. Biochem. Zool.* **75**, 498-512.

Kvadsheim, P. H., Folkow, L. P. and Blix, A. S. (1994). A new device for measurement of the thermal conductivity of fur and blubber. *J. Therm. Biol.* **19**, 431-435.

Kvadsheim, P. H., Folkow, L. P. and Blix, A. S. (1996). Thermal conductivity of minke whale blubber. *J. Therm. Biol.* **21**, 123-128.

Le Boeuf, B. J., Naito, Y., Huntley, A. C. and Asaga, T. (1989). Prolonged, continuous, deep diving by northern elephant seals, *Mirounga angustirostris. Can. J. Zool.* **67**, 2514-2519.

Liley, P. E. (1996). *Marks' Standard Handbook for Mechanical Engineers.* New York: McGraw-Hill.

Lockyer, C. (1991). Body composition of the sperm whale, *Physeter catodon*, with special reference to the possible functions of fat depots. *J. Mar. Res.* **12**, 1-24.

Lockyer, C. (1993). Seasonal changes in body fat condition of Northeast Atlantic pilot whales, and their biological significance. *Rep. Int. Whal. Comm. Spec. Issue* **14**, 325-350.

McLellan, W. A., Koopman, H. N., Rommel, S. A., Read, A. J., Potter, C. W., Nicolas, J. R., Westgate, A. J. and Pabst, D. A. (2002). Ontogenetic allometry and body composition of the harbor porpoises (*Phocoena phocoena*) from the western north Atlantic. *J. Zool.* **257**, 457-471.

Montie, E. W., Garvin, S. R., Fair, P. A., Bossart, G. D., Mitchum, G. B., McFee, W. E., Speakman, T., Starczak, V. R. and Hahn, M. E. (2008). Blubber morphology in wild bottlenose dolphins (*Tursiops truncatus*) from the Southeastern United States: influence of geographic location, age class, and reproductive state. *J. Morphol.* **269**, 496-511.

Norris, K. S. (1961). Standardized methods for measuring and recording data on the smaller cetaceans. *J. Mammal.* **42**, 471-476.

Pabst, D. A., Rommel, S. A. and McLellan, W. A. (1999). The functional morphology of marine mammals. In *Biology of Marine Mammals* (ed. J. E. Reynolds III and S. A. Rommel), pp. 15-72. Washington, DC: Smithsonian Institution Press.

Parry, D. A. (1949). The structure of whale blubber, and a discussion of its thermal properties. *Q. J. Microsc. Sci.* **90**, 13-25.

Payne, P. M. and Heinemann, D. W. (1993). The distribution of pilot whales (*Globicephala* spp.) in shelf/shelf-edge and slope waters of the Northeastern United States, 1978-1988. In *Biology of Northern Hemisphere Pilot Whales* (ed. G. P. Donovan, C. H. Lockyer and A. R. Martin), pp. 53-68. Cambridge, MA: International Whaling Commission.

Piscitelli, M. A., McLellan, W. A., Rommel, S. A., Blum, J. E., Barco, S. G. and Pabst, D. A. (2010). Lung size and thoracic morphology in shallow- and deep-diving cetaceans. *J. Morphol.* **271**, 654-673.

Rommel, S. A., Pabst, D. A., McLellan, W. A., Williams, T. M. and Friedl, W. A. (1994). Temperature regulation of the testes of the bottlenose dolphin (*Tursiops truncatus*): evidence from colonic temperatures. *J. Comp. Physiol. B* **164**, 130-134.

Sargent, J. R., Lee, R. F. and Nevenzel, J. C. (1976). Marine waxes. In *Chemistry and Biochemistry of Natural Waxes* (ed. P. E. Kolattukudy), pp. 49-91. Oxford: Elsevier.

Sari, A. (2003). Thermal reliability test of some fatty acids as PCMs used for solar thermal latent heat storage applications. *Energy Convers. Manage.* **44**, 2277-2287.

Sari, A. and Kaygusuz, K. (2001). Thermal energy storage system using some fatty acids as latent heat energy storage materials. *Energy Sources* **23**, 275-285.

Sari, A., Sari, H. and Onal, A. (2004). Thermal properties and thermal reliability of eutectic mixtures of some fatty acids as latent heat storage materials. *Energy Convers. Manage.* **45**, 365-376.

Schmidt-Nielsen, K. (1997). *Animal Physiology: Adaptation and Environment.* New York: Cambridge University Press.

Struntz, D. J., McLellan, W. A., Dillaman, R. M., Blum, J. E., Kucklick, J. R. and Pabst, D. A. (2004). Blubber development in bottlenose dolphins (*Tursiops truncatus*). *J. Morphol.* **259**, 7-20.

Suppes, G. J., Goff, M. J. and Lopes, S. (2003). Latent heat characteristics of fatty acid derivatives pursuant phase change material applications. *Chem. Eng. Sci.* **58**, 1751-1763.

Swaim, Z. T., Westgate, A. J., Koopman, H. N., Rollan, R. M. and Kraus, S. D. (2009). Metabolism of ingested lipids by North Atlantic right whales. *Endanger. Species Res.* **6**, 259-271.

Tabachnick, B. G. and Fidell, L. S. (1996). *Using Multivariate Statistics*, 3rd edn. New York: Harper Collins College Publishers.

Tan, C. P. and Che Man, Y. B. (2000). Differential scanning calorimetric analysis of edible oils: comparison of thermal properties and chemical composition. *J. Am. Oil Chem. Soc.* **77**, 143-155.

Weast, R. C. (1989). *Handbook of Chemistry and Physics,* 69th edn. Boca Raton, FL: CRC Press.

Worthy, G. A. J. and Edwards, E. F. (1990). Morphometric and biochemical factors affecting heat loss in a small temperate cetacean (*Phocoena phocoena*) and a small tropical cetacean (*Stenella attenuata*). *Physiol. Zool.* **63**, 432-442.

Yasui, W. Y. and Gaskin, D. E. (1986). Energy budget of a small cetacean, the harbor porpoise (*Phocoena phocoena*) (L.). *Ophelia* **25**, 183-197.