Ontogenetic Changes in the Thermal and Buoyant Properties of Atlantic Bottlenose Dolphin (*Tursiops truncatus*) Blubber

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CHAPTER 1

The thermal properties of cetacean blubber are influenced by its lipid content and thickness. In Atlantic bottlenose dolphins (*Tursiops truncatus*), both these features vary across ontogeny and with reproductive and nutritional status and, thus, may result in ontogenetic differences in blubber's insulative quality. Lipid and water contents, and thermal conductivity and thermal insulation values of Atlantic bottlenose dolphin blubber were measured across fetal through adult life history categories (n = 36), and in pregnant females (n=4) and emaciated animals (n = 5). The thermal conductivities of deep and superficial blubber layers were also measured.

Thermal conductivity varied significantly across ontogeny. Fetal through sub-adult life history categories had significantly lower mean thermal conductivity values (0.11 to 0.13 ± 0.01 W/m°C) than adults (0.18 ± 0.02 W/m°C). The conductivity of blubber from pregnant females was similar to non-adult categories, while that of emaciated animals was significantly higher than all other categories. The conductivity of superficial blubber was 37% higher than that of deep blubber. Across life history categories, the conductivity of superficial blubber was similar, while that of deep blubber was significantly greater in emaciated animals.

Thermal insulation varied significantly across life history categories. Sub-adults and pregnant females had the highest insulation while fetuses and emaciated animals had the lowest insulation across life history categories. The insulation of neonates and juveniles was similar to that of adult dolphins.

Heat flux measurements at the deep blubber surface were significantly higher than that at the superficial surface and this difference in heat flux was significantly correlated with blubber thickness. This pattern was not observed in control materials, polystyrene foam and white pine wood.

In nutritionally dependant life history categories, changes in blubber's thermal insulation resulted from changes in blubber thickness (i.e. quantity) and not thermal conductivity (i.e. quality). Conversely, in nutritionally independent animals, blubber quantity remained stable while blubber quality varied. Differences in conductivity through the blubber depth support the characterization of deep blubber as more insulative and metabolically active layer of lipid deposition and mobilization. Finally, blubber's composition and its ability to absorb heat suggest that it likely is a phase change material.

ABSTRACT
CHAPTER 2

Blubber is the hypertrophied hypodermis of cetaceans composed primarily of adipocytes and structural fibers. Because the density of lipid is less than that of seawater, blubber has the potential to contribute to positive buoyancy. The blubber of Atlantic bottlenose dolphins (*Tursiops truncatus*) varies both in thickness and lipid content across ontogeny and with reproductive and nutritional status. This variation in blubber's quantity and quality may significantly influence its contribution to buoyancy. To measure blubber's buoyant force, its density was measured volumetrically and its volume was calculated at two body sites (trunk and tailstock), across an ontogenetic series of bottlenose dolphins and in pregnant females and emaciated animals. Lipid and water content were measured to correlate compositional changes with differences in blubber's buoyant force.

The density of blubber from the trunk region (mean ± standard error = 1043.1 ± 13.18 kg/m^3) was similar to that of the tailstock (mean = 1077.1 ± 24.17 kg/m^3) and these were not significantly different than the density of seawater (1026 kg/m^3). Density in these regions was also similar between life history categories. Blubber volume in the trunk and tailstock regions increased over two orders of magnitude between fetuses and adults. The buoyant force of trunk blubber was similar across categories (mean = –0.91 ± 8.85N) and was not significantly different from neutral buoyancy (0N). Trunk blubber of emaciated animals was twelve times more negatively buoyant than that of adults. The buoyant force of tailstock blubber was similar between life history categories (mean = –0.30 ± 1.83 N). For groups with a sufficient sample size for statistical analyses (fetus,

neonate, and juvenile), mean total buoyant force of blubber was 0.61 ± 7.45 N and was not significantly different between these groups.

Despite significant differences in lipid content and volume across life history categories, blubber's contribution to buoyancy remained neutral. Because this body compartment is nearly 25% of total body mass, it may be essential for this tissue to be neutrally buoyant. Pregnancy and emaciation can significantly influence blubber's contribution to buoyancy and may impose additional locomotor costs associated with overcoming a positive or negative vertical force.

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DEDICATION

I would like to dedicate this work to my parents and sister. They have always encouraged me to pursue my interests in science and marine biology, even when it meant moving across the country. I appreciate their confidence in me and enthusiasm for my interests and I can't express enough gratitude for their support.

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INTRODUCTION

Blubber is the specialized hypodermis of cetaceans that streamlines the body, acts as a metabolic energy storage site, contributes to positive buoyancy, and provides insulation for the body core (e.g. Kipps et al., 2002; Koopman, 1998; Ling, 1974; McLellan et al., 2002; Pabst, 2000; Pabst et al., 1999b; Parry, 1949; Ryg, 1988; Worthy and Edwards, 1990). This multifunctional tissue is formed by adipocytes bound within a highly organized, three-dimensional weave of structural fibers (Hamilton et al., *in press;* Koopman, 1998; Pabst et al., 1999a; Parry, 1949). Several features of blubber, including adipocyte size, thickness, and lipid content, vary significantly across ontogeny in Atlantic bottlenose dolphins (*Tursiops truncatus*) (Struntz et al., 2004). These morphological and compositional changes may result in differences in the insulative quality of blubber throughout development.

Insulation may be particularly important to neonatal bottlenose dolphins that are born into a fluid medium that conducts heat away from a body 25 times faster than air at the same temperature (Schmidt-Nielsen, 1997). Because a neonatal dolphin has a larger surface area to volume ratio than an adult, heat loss to the environment may be high (McLellan et al., 2002; Worthy and Edwards, 1990). Struntz et al. (2004) suggested that the blubber of neonatal dolphins may be specialized to provide enhanced insulation. To date, however, no study has measured changes in the thermal properties of blubber across an ontogenetic series.

Blubber's thermal conductivity, k (W/m°C), and thermal conductance, C (W/m^2°C), have been measured across a phylogenetically diverse sample of cetaceans (Table 1). Thermal conductivity, a constant material property, is a quantitive measure of

how well heat moves through a material (McNab, 2002; Schmidt-Nielsen, 1997) and is, thus, useful for comparing the insulative quality of blubber across species (Worthy and Edwards, 1990). Thermal conductance, which is dependent upon the material thickness, or quantity of blubber, provides an absolute value of heat transfer across this thermal barrier. Conductivity can be calculated using the Fourier equation:

(I) $$k = \frac{dQ}{A(T_2 - T_1)}$$

where d is the thickness of the blubber (m), Q is the rate of heat transfer in Watts (W), A is the surface area across which heat flows (m^2), and ($T_2 - T_1$) is the temperature difference (°C) across the thickness of the blubber (Kvadshiem et al., 1994; Schmidt-Nielsen, 1997). Conductance can be calculated as:

(II) $$C = \frac{H}{(T_2 - T_1)}$$

where H is heat flux (W/m^2) (Kvadshiem et al., 1994; Worthy and Edwards, 1990). Another often reported value is thermal insulation, R (m^2°C/W), a measure of thermal resistance to heat flow, which is simply the inverse of thermal conductance.

Across cetacean species, blubber's thermal conductivity can vary by more than four-fold, from 0.06 W/m°C in harbor porpoises (*Phocoena phocoena*) to as high as 0.28 W/m°C in minke whales (*Balaenoptera acutorostrata*) (Table 1). These differences in blubber's quality as a conductive material are likely the result of differences in lipid and water content, which are highly variable among species. The lipid content of harbor porpoise blubber can range between 76 and 88% (Worthy and Edwards, 1990), while that of minke whales can range between 42 and 96% lipid (Kvadshiem et al., 1996). In minke whale blubber, there is a significant inverse relationship between lipid content and

Table 1: Thermal conductivity values for blubber from a variety of marine mammals and other substances.

Species	k (W/m^{o}C) (Reported Mean)	Source	Method
Cetaceans			
Balaenoptera acutorostrata	0.20-0.28*	Kvadshiem *et al*., 1996	Standard material method
Balaenoptera acutorostrata	0.18	Folkow and Blix, 1992	Heat flux disc method
Balaenoptera physalus	0.21	Parry, 1949	Hot plate method
Delphinapterus leucas (blubber)	0.102	Doige, 1990	Heat flux plate method
Delphinapterus leucas (epidermis)	0.249	Doige, 1990	Heat flux plate method
Phocoena phocoena	0.06	Yasui and Gaskin, 1986	Heat flux disc method
Phocoena phocoena	0.1	Worthy and Edwards, 1990	Heat flux disc method
Stenella attenuata	0.2	Worthy and Edwards, 1990	Heat flux disc method
Pinnipeds			
Mirounga leonina	0.07	Bryden, 1964	Unknown
Phoca groenlandica	0.18	Worthy, 1985	Heat flux disc method
Phoca groenlandica	0.19	Kvadshiem *et al*., 1994	Standard material method
Phoca hispida	0.2	Scholander *et al*., 1950	Hot plate method
Phoca vitulina	0.18	Worthy, 1985	Heat flux disc method
Halichoerus grypus	0.18	Worthy, 1985	Heat flux disc method
Fatty Acids			
stearic acid (C18:0)	0.16	CRC 1967 in Doige, 1990	Unknown
palmitic acid (C16:0)	0.17	CRC 1967 in Doige, 1990	Unknown
oleic acid (C18:1)	0.23	CRC 1967 in Doige, 1990	Unknown
Miscellaneous Materials			
air	0.024	Schmidt-Nielson, 1997	Unknown
white pine wood	0.104	Marks Standard Handbook	Unknown
human fat	0.21	Hensel *et al*., 1973 in Doige, 1990	Unknown
iron	80	Schmidt-Nielson, 1997	Unknown

This range of values is mean thermal conductivity across four body sites.

conductivity as well as a strong positive relationship between conductivity and water content (Kvadshiem et al., 1996).

Blubber's thermal conductance, which is reliant upon both its conductive quality and quantity (i.e. thickness), also varies widely across species. For example harbor porpoise blubber has a lower conductivity than that of a pan-tropical spotted dolphin (*Stenella attenuata*), and is twice as thick (Table 1) (Worthy and Edwards, 1990). Spotted dolphin blubber, thus, has a conductance value four times greater than, or an insulative value one quarter of, harbor porpoise blubber.

In Atlantic bottlenose dolphins, blubber thickness and lipid content vary significantly throughout ontogeny. Blubber lipid content doubles between fetal (37%) and adult animals (68%) and mean blubber thickness increases over three fold between these life history categories (Struntz et al., 2004). These significant changes in quality and quantity suggest that changes in blubber's thermal properties across ontogeny may be equal to or greater than differences reported among species.

Multiple methods have been used to measure blubber's thermal conductivity and thermal conductance. Parry (1949) and Scholander et al. (1950) measured thermal conductivity by placing two pieces of blubber on either side of a hot plate and measuring the rate of energy (W) used to maintain the plate at a constant temperature. The surface area and thickness of the blubber sample, and the temperature differential between the hotplate and environment were used to calculate thermal conductivity (Equation I). A more recent method of measuring thermal conductivity relies upon the use of heat flux discs. A heat flux disc is placed in series with, and usually between, a constant heat source and the blubber sample. Once steady state is achieved, Equation I can be used to

calculate conductivity (Doidge, 1990; Worthy and Edwards, 1990; Yasui and Gaskin, 1986). Kvadshiem et al. (1994) introduced a method to calculate conductivity that does not rely upon a direct measure of heat flux. Instead, this method uses a standard material, with a known thermal conductivity, aligned in series with a heat source and blubber sample. Once the system reaches steady state, the heat flow rate through each material is equal (Kreith, 1958; Kvadshiem et al., 1994). Equation I can then be used to calculate the thermal conductivity of blubber by setting equal the heat flow through the standard material and blubber sample.

Each of these more recent methods has advantages and disadvantages. Heat flux discs are relatively affordable, convenient to use, and the results are directly comparable to many previous measurements of blubber's thermal properties (see Table 1). However, Ducharme et al. (1990) identified a potential source of error associated with heat flux disc measurements. The placement of the disc on the surface of interest will cause a local increase in insulation, which may result in measured heat flux values that are lower than actual values. This "reactive error" varies with both the insulative quality of the material relative to that of the heat flux disc, and the insulative quality of the media overlying the disc (usually air or water) (Ducharme et al., 1990; Frim and Ducharme, 1993). Reactive errors are minimized when the disc's insulation is equal to or lower than that of the material being tested and when the experiments are conducted in air (Frim and Ducharme, 1993; Willis, 2003). The standard material method avoids these potential heat flux disc errors and, as reported by Kvadshiem et al. (1994), is accurate to within ± 4.0%. Because it is a relatively new technique however, there are fewer studies that have measured blubber's thermal properties using this method. In the present study, both the

heat flux disc and standard material methods were used simultaneously, permitting cross-calibration of these methods as well as an enhanced ability to compare results from previous studies.

The goals of this study were to (1) to measure the thermal conductivity and thermal conductance of Atlantic bottlenose dolphin blubber across an ontogenetic series, (2) correlate these thermal conductivity and conductance values with measures of lipid and water content of blubber, and (3) compare the results of the heat flux disc and standard material methods to permit comparison with previous studies. Measurements were made across life history categories from fetus through adult. Pregnant females and emaciated adults were also included to investigate how blubber's thermal properties vary with the reproductive and nutritional status of the dolphin.

METHODS

Specimens

Blubber samples were acquired from 40 robust and 3 emaciated Atlantic bottlenose dolphins (*Tursiops truncatus*) that either stranded or were incidentally killed in fisheries in North Carolina and Virginia (Table 2). The sample set also included one emaciated adult from Florida and one from New Jersey. Body condition was scored based upon a suite of characters defined in Cox et al. (1998). Twenty-four of the individuals used in this study were also investigated by Struntz et al. (2004) (Table 2). Only animals with a Smithsonian Institution Code of 1 (live stranded and died naturally or by euthanasia) or 2 (fresh dead) (Geraci and Lounsbury, 1993) were used in this study. Seven life history categories were defined based upon a suite of morphological characters

Table 2: *T. truncatus* specimens grouped by life history category and length.

Animal	Life History Category	Sex	Body Length (cm)
PTM 109f*	fetus	F	58
WAM560f*	fetus	F	63.5
PTM114f	fetus	F	82
ASF 033f*	fetus	M	82
WJW007f*	fetus	M	86.5
WAM 545f*[y]	fetus	F	92
WAM 535f*	fetus	M	96.5
EMM 010*	neonate	M	106
VMSM2000 1020*	neonate	M	106.5
CALO99-13*	neonate	F	109.5
VMSM2001 1080*	neonate	M	110.3
VMSM2002 1042	neonate	M	111
MMB 003[x]	neonate	M	113
WAM 550*	neonate	M	114.5
WAM 584	neonate	F	117
VMSM2000 1031*	juvenile	M	127
DJS 001*	juvenile	F	129.5
VMSM2001 1087*	juvenile	M	129.7
VMSM 2003 1082	juvenile	M	142
VMSM2002 1089	juvenile	F	142.5
ASF 042	juvenile	F	143.5
WAM 569*	juvenile	F	150
DAP 034	sub-adult	M	171
SAE 003	sub-adult	M	173
PTM 117	sub-adult	M	189
WAM 585	sub-adult	F	192
EMM 006	sub-adult	M	197
KMT 013	sub-adult	F	204
VMSM2000 1049*[y]	sub-adult	M	207
WAM 553*	sub-adult	M	232
WAM 574*‡	adult	F	223
MMB 002	adult	M	235
WAM 579	adult	M	237
WAM 559*	adult	F	237
SDZ 005	adult	M	262
REL 014	adult	M	265
WAM 545*	pregnant female	F	246
ASF 041*	pregnant female	F	260
ASF 038*	pregnant female	F	263
WJW 007*	pregnant female	F	273
WAM 591	emaciated	M	222.8
WAM 533*	emaciated	F	261
NJ00-111*	emaciated	F	262
MMB 004[x]	emaciated	M	275
MMSN FB 192	emaciated	M	278

* Animals investigated in Struntz et al. (2004). ‡WAM 574 was a fisheries-take animal and was missing her flukes, resulting in a reduced total length. ([x]) Animals excluded from lipid analysis. ([y]) Animals excluded from thermal measurements because of superficial damage.

described in Struntz et al. (2004) (Table 3). These categories include fetus (n=7), neonate (n=8), juvenile (n=7), sub-adult (n=8), adult (n=6), pregnant female (n=4), and emaciated animals (n=5).

Each animal was first weighed to the nearest kilogram (Dillon, 2000kg capacity scale, Brooklyn, NY, USA) and measured using a standard set of morphometrics. The carcass was then systematically dissected to yield masses of the complete integument, individual muscle groups, internal organs, and skeletal elements (McLellan et al., 2002). Full depth integumental samples, including epidermis, dermis, and hypodermis (subsequently referred to as blubber samples), were taken from a dorsal, mid-thoracic site, just caudal to the pectoral flipper (Figure 1). After removal, the blubber samples were notched at the dorso-cranial margin to maintain orientation and were then either vacuum sealed (Koch, 1700, Kansas City, MO, USA) or wrapped in Saran wrap® and sealed in freezer bags to prevent desiccation. Samples were stored at -20°C until analyzed.

Lipid and Water Content

Lipid content was determined using procedures similar to those of Struntz et al. (2004). Briefly, an approximately 1g full-depth blubber sample (excluding the epidermis) was weighed to the nearest 0.001g , macerated, and dried with approximately 30g of sodium sulfate (Na_2SO_4). The lipid was then extracted using an accelerated solvent extractor (Dionex, Salt Lake City, UT, USA). The excess solvent was evaporated (Turbo Vap II, Zymark, Hopkinton, MA) and the extracted lipid was then reweighed to the nearest 0.001g.

Table 3: Definitions of life history categories based on Struntz et al. (2004).

Life History Category	Code	Defining Characters
Fetus	**1**	Position *en utero.*
Neonate	**2**	Possessed four of the following six characters: presence of rostral hairs, floppy or folded dorsal fin, unhealed umbilicus, prominent fetal folds, floppy or folded dorsal fin, floppy or folded dorsal keel.
Juvenile	**3**	Absence of neonatal characters and estimated to be less than 1 year of age based on total length (≤150 cm).
Sub-adult	**4**	Absence of milk in the stomach, immature reproductive tissues, and total length (>150 cm).
Adult	**5**	Mature reproductive tissues as indicated by obvious ovarian scars in females and the size of the testis and/or presence of sperm in males.
Pregnant female	**6**	Presence of a fetus.
Emaciated animal	**7**	Skeletal elements such as ribs, scapula, vertebral transverse processes, and/or skull prominently visible under blubber layer; atrophy of epaxial musculature and/or the nuchal fat pad.

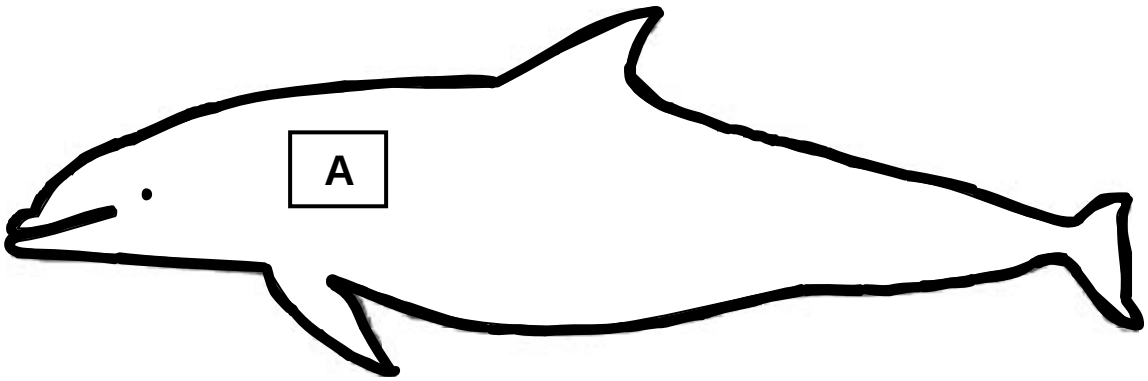

Figure 1 - A is the blubber sample site used for thermal measurements on an ontogenetic series of Atlantic bottlenose dolphins (*Tursiops truncatus*).

Water content was determined by excising an approximately 1x1cm square through the depth of the sample and weighing it prior to and after freeze-drying (Labconco 4.5, Kansas City, MO, USA). Samples were weighed each day until the mass of the sample was stable (± 0.005g) for two consecutive days (total time = 5 days).

Measurement of Thermal Properties

Blubber's thermal properties were measured using an experimental set-up that integrated both the standard material (Kvadshiem et al., 1994) and heat flux disc (e.g. Worthy and Edwards, 1990) methods. Tests were conducted in a dual compartment heat flux chamber (68 quart, Coleman Cooler, Albany, NY, USA) with a lower, highly insulated compartment, and an upper, chilled compartment, which were separated by a wood platform (Figure 2). The heat source consisted of a two-part aluminum box. The lower portion was a sealed, hollow box into which heated water (35°C) from a water bath (RE-120 Lauda Ecoline, Brinkmann Instruments, Inc., Toronto, Ontario, Canada) was circulated to provide a constant heat source. The upper portion was an open platform upon which the standard material and blubber sample were placed. The insulated lower chamber ensured a constant water temperature and unidirectional heat flow through the standard material. The upper chamber was cooled to a constant 15°C with ice packs stacked upon the wood platform.

An elastomer (Plastisol vinyl, Carolina Biological Supply, Burlington, NC, USA) ($k = 0.109 \pm 0.01 W/m°C$) was used as the standard material and was placed flush against the heated surface of the aluminum box. Depending upon the size of the available blubber sample, an approximately 4x4 to 15x15cm blubber sample was used. The thickness of the blubber sample including epidermis, dermis, and hypodermis was

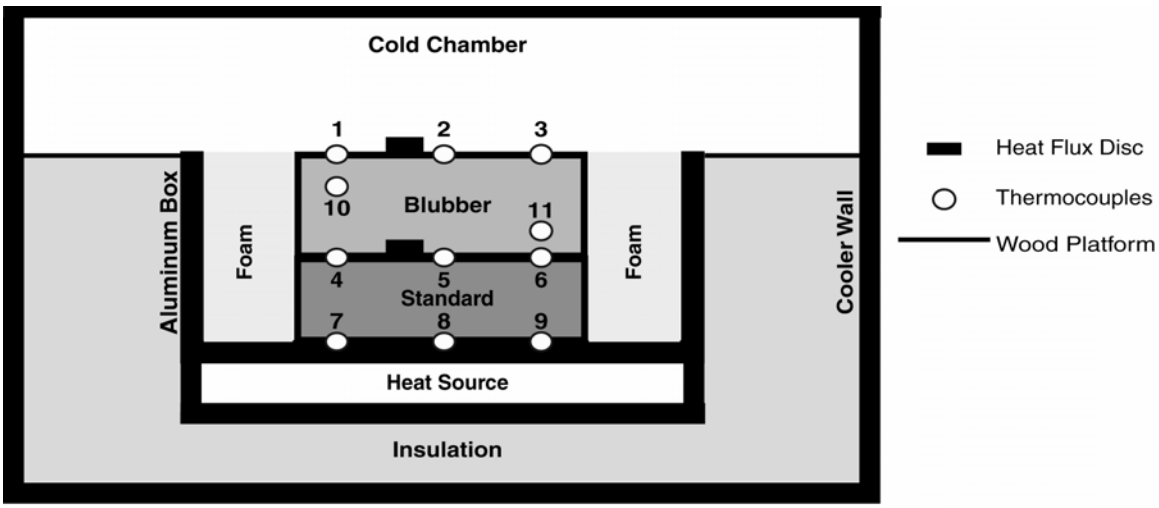

Figure 2 - Heat flux chamber with placement of thermocouples and heat flux discs. Figure is not drawen to scale and the size of the thermocouples and heat flux discs are exaggerated for clarity.

measured on each of its four sides (Absolute Digimatic calipers, Mitutoyo, Tylertown, MS, USA) and the mean of these values was used in thermal calculations. The blubber sample was placed in series, with the deep hypodermis in contact, with the elastomer. The standard material and blubber were surrounded by insulating foam plates to ensure unidirectional heat flow through these materials (Figure 2).

Temperatures were measured using copper-constantan (T-Type) thermocouples (Omega, Stamford, CT, USA) placed on the superficial surface of the epidermis (probes 1-3), between blubber and the standard material (probes 4-6), and between the standard material and the surface of the heat source (probes 7-9) (Figure 2). The mean temperature of the three probes at each surface was used in the thermal calculations. Additionally, two thermocouples (probes 10 and 11) were placed in the superficial 1/3 and deep 1/3 of the blubber sample, respectively. Blubber is non-uniform in composition through its depth, and other studies have characterized the superficial 1/3 of the blubber as primarily structural and the deep 1/3 as more metabolically active (Koopman, 1998; Struntz et al., 2004). Placing probes in these positions allowed for measurement of possible differences in thermal conductivity in these two regions. The fetal samples were too thin (usually less than 0.2 cm) to permit measuring temperatures within the blubber.

Heat flux was directly measured using two heat flux discs [(HA 13-18-19-P (C), Thermonetics Corp., San Diego, CA, USA]. One disc was placed on the superficial surface of the epidermis and the other was placed between the standard material and hypodermis (Figure 2). The discs will be identified as the superficial and deep discs, respectively. To ensure complete contact between the superficial heat flux disc and the sample, thin strips of medical adhesive tape (Nexcare Advanced Holding Power, 3M, St.

Paul, MN, USA) were used to secure the disc. The tape was only in contact with the outer silicone edge of the disc and did not touch the thermopile surface.

All eleven thermocouples and the two heat flux discs were wired to a Fluke Hydra data logger (model 2625A, Fluke Inc., Everett, WA, USA) and the outputs in °C and millivolts, respectively, were recorded at 1 minute intervals. These data were downloaded to a laptop computer for later analysis. The experiment was concluded once the heat flux values at the superficial and deep surfaces were stable (± 5 W/m^2) for 30 minutes. Heat flux readings were converted into W/m^2 using the calibration coefficient provided by the manufacturer.

The experimental set-up was calibrated using control materials [white pine wood, polystyrene foam (Dow Chemical, Midland, MI, USA)] with known thermal conductivities. Additionally, experiments with the control materials were performed to determine if sample depth or surface area influenced thermal measurements.

Statistics

For thermal conductivity, conductance, and insulation values, an ANCOVA (SAS Inc., Cary, NC, USA) (alpha = 0.05) was used with life history category and sample area as factors. Sample area was included to account for variation in the measurements that was a result of differences in the dimensions of the blubber sample. A one-way ANOVA (JMP 5.1, SAS Inc., Cary, NC, USA) (alpha = 0.05) was performed to determine if there were significant differences between life history categories in blubber thickness, lipid content, and water content. If significant differences were present, a Tukey-Kramer Honestly Significant Difference Test or Ryan's Q Test was performed to determine which groups were different from one another.

RESULTS

Morphology and Composition

Blubber thickness steadily increased between fetal and adult life history categories with maximal thickness reached in adult animals (Table 4). Fetal animals had significantly thinner blubber than all other life history categories ($F=22.09$; $p<0.001$) and within the fetal life history category, blubber thickness linearly increased with body length ($r^2=0.74$; $p=0.027$) (Figure 3a). Blubber thicknesses of juveniles, sub-adults, adults, pregnant females, and emaciated adults were not significantly different (Table 4). Mean blubber thickness of emaciated dolphins decreased by 26% compared to adults and was similar to the mean blubber thickness of neonatal and juvenile animals.

Blubber lipid content increased linearly in fetuses and increased steadily from fetal through juvenile life history categories ($r^2=0.65$; $p=0.028$) (Figure 3b, Table 4). Although not a significant trend, lipid content declined between juvenile, sub-adult, and adult life history categories. The blubber of pregnant females had a lipid content similar to that of juvenile animals, which represented an increase of 27% compared to adults. The blubber of emaciated adults contained significantly less lipid than all life history categories except fetuses (Table 4).

Across life history categories, blubber thickness was not a good predictor of lipid content (Figure 4). Rather, the relationship between lipid content and blubber thickness displayed life history category-specific trends. In fetal and adult animals, lipid content increased linearly with blubber thickness ($r^2=0.91$; $p=0.0034$ and $r^2=0.96$; $p=0.0008$, respectively). Although not a significant trend, blubber lipid content of sub-adults tended

Table 4: Thermal data for blubber from each life history category in Atlantic bottlenose dolphins reported as mean ± standard error.

	Fetus	Neonate	Juvenile	Sub-Adult	Adult	Pregnant Female	Emaciated Adult
	n=6	n=8	n=7	n=7	n=6	n=4	n=5
Blubber thickness d (cm)	0.49 ± 0.001 c	1.23 ± 0.002 b	1.61 ± 0.001 a, b	2.0 ± 0.003 a	2.13 ± 0.004 a	2.05 ± 0.004 a	1.57 ± 0.003 a, b
Lipid weight/wet weight (%)	35.06 ± 6.09 b, c	55.82 ± 2.86 a, b	69.72 ± 4.08 a	62.59 ± 2.31 a	54.31 ± 4.88 a, b	69.20 ± 5.73 a	28.22 ± 9.14 c
Water weight/wet weight (%)	50.3 ± 3.52 a	33.06 ± 1.97 b	29.9 ± 0.96 b	30.16 ± 2.35 b	32.78 ± 1.08 b	31.66 ± 1.57 b	59.34 ± 6.63 a
Conductivity k (W/m°C) Whole blubber	0.12 ± 0.01 c	0.13 ± 0.01 c	0.12 ± 0.01 c	0.11 ± 0.01 c	0.18 ± 0.02 b	0.12 ± 0.01 c	0.24 ± 0.04 a
Conductivity k (W/m°C) Deep blubber	NE	0.12 ± 0.03 b	0.10 ± 0.02 b	0.09 ± 0.01 b	0.12 ± 0.01 b	0.09 ± 0.01 b	0.23 ± 0.02 a
Conductivity k (W/m°C) Superficial blubber	NE	0.13 ± 0.02 a	0.17 ± 0.03 a	0.14 ± 0.02 a	0.17 ± 0.003 a	0.11 ± 0.01 a	0.22 ± 0.03 a
Conductance C ($W/m^2°C$)	25.69 ± 6.04 a	10.44 ± 0.69 b, c	7.30 ± 0.61 c, d	5.74 ± 0.44 d	8.44 ± 1.03 c, d	5.73 ± 0.82 d	14.79 ± 2.64 b
Insulation R ($m^2°C/W$)	0.05 ± 0.01 d	0.10 ± 0.005 b, c, d	0.14 ± 0.01 a, b	0.18 ± 0.012 a	0.12 ± 0.016 b, c	0.18 ± 0.03 a	0.07 ± 0.015 c, d

For all measurements, life history categories with the same letter are not significantly different ($p>0.05$).

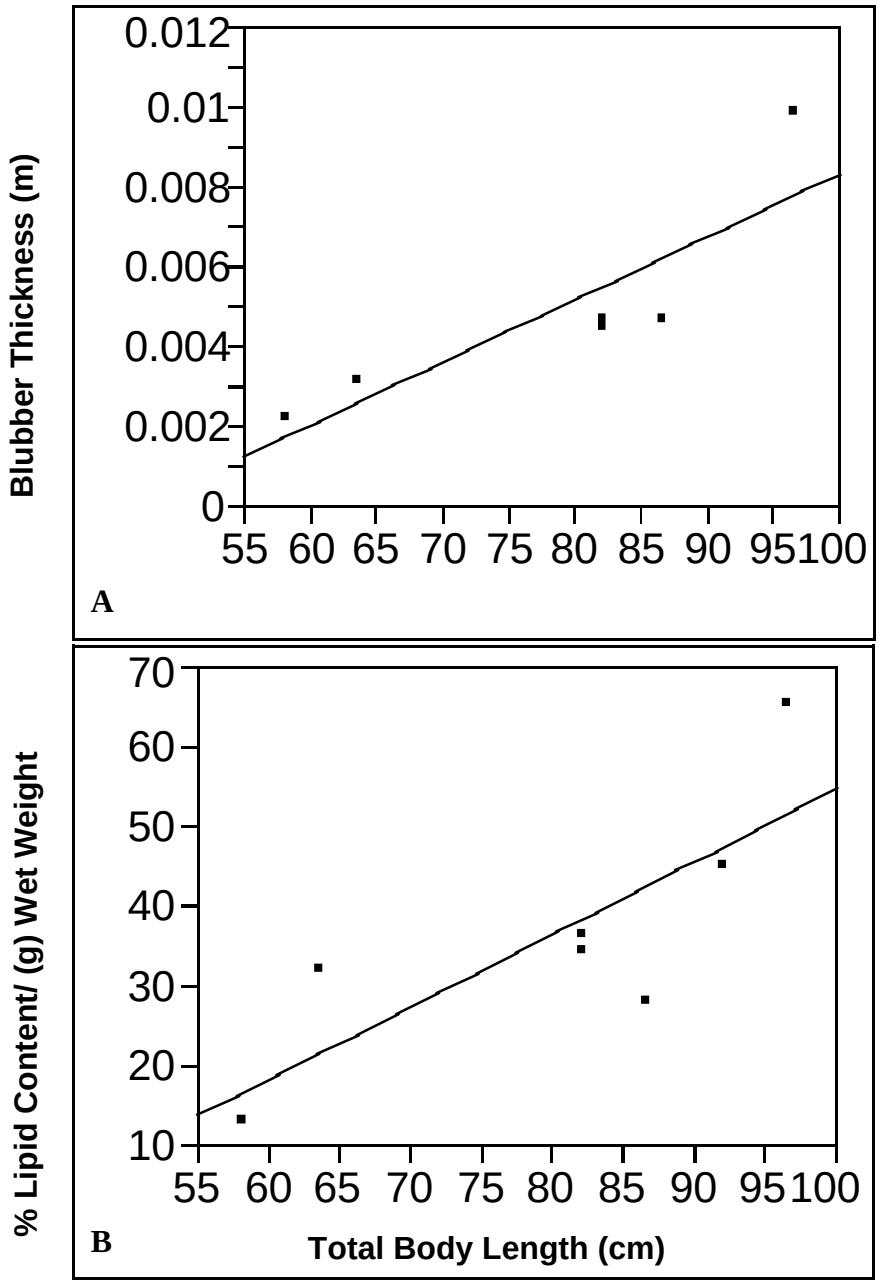

Figure 3 - (A) fetal blubber thickness and (B) fetal blubber lipid content plotted against fetal total body length in Atlantic bottlenose dolphins (*Tursiops truncatus*).

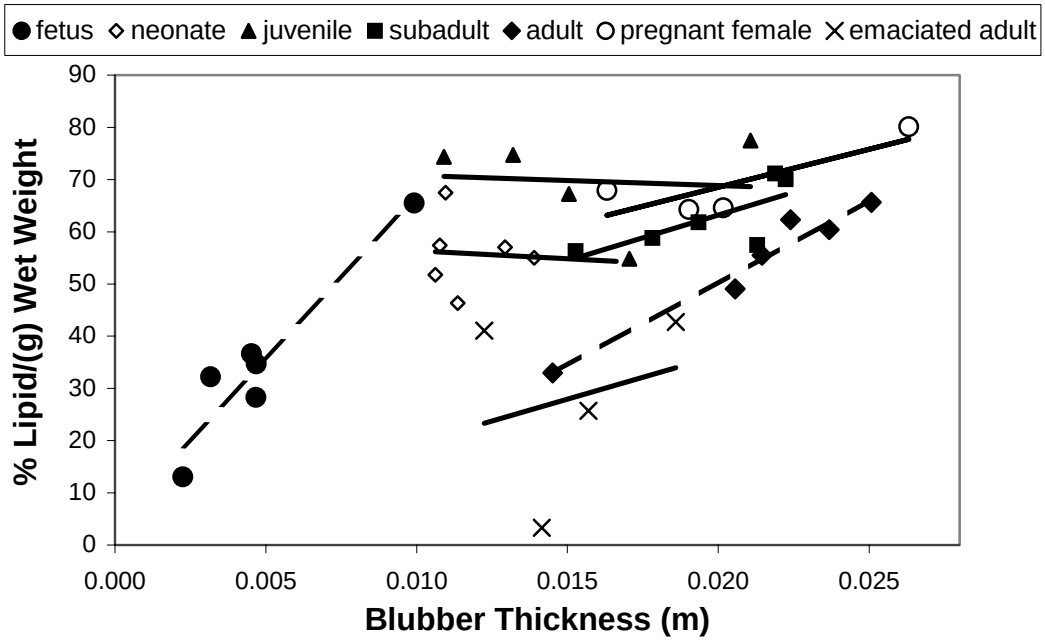

Figure 4: Percent lipid content as a function of blubber depth in *T. truncatus*. Broken trend lines indicate life history categories where there was a significant linear relationship between lipid content and blubber thickness (fetus and adult). Solid trend lines indicate categories in which there was not a significant relationship between lipid content and blubber thickness.

to increase with blubber thickness (r^2=0.54; p=0.097). Blubber thickness and lipid content were not correlated in neonatal, juvenile, or pregnant animals. There was no clear relationship between lipid content and blubber thickness in emaciated adults, however, both of these measures were highly reduced from adult values (Figure 4, Table 4).

Water content was less variable across life history categories (Table 4). The blubber of fetuses and emaciated adults, which had significantly lower lipid contents, contained significantly more water than all other life history categories (F=14.84; p<0.001).

Comparison of Standard Material and Heat Flux Methods

Both the standard material method and the heat flux method (using outputs of either the superficial or deep disc) yielded thermal conductivity values for the control materials that were similar to their commercially reported values. For polystyrene foam and white pine wood, thermal conductivity values, reported as mean ± standard error, were determined to be 0.033 ± 0.0014 W/m°C (reported value 0.03 W/m°C; Dow Chemical Company) and 0.11 ± 0.0025 W/m°C (reported value 0.104 W/m°C; Liley, 1996) respectively. These values indicate a maximum error of 10% for conductivity values in the range of polystyrene foam, but error was minimized to 6% for materials with conductivity values similar to wood.

Blubber thermal conductivity values calculated with the standard material method and with the output of the superficial heat flux disc were similar (F=0.05; p=0.81) and yielded overall mean conductivity values that were within 2.0% of each other (Figure 5a,b). The results of both of these methods though, were significantly different from the

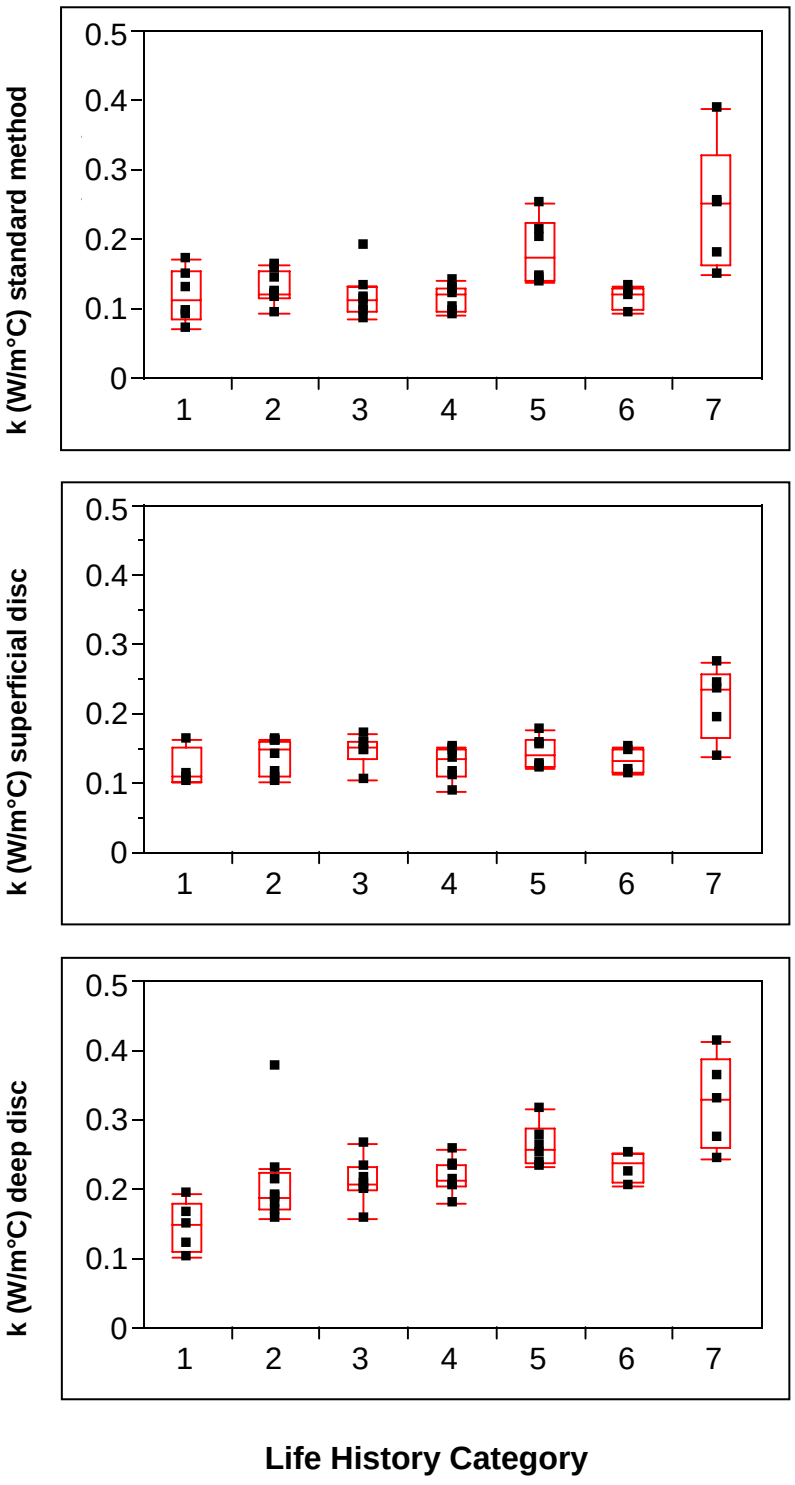

Figure 5 – Blubber thermal conductivity values for *T. truncatus* calculated with (A) standard material method, (B) heat flux values from the superficial disc, and (C) heat flux values from the deep disc. Life history categories are (1) fetus, (2) neonate, (3) juvenile, (4) sub-adult, (5) adult, (6) pregnant female, and (7) emaciated animals. Box plots represent the upper and lower quartiles for each life history category.

values obtained with the deep heat flux disc measurements ($F=31.8$; $p<0.001$) (Figure 5c). On average, conductivity values of whole blubber calculated with the deep disc were 57% higher than those obtained with the other two methods. The differences between the deep and superficial heat flux measurements are described in more detail below.

For all subsequent analyses, the conductivity values obtained by the standard material method were used. The standard material method was chosen because recent studies (Kvadshiem et al., 1994; Kvadshiem et al., 1996) have extensively calibrated a similar system and because it was the only method by which the conductivity of the deep and superficial blubber could be separately calculated.

Thermal Properties of Blubber

Thermal conductivity of blubber remained similar in fetal through sub-adult life history categories but increased significantly in adult animals ($F=4.39$; $p=0.0067$) (Table 4; Figure 5a). The conductivity of blubber from pregnant females was significantly less than that of adults while that of emaciated adults was significantly greater than all other life history categories ($F=6.93$; $p<0.001$) (Table 4, Figure 5a).

There was a significant difference in thermal conductivity between deep and superficial blubber ($F=10.06$; $p=0.0026$). The mean thermal conductivity of superficial blubber was 32% higher than that of the deep blubber layer (Figure 6a). Deep blubber conductivity values were similar across all life history categories except in emaciated adults, which had significantly higher values ($F= 5.13$; $p=0.002$) (Figure 6b). Across life history categories, superficial blubber conductivity values were similar ($F= 2.23$; $p=0.08$) (Figure 6c).

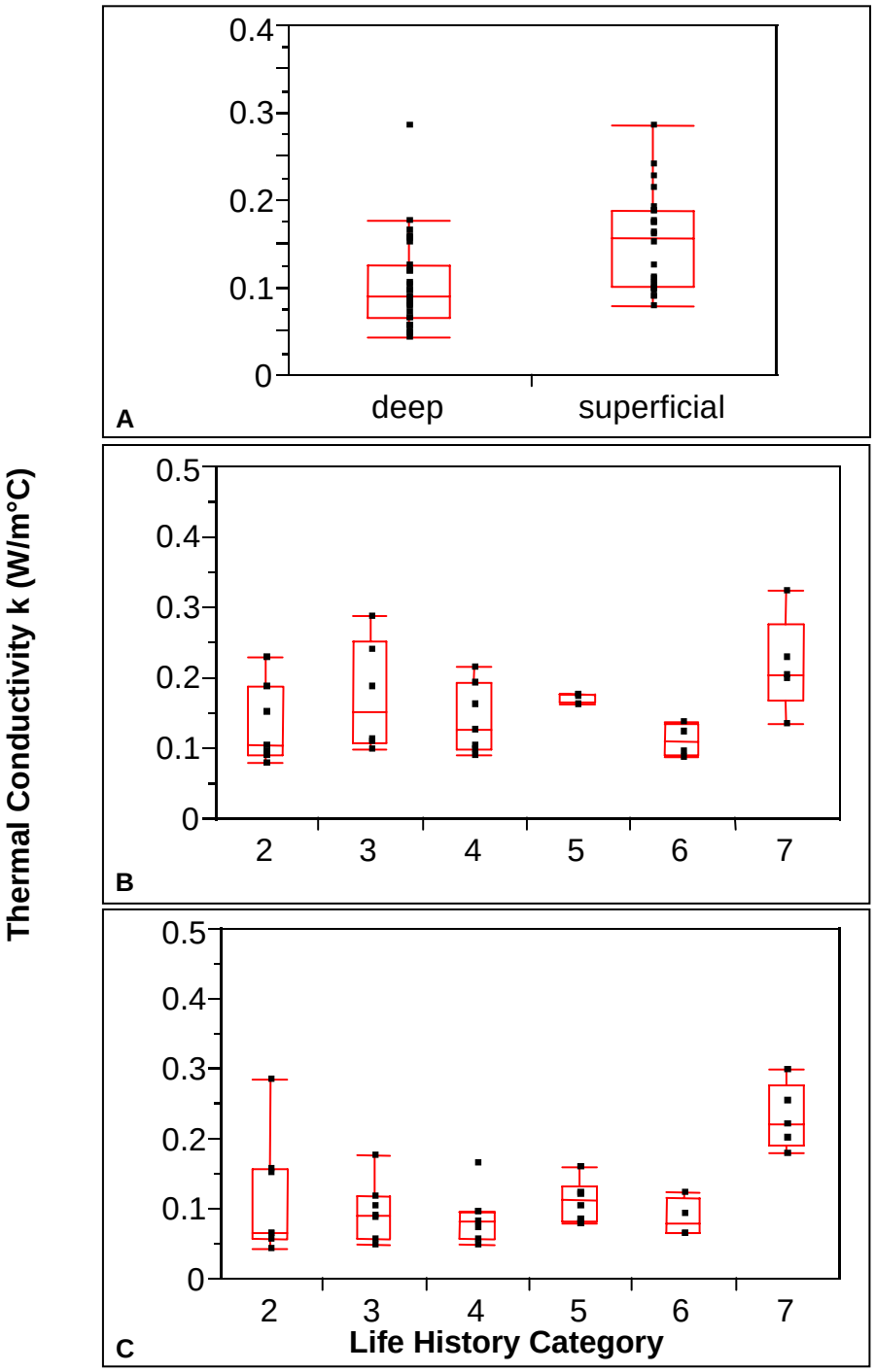

Figure 6 – (A) Overall mean conductivity difference between deep and superficial blubber layers for combined neonatal through adult life history categories in *T. truncatus*. B and C are superficial and deep blubber conductivity values respectively, across life history categories. Life history categories are (2) neonate, (3) juvenile, (4) sub-adult, (5) adult, (6) pregnant female, (7) emaciated animals. Fetuses were excluded because of their thin blubber layer. Box plots represent the upper and lower quartiles for each life history category.

The mean thermal insulation (inverse of conductance) between life history categories varied significantly ($F=12.66$; $p<0.001$). Insulation increased from fetal through sub-adult categories but declined in adult animals. Pregnant females had a significantly higher mean insulation value compared to fetuses, neonates, adults, and emaciated adults. Emaciated animals had significantly less insulation than juveniles, sub-adults, and pregnant females (Table 4).

Differences in Heat Flux Values Across Blubber Thickness

For blubber samples, there was a substantial difference between heat flux values recorded by the deep and superficial heat flux discs. The deep disc consistently recorded higher values (mean of difference = 46.8 W/m^2; range = 9.9 to 87.2 W/m^2) than the superficial disc, and, thus, yielded thermal conductivity values that were higher than those reported for the other two methods (Figure 4). This result is in contrast to that for the control materials, polystyrene foam and white pine wood, where deep and superficial heat flux values were similar to each other. For the foam, the mean difference between the superficial and deep discs was 3.45 W/m^2 (range = 3.5 to 6.2 W/m^2) and for the wood, the mean was 10.25 W/m^2 (range = 5.3 to 14.7 W/m^2).

In blubber, sample thickness was significantly correlated with the difference in heat flux between the deep and superficial discs ($F=11.91$; $p=0.0014$) (Figure 7). To determine if this difference was due simply to heat loss to the sides of the blubber sample, experiments with increasing layers of polystyrene foam and wood were performed. For these control materials, there was no pattern of increased heat loss with increased material depth (foam: $F=0.85$; $p=0.42$; wood: $F=4.26$; $p=0.28$) (Figure 7).

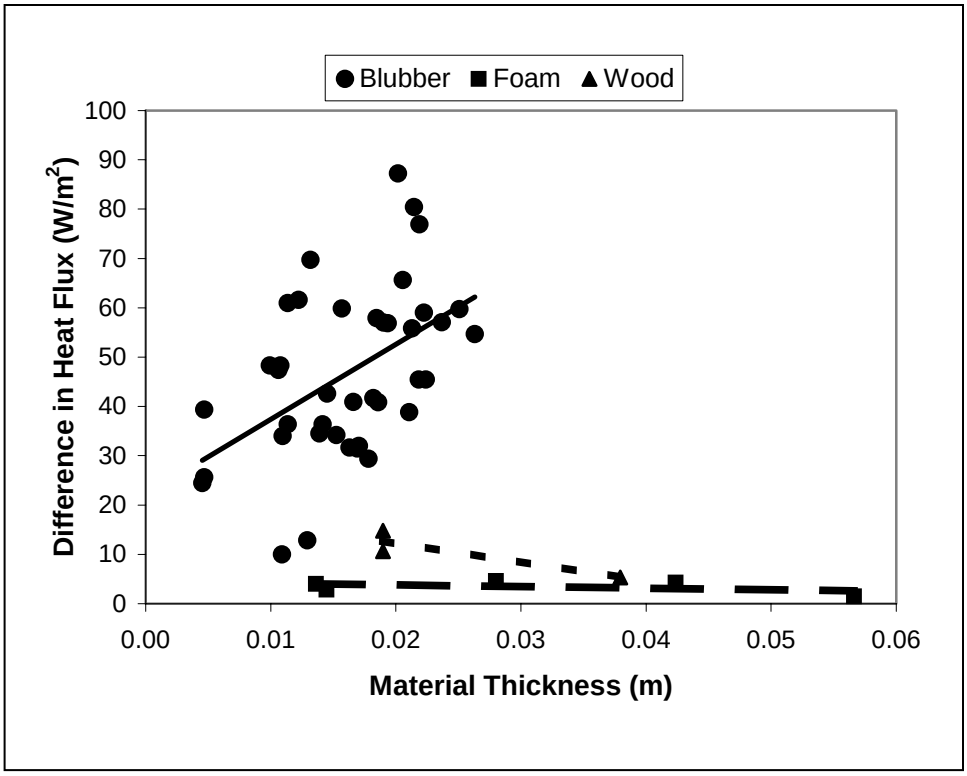

Figure 7 – The difference between the deep and superficial heat flux disc measurements plotted against the material thickness for *T. truncatus* blubber, foam, and wood.

There was a weak, non-significant relationship between the surface area of the blubber sample and the heat flux difference ($F=3.54$; $p=0.067$). There was no relationship between the surface area of the foam sample and the difference in heat flux ($F=5.8$; $p=0.137$). Thus, in blubber, there existed a substantial difference between the energy entering the deep surface of the blubber and that leaving the sample at its superficial surface. There was no relationship between the magnitude of this difference in heat flux and life history category ($F=1.74$; $p=0.14$), lipid content ($F=1.49$; $p=0.23$), or water content ($F=0.06$; $p=0.81$).

DISCUSSION

The goals of this study were to measure the thermal properties of blubber across an ontogenetic series of bottlenose dolphins and to correlate these properties with changes in blubber morphology and composition that vary across development. Specifically, it was hypothesized that neonatal dolphins may have blubber that is specialized to provide enhanced insulation compared to other life history categories. To permit comparison of these results with previously reported values, two distinct methods were used to measure blubber's thermal properties.

Blubber's Quality, Quantity, and Thermal Properties

Fetal blubber underwent continuous growth throughout gestation, with both thickness and lipid content increasing rapidly. This growth pattern, similar to that observed by Struntz et al.(2004), prepares the animal for birth into a highly conductive fluid medium. In contrast with pinnipeds, which are born on land, cetaceans must be fully capable of maintaining thermal homeostasis in water at the time of birth. Thus,

blubber, their primary thermal barrier, must be of an appropriate thickness and quality to minimize heat loss.

Figure 8 illustrates how blubber's thickness, lipid content, conductivity, and insulation values varied across life history categories. Between fetal and juvenile life history categories, both lipid content and blubber thickness increased. Blubber's thermal conductivity, which is independent of thickness, remained stable between these life history categories. In contrast, thermal insulation, a measure of both blubber quality and quantity, increased three-fold. It was hypothesized that thickness and/or lipid content would be relatively greater in neonates compared with other life history categories. The results of this study indicate that instead, fetal, neonatal, and juvenile life history categories represented a period of continual blubber growth - blubber's thermal conductivity remains static but its thermal insulation increases as a result of increased blubber quantity.

The juvenile life history category represented a transitional period in blubber's development in which lipid content peaked and blubber thickness values were similar to those of adults. Between juvenile and adult life history categories, blubber lipid content decreased steadily and adult blubber had a significantly higher conductivity than all non-emaciated categories. The insulation of adult blubber was also significantly less than that of sub-adults, due to a decrease in blubber quality, rather than quantity. Thus, two distinct patterns describe ontogenetic changes in the thermal properties of blubber. Nutritionally dependent animals increased blubber *quantity* but maintained similar blubber *quality*. Nutritionally independent animals maintained relatively stable blubber *quantity*, and rather, varied the *quality* of the blubber layer.

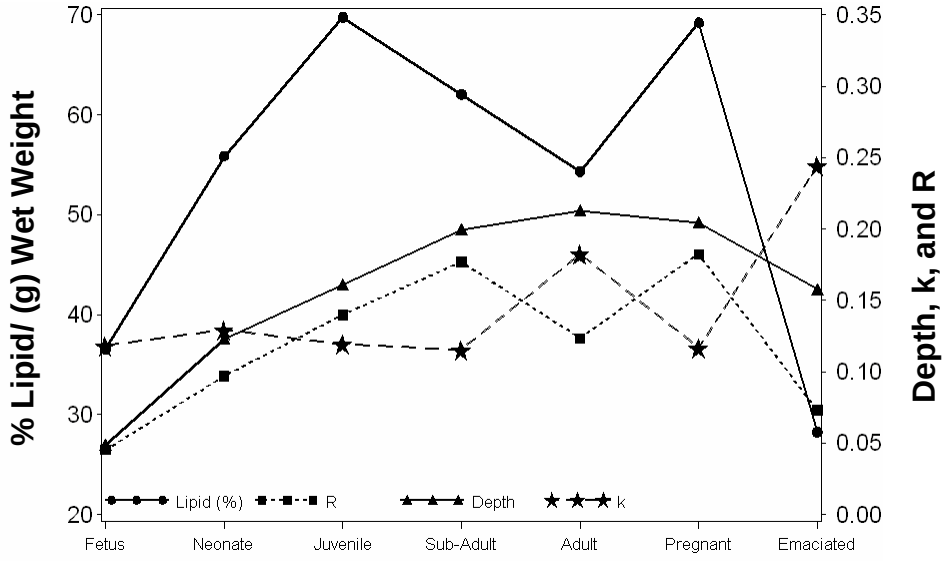

Figure 8 - Blubber thickness, lipid content, thermal conductivity, and insulation values for *T. truncatus* plotted against life history category. Thickness is in decimeters; all other values are in units shown with Table 4.

Interestingly, the blubber of neonatal and juvenile animals had the same insulation value as that of adult dolphins. This result suggests that the mass specific metabolic rates of these young animals must be higher than that of adult dolphins to compensate for the relatively higher rates of heat loss resulting from their larger surface area to volume ratios. Mass specific metabolic rates scale to body mass raised to the –0.25 (Kleiber, 1961) and young animals are known to have relatively higher mass specific metabolic rates compared to adult animals of the same species (reviewed in Lavigne et al., 1986).

To estimate the relative metabolic rates of neonatal and adult dolphins in this study, the heat flux value (from the superficial disc) for each dolphin was multiplied by the surface area of the dolphin (see Table 5 for methods of estimating surface area). The resulting metabolic rate was then divided by the mass of the animal to obtain a mass specific metabolic rate. The results of these calculations indicate that the mass specific metabolic rate of neonatal bottlenose dolphins is approximately three times higher than that of adult dolphins. This result is consistent with experimentally derived values for newborn harbor seals (*Phoca vitulina*) (Miller and Irving, 1975), and pups of California sea lions (Thompson et al., 1987), northern fur seals (Callorhinus ursinus) (Donohue et al., 2000), and northern elephant seals (*Mirounga angustriostris*) (Noren, 2002), which had mass specific metabolic rates that ranged between 1.4 and 4 times higher than that of adults. Additionally, the estimated mass specific metabolic rate for neonates in this study was 5 % lower, and the adult value 38% lower, than those values predicted by Kleiber (1961). The calculated adult metabolic rate is also considerably lower than the resting metabolic rate for this species measured by Williams et al. (2001) (0.392 ± 0.01 LO_2/hr·kg).

Table 5: Calculated mass specific metabolic rates for neonatal and adult bottlenose dolphins. Values are presented as the mean for each life history category ± standard error.

	Neonates	**Adults**
Body Mass (kg)	18.23 ± 11.4	194.35 ± 13.92
Insulation (R) (m^2°C/W)	0.10 ± 0.01	0.12 ± 0.01
Surface Area (m^2)*	0.308 ± 0.13	1.759 ± 0.16
Surface Area/ Volume	27.48 ± 1.27	12.48 ± 1.92
Mass Specific Metabolic Rate (LO_2/hr·kg)	0.301 ± 0.017	0.111± 0.02
Predicted Kleiber Mass Specific Metabolic Rate (LO_2/hr·kg)	0.331 ± 0.01	0.182 ± 0.005

* Surface area and volume were estimated by modeling the trunk (nuchal crest to anus) as a cylinder and the tailstock (anus to fluke insertion) as a truncated cone. The head and appendages were excluded from these calculations.

There are multiple reasons why the mass specific metabolic rates measured in this study may be low. First, only the post-cranial body surface area was calculated - the appendages and head were excluded. Second, the calculated metabolic rates in this study used heat flux values that were measured in air and on inert tissue in the absence of blood flow. They do, however, illustrate the relative differences in the cost of endothermy in animals of varying body size as well as represent an estimated minimum metabolic rate for bottlenose dolphins.

The thermal properties of blubber were also influenced by morphological and compositional differences associated with changing reproductive and nutritional status. Pregnant female blubber had a lipid content that was similar to juveniles, and blubber thickness that was similar to adults, which resulted in an overall insulation value that was higher than that of adults. Sub-adult and pregnant female blubber layers have the highest insulation values of any life history category, suggesting that these categories may represent a maximal insulation value for bottlenose dolphins.

Emaciation profoundly impacted blubber's thermal properties. The insulation value of emaciated blubber was substantially lower than that of adults. With a low blubber insulation value, emaciated animals likely experience relatively higher rates of heat loss to the environment compared to non-emaciated adults, and their metabolic rates may, thus, be higher. In the emaciated state, blubber's dual roles of providing insulation and storing metabolic energy are in direct opposition to one another. As lipid is depleted for utilization as energy, the thermal insulation of the blubber layer is compromised and, therefore, the rate of heat loss to the environment is increased. The metabolic rate of the animal may increase to compensate for the increased heat loss and in turn, more lipid is

depleted to meet the increased demand. In this way, the potential for a positive feed-back loop exists and declining nutritional and health status is potentially accelerated by the opposing thermal and metabolic demands on the blubber.

Across life history categories, blubber thickness was generally not a good predictor of lipid content. For a given blubber thickness, lipid content could vary by more than 50%. In an extreme example, emaciated individuals, WAM 533 and WAM 591, had similar blubber thicknesses, however, their blubber lipid content was 41% and 3.3% respectively. The highly organized three-dimensional matrix of structural fibers in blubber is vital to its role as a structural and locomotor tissue (Hamilton et al., *in press;* Pabst et al., 1999a). Reducing the thickness of this tissue may be detrimental to blubber's other functions such as streamlining the body or aiding in locomotion. Interestingly, in the life history categories where lipid content was highly reduced (fetuses and emaciated animals), water content was significantly higher. The replacement of lipid with water may be a potential mechanism for maintaining the structural integrity of the tissue despite fluctuations in lipid content.

In addition to ontogenetic differences in blubber's thermal properties, there were also differences in thermal conductivity across the depth of the blubber layer. The conductivity of deep blubber was consistently lower than that of superficial blubber. Deep blubber is more metabolically labile (e.g. Ackman et al., 1975a; Ackman et al., 1975b; Aguilar and Borrell, 1990; Koopman, 1998; Koopman et al., 1996; Lockyer et al., 1984) and undergoes larger morphological changes during development and emaciation than does superficial blubber (Koopman et al., 1996; Struntz et al., 2004). Superficial blubber contains smaller adipocytes and more densely packed structural fibers (Hamilton

et al., *in press;* Struntz et al., 2004). Thus, these results support the observation that blubber is not a homogenous material throughout its depth and that the superficial and deep blubber may differ in their functional roles. Deep blubber is more metabolically active and contributes more to insulation than does superficial blubber. Superficial blubber has a more structural role and is a better thermal conductor than the deep blubber.

Phylogenetic and Methodological Comparisons of Blubber's Thermal Properties

The ontogenetic changes in blubber's thermal properties observed in this study are nearly as great as those observed across a broad range of cetaceans (Table 1). For all non-adult bottlenose dolphins, blubber thermal conductivity values were similar to that of harbor porpoise and beluga whale (*Delphinapterus leucus*) blubber. Thus, relatively small bodied, young dolphins possessed blubber of the same thermal quality as northern temperate to polar species. In contrast, conductivity of adult bottlenose dolphin blubber was more similar to that of large baleen whales and tropical delphinids (Table 1). However, direct comparisons of absolute thermal conductivity values may be complicated by differences in experimental methods. The thermal conductivity values calculated using the standard material method and the heat flux values from the superficial disc, yielded values that were very similar. However, conductivity values calculated using heat flux values from the deep disc were more than 50% higher. In most previous studies that have used the heat flux method, the disc has been placed deep to the blubber (Worthy, 1991; Worthy and Edwards, 1990; Yasui and Gaskin, 1986). Thus, both absolute and relative comparisons between values obtained with deep heat flux measurements and those obtained by either the standard material method or superficial

heat flux, must be made with caution. A discussion of the potential explanation for this pattern is presented here.

The observed difference in the rate of energy entering and leaving the blubber surfaces may be attributable to several factors. First, heat loss to the sides of the blubber could cause a reduction in heat flux measured at the blubber surface. The results of calibrations with foam and wood, however, suggest that changing the thickness of the sample did not affect the difference between superficial and deep heat flux values. Second, the reactive error (Ducharme et al., 1990) of the superficial heat flux disc may reduce the heat flux value at this surface. However, as discussed previously, this error is expected to be low because the ratio between the insulative quality of the tissue and disc is low ($R_{blubber}/R_{heat\ flux\ disc}$ ranged between 6.02 and 21.7) (Frim and Ducharme, 1993). The maximum reactive error can be calculated using the correction factors provided by Frim and Ducharme (1993) and a maximum heat flux value (in this study maximum heat flux = 142.7 W/m^2). The maximum error attributable to reactive error in this study was calculated as 8 W/m^2. Because the difference between the deep and superficial heat flux measurements could be as high as 87.2 W/m^2 and was usually near 50 W/m^2, it is unlikely that the observed difference in heat flux values is the result of this source of experimental error. Instead, the difference in heat flux may be indicative of a previously undescribed property of blubber – its capacity to absorb heat by undergoing a phase change.

Phase change materials (PCM) are defined as latent thermal storage materials that use chemical bonds to store and release heat (Suppes et al., 2003). These materials are currently being investigated for use in residential and commercial buildings as a means of

increasing energy efficiency (Nikolic et al., 2002; Sari, 2003; Sari and Kaygusuz, 2001; Sari et al., 2003; Suppes et al., 2003). For example, solar heat may be absorbed during the day, by causing the material to melt or soften. When temperatures drop at night, the material will undergo a phase change to a solid and release the stored energy. For a phase change material to efficiently store and release heat, four requirements must be met (Nikolic et al., 2002; Sari, 2003; Sari and Kaygusuz, 2001; Sari et al., 2003; Suppes et al., 2003) First, the melting point of the material must be in an appropriate temperature range for the desired application (e.g. near room temperature for building materials). Second, the material must have a relatively large latent heat plateau (i.e. the range of temperatures over which a material will change phase), to maximize the amount of heat that may be stored. Third, the material must not stratify in the liquid phase, which would result in an inability to properly harden when the environmental temperature is reduced. Finally, an intermittent heat load must be present to deliver and absorb heat from the material.

There is substantial evidence to support the classification of blubber as a phase change material. First, many of the fatty acids found in blubber are classified as phase change materials and have melting points in the range of mammalian body temperatures (Sari, 2003; Sari and Kaygusuz, 2001; Sari et al., 2003; Suppes et al., 2003). Suppes et al. (2003) classified palmitic (C16:0), steric (18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), and arachidic (C20:0) fatty acids as excellent phase change materials. All of these fatty acids have been identified in cetacean blubber (Koopman et al., 1996). Mixtures of these fatty acids yield phase change materials with melting points between 29° and 38°C (Suppes et al., 2003), which include the range of mammalian body temperatures. Second, these fatty acids also satisfy the requirement that the material have

a relatively large latent heat plateau, with latent heat values generally greater than 180 J/g (Suppes et al., 2003). Third, their stratification in blubber may be prevented by their containment in adipocytes as well as the highly structured nature of adipocytes in the blubber tissue. Finally, cetaceans are known to have fine vascular control to their appendages and to the periphery of their body (Elsner et al., 1974; Kvadshiem and Folkow, 1997; Ling, 1974; Meagher et al., 2002; Pabst et al., 1999b; Scholander and Schevill, 1955). Intermittent heat loads could be applied to the blubber through shunting of warm blood to the blubber layer, followed by periods of vasoconstriction. Future studies are needed to fully characterize blubber's potential phase change properties as well as investigate the possible functions that may be associated with such a property.

Conclusion

Blubber's thermal properties were influenced by morphological and compositional changes that occurred across ontogeny, and in individuals of differing reproductive and nutritional status. In nutritionally dependant life history categories, changes in blubber's thermal properties were characterized by stable blubber quality and increased blubber quantity. In nutritionally independent animals, blubber quantity remained stable while blubber quality varied. The ontogenetic differences in thermal conductivity and thermal insulation were as large as those reported across temperate to tropical cetacean species. Differences in conductivity also occurred through the depth of the blubber. Deep blubber had a lower thermal conductivity than superficial blubber supporting the characterization of the deep blubber as the more insulative and metabolically active site of lipid deposition and mobilization. Finally, blubber's composition and its ability to absorb heat suggest that it likely is a phase change material.

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CHAPTER 2. ONTOGENETIC CHANGES IN THE BUOYANCY PROPERTIES OF ATLANTIC BOTTLENOSE DOLPHIN (*TURSIOPS TRUNCATUS*) BLUBBER

INTRODUCTION

Atlantic bottlenose dolphins (*Tursiops truncatus*) possess many adaptations for a fully aquatic lifestyle including a streamlined body shape, axial locomotor style, enhanced breath-hold capabilities, and a specialized integumental layer called blubber (reviewed in Costa and Williams, 1999; Ling, 1974; McLellan et al., 2002; Pabst et al., 1999b). Blubber, the hypertrophied hypodermal layer, is composed primarily of adipocytes and structural fibers (Ackman et al., 1975; Hamilton et al., *in press;* Koopman, 1998; Ling, 1974; Lockyer et al., 1984; Pabst et al., 1999a; Parry, 1949). It is a multifunctional tissue that acts to store metabolic energy, streamline the body, insulate the body core, and contribute to positive buoyancy (e.g. Hamilton et al., *in press;* Kipps et al., 2002; Koopman, 1998; Ling, 1974; Parry, 1949; Worthy and Edwards, 1990). For a neonatal bottlenose dolphin these last two functions may be particularly critical. A neonate has a larger surface area to volume ratio than an adult, and, thus, may experience high rates of heat loss to the environment (McLellan et al., 2002; Struntz et al., 2004; Worthy and Edwards, 1990). Additionally, neonates have immature locomotor capabilities and axial locomotor muscles that are not poised for high aerobic output (Dearolf et al., 2000; Noren et al., 2001). Thus, unlike an adult, a neonate may be more reliant on hydrostatic (i.e. positive buoyancy) rather than hydrodynamic mechanisms to maintain its position at the water's surface to breath (Taylor, 1994).

Blubber's contributions to insulation and positive buoyancy are reliant on its quantity and quality, both of which can vary significantly across ontogeny in bottlenose dolphins. Struntz et al. (2004) demonstrated that blubber thickness, a measure of blubber quantity, more than doubled between neonatal and adult animals and that lipid content, or

blubber quality, varied by as much as 37% between life history categories. These changes in quantity and quality resulted in ontogenetic differences in blubber's thermal properties that were as great as those reported across a phylogenetically diverse sample of cetaceans (Dunkin, 2004). For example, the blubber of young bottlenose dolphins had thermal conductivity values that were similar to that of harbor porpoises (*Phocoena phocoena*), while the thermal conductivity of adult blubber was significantly higher, and similar to that of minke (*Balaenoptera acutorostrata*) and fin (*Balaenoptera physalus*) whales (Dunkin, 2004).

Reproductive and health status also influence blubber's quality and quantity and, thus, influence blubber's thermal properties. For example, the blubber of pregnant bottlenose dolphins contained 27% more lipid, and had a significantly lower thermal conductivity than blubber of other adults (Dunkin, 2004). In contrast, the blubber of emaciated dolphins contained 48% less lipid, and its conductivity was significantly greater than adults. Variation in blubber's quality and quantity may also significantly influence it's buoyant properties, however, to date, no study has investigated blubber's contribution to buoyancy across an ontogenetic series or between individuals of different reproductive and nutritional status.

Buoyancy, a force exerted on an object by the fluid surrounding it, can be calculated with the following equation:

(I) $$B = (\rho_f - \rho_0)V_0 g$$

where B is the buoyant force (N), ρ_f is the density of the fluid (kg/m^3), ρ_0 is the density of the object, V_0 is the volume of the object (m^3), and g is acceleration due to gravity (m/s^2) (reviewed in Denny, 1993). Whether an object will be negatively, neutrally, or positively

buoyant is, thus, directly related to its density relative to that of the surrounding fluid. A dolphin's overall density is dependant upon its body composition. While many tissues (e.g. muscle and bone) are more dense than seawater (1,026 kg/m^3), air (1.3 kg/m^3) in the lungs, and lipid (~900 kg/m^3) in blubber, are less dense (reviewed in Kipps et al., 2002).

Taylor (1994) identified two alternative buoyancy control strategies for marine tetrapods. A tetrapod can control its position in the water column hydrostatically, by adjusting the relative volume of low and high density body compartments. Alternatively, it can hydrodynamically adjust its depth by using locomotor energy (Taylor, 1994). Although an individual can use both strategies, one may dominate over another depending on habitat preference, locomotor style, or preferred diving depth. An animal that is a fast swimmer or deep diver is more likely to adjust its position in the water column hydrodynamically, while an animal that is a bottom dwelling or shallow diver is more likely to use hydrostatic control (e.g. Kipps et al., 2002; Taylor, 1994; Williams, 1999; Williams et al., 2000; Williams et al., 1999).

An adult bottlenose dolphin is a fast swimmer and a fairly deep diver and would, thus, be expected to control its buoyancy hydrodynamically (Ridgway, 1971; Skrovan et al., 1999; Taylor, 1994; Wells and Scott, 1999; Williams et al., 1999). A neonatal dolphin however, has both immature locomotor capabilities and decreased aerobic stamina (Dearolf et al., 2000; Noren et al., 2001) but like adults, must be able to reach the surface to breath. Thus, neonatal dolphins may be more reliant than adults on hydrostatic buoyancy control. Specifically, neonates may need to be positively buoyant to remain at the water's surface, without expending locomotor energy. To achieve positive buoyancy,

the overall body density of neonatal dolphins must be relatively less than that of adult animals.

Cockcroft and Ross (1990) qualitatively described changes in buoyancy in a captive bottlenose dolphin calf in its first two years of life. The calf was observed to be positively buoyant at birth and unable to fully control its position in the water column until approximately six months of age. These authors attributed these apparent changes in buoyancy to changes in body composition. Until bottlenose dolphins reached a mass of 22kg, at approximately four to five weeks of age, blubber mass was greater than muscle mass (Cockroft and Ross, 1990). Although no direct measure of buoyancy was made, and no discussion of the role of the developing lung was presented, these observations suggested that in these young animals, blubber was contributing relatively more to overall positive buoyancy than in adult animals (Cockroft and Ross, 1990). In contrast, Struntz et al. (2004) found no significant difference in the percent of total body mass invested in blubber across life history categories in Atlantic bottlenose dolphins.

The goal of this study was to measure blubber's buoyant force across an ontogenetic series of bottlenose dolphins. Specifically, the hypothesis that neonatal blubber contributes relatively more to positive buoyancy than in other life history categories was tested. To calculate blubber's buoyant force, its density was measured and its volume was calculated for fetal, neonatal, juvenile, sub-adult, and adult bottlenose dolphins. In addition, pregnant females and emaciated adults were investigated to determine how blubber's contribution to buoyancy may change with reproductive and nutritional status. The lipid and water content of blubber (Dunkin, 2004), were also

measured across these life history categories to correlate measures of blubber quality with buoyant force.

METHODS

Specimens

Blubber samples were acquired from 40 robust Atlantic bottlenose dolphins (*Tursiops truncatus*) and three emaciated dolphins that either stranded or were incidentally killed in fishing operations in North Carolina and Virginia (Table 1). The data set also included one emaciated adult from Florida and one from New Jersey. The thermal properties of the blubber of these individuals was examined by Dunkin (2004). Only animals with a Smithsonian Institution Code of 1 (live stranded and died naturally or by euthanasia) or 2 (fresh dead) (Geraci and Lounsbury, 1993) were used in this study. Seven life history categories were defined based upon a suite of morphological characters described in Struntz et al. (2004) (Table 2). These categories include fetus (n=7), neonate (n=8), juvenile (n=7), sub-adult (n=8), adult (n=6), pregnant female (n=4), and emaciated animal (n=5).

Each animal was first weighed to the nearest kilogram (Dillon, 2000kg capacity scale, Brooklyn, NY, USA) and measured using a standard set of morphometrics. The animal was divided into two post-cranial regions, the trunk (defined here from the leading edge of the pectoral flippers to the anus) and tailstock (from the anus to the fluke insertion), and the length of each of these regions was measured (Figure 1a). Previous studies have suggested that the blubber in these two regions is functionally specialized. Trunk blubber is more metabolically labile and tailstock blubber is more structural in function (Hamilton et al., *in press;* Koopman, 1998; Koopman et al., 1996; Pabst, 1990; Pabst et al., 1999a; Pabst et al., 1999b). At each body region, the integument was

Table 1: *T. truncatus* specimens grouped by life history category and length.

Animal	Life History Category	Sex	Body Length (cm)
PTM 109f*	fetus	F	58
WAM560f*	fetus	F	63.5
PTM114f	fetus	F	82
ASF 033f*	fetus	M	82
WJW007f*	fetus	M	86.5
WAM 545f*	fetus	F	92
WAM 535f*	fetus	M	96.5
EMM 010*	neonate	M	106
VMSM2000 1020*	neonate	M	106.5
CALO99-13*	neonate	F	109.5
VMSM2001 1080*	neonate	M	110.3
VMSM2002 1042[y]	neonate	M	111
MMB 003[xy]	neonate	M	113
WAM 550*	neonate	M	114.5
WAM 584[y]	neonate	F	117
VMSM2000 1031*	juvenile	M	127
DJS 001*	juvenile	F	129.5
VMSM2001 1087*	juvenile	M	129.7
VMSM 2003 1082	juvenile	M	142
VMSM2002 1089	juvenile	F	142.5
ASF 042	juvenile	F	143.5
WAM 569*	juvenile	F	150
DAP 034	sub-adult	M	171
SAE 003	sub-adult	M	173
PTM 117	sub-adult	M	189
WAM 585	sub-adult	F	192
EMM 006	sub-adult	M	197
KMT 013	sub-adult	F	204
VMSM2000 1049*	sub-adult	M	207
WAM 553*	sub-adult	M	232
WAM 574*‡	adult	F	223
MMB 002	adult	M	235
WAM 579	adult	M	237
WAM 559*	adult	F	237
SDZ 005	adult	M	262
REL 014	adult	M	265
WAM 545*	pregnant female	F	246
ASF 041*	pregnant female	F	260
ASF 038*	pregnant female	F	263
WJW 007*	pregnant female	F	273
WAM 591	emaciated	M	222.8
WAM 533*	emaciated	F	261
NJ00-111*	emaciated	F	262
MMB 004[x]	emaciated	M	275
MMSN FB 192	emaciated	M	278

* Animals investigated in Struntz et al. (2004). ‡WAM 574 was a fisheries-take animal and was missing her flukes, resulting in a reduced total length. ([x]) Animals excluded from lipid analysis. ([y]) Animals sub-sampled for regional density analysis.

Table 2: Defining life history characters based on Struntz et al. (2004).

Life History Category	Code	Defining Characters
Fetus	**1**	Position *en utero.*
Neonate	**2**	Possessed four of the following six characters: presence of rostral hairs, floppy or folded dorsal fin, unhealed umbilicus, prominent fetal folds, floppy or folded dorsal fin or floppy or folded dorsal keel.
Juvenile	**3**	Absence of neonatal characters and estimated to be less than 1 year of age based on total length (≤150 cm).
Sub-adult	**4**	Absence of milk in the stomach, immature reproductive tissues, and total length (>150 cm).
Adult	**5**	Mature reproductive tissues as indicated by obvious ovarian scars in females and the size of the testis and presence of sperm in males.
Pregnant female	**6**	Presence of a fetus in the uterus.
Emaciated animal	**7**	Skeletal elements such as ribs, scapula, transverse processes, or skull were prominent under the blubber layer or there was atrophy of the epaxial musculature and/or the nuchal fat pad.

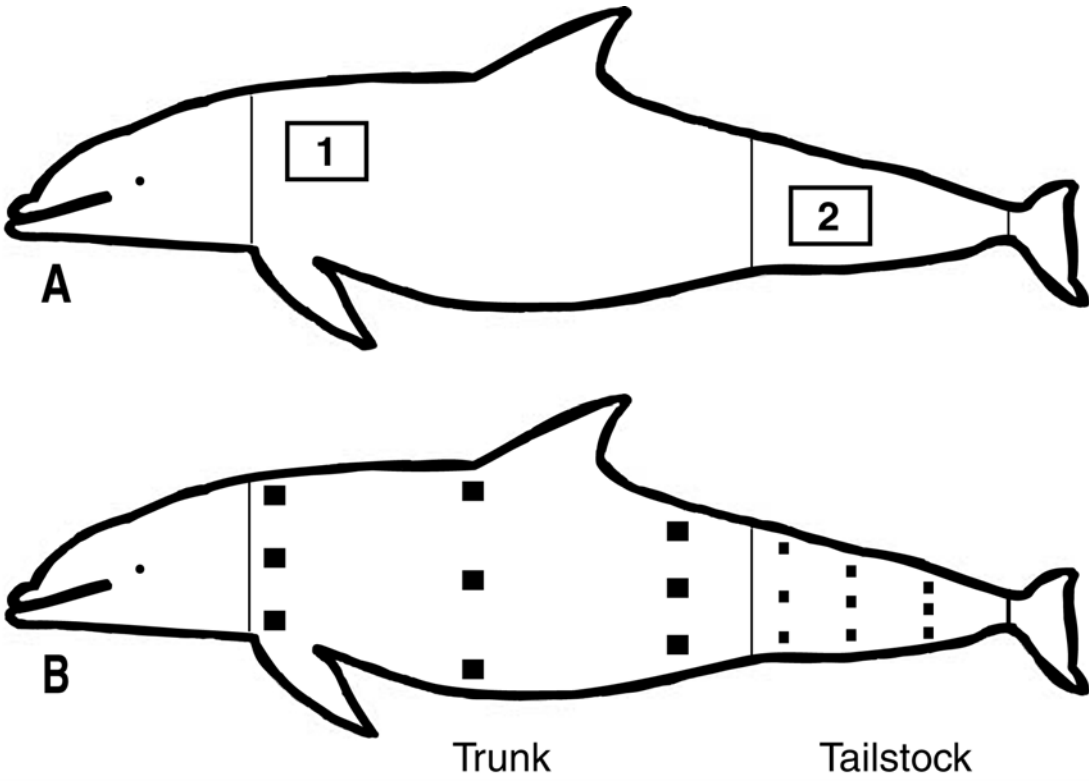

Figure 1 – (A) The sample locations for the trunk (1) and the tailstock (2) blubber from *T. truncatus*. (B) The sites for the intra-regional density measurements.

dissected cleanly from the underlying subdermal connective sheath (Pabst, 1990) and weighed separately to the nearest 0.1g (Ohaus I10, Bradford, MA, USA). Integument mass, as a percentage of total body mass, was calculated from these measurements and recorded. The integument of the head and appendages was excluded from this analysis.

Trunk blubber (n = 45) was sampled at a dorso-lateral position, just caudal to the pectoral flipper, and tailstock blubber (n = 20) was sampled at a lateral position just caudal to the anus (Figure 1a). Samples were between 5x5cm and 15x15cm and included the entire integumental layer (subsequently referred to as blubber). Blubber samples were then either vacuum sealed (Koch, 1700, Kansas City, MO, USA) or wrapped in Saran wrap® and sealed in freezer bags to prevent desiccation. Samples were stored at -20°C until analyzed.

Blubber Lipid and Water Content

Lipid content of trunk blubber was determined using procedures similar to those of Struntz et al. (2004). Briefly, an approximately 1g full depth blubber sample (excluding the epidermis) was weighed to the nearest 0.001g , macerated, and dried with approximately 30g of sodium sulfate (Na_2SO_4). The lipid was then extracted using an accelerated solvent extractor (Dionex, Salt Lake City, UT, USA). The excess solvent was evaporated (Turbo Vap II, Zymark, Hopkinton, MA) and the extracted lipid was then reweighed to the nearest 0.001g.

For both trunk and tailstock blubber samples, water content was determined by excising an approximately 1x1cm square sample and weighing it prior to and after freeze-drying (Labconco 4.5, Kansas City, MO, USA). Samples were weighed each day until

the mass of the sample was stable (within 0.001g) for two consecutive days (total time = 5 days).

Blubber Density and Volume Measurements

To calculate blubber's buoyant force, it was necessary to determine both its density and volume. Density was measured volumetrically using the methods of Kipps et al. (2002). Briefly, three approximately 1x1cm full depth subsamples were taken from each of the trunk and tailstock blubber samples. Each subsample was weighed to the nearest 0.001g (Satorius, PT-6, Bradford, MA, USA) and then placed in room temperature distilled water, in a 25mL graduated cylinder. Its volume (to the nearest 0.1ml) was measured by displacement. The density of each subsample was calculated by dividing its mass by its volume, and the mean of the three measurements was reported as the density for that blubber sample (standard error of measurements was ± 5.8%).

To ensure that the density measurements of the subsamples were representative of the entire trunk or tailstock blubber, additional density measurements were performed. On a subset of animals (total n = 3, see Table 1) across several life history categories, blubber density was measured at nine additional positions within the trunk and tailstock regions (Figure 1b).

Total blubber volume was calculated by dividing the blubber mass from each region (trunk and tailstock) by the mean blubber density at each region. For non-pregnant, non-emaciated animals, trunk blubber mass (n = 16) or tailstock blubber mass (n = 1) was unavailable. For these animals an interpolated blubber mass was determined based on a linear regression of total body length and blubber mass of all animals for which mass was available (trunk n=19, r^2=0.972; $p<0.0001$; tailstock n=20, r^2=0.969;

$p<0.0001$). Pregnant and emaciated animals were not included in this analysis because the sample size in these groups was not large enough to permit interpolation of blubber mass. The buoyant force of the trunk and tailstock blubber was calculated separately using Equation I, and these values then summed to obtain the total blubber buoyant force.

Statistics

For comparisons across life history category, a one-way ANOVA (JMP 5.1, SAS Inc., Cary, NC, USA) (alpha = 0.05) was performed to determine if there were significant differences in blubber mass, volume, density, or buoyant force. The same analysis was used to evaluate these factors between body sites. When a significant result was present, a Tukey-Kramer Honestly Significant Difference test or a Ryan's Q test was performed to determine which groups were significantly different from one another. For each body region, t-tests were used to determine if the mean blubber density values for each life history category were significantly different than the density of seawater or if the buoyant force of blubber was significantly different from neutral buoyancy. Life history categories with less than three samples for a particular analysis were excluded from the statistical tests.

RESULTS

Lipid and Water Content

The results of the lipid content analysis of trunk blubber from Dunkin (2004) and Struntz et al. (2004) are briefly summarized below. Blubber lipid content increased consistently from fetal through juvenile life history categories (Table 3). Although not a significant trend, across juvenile, sub-adult, and adult life history categories, lipid content

Table 3 – Summary of blubber's density, buoyancy, composition, and contribution to total body mass across life history categories in Atlantic bottlenose dolphins reported as the mean ± standard error

	Fetus	**Neonate**	**Juvenile**	**Sub-adult**	**Adult**	**Pregnant Female**	**Emaciated Adult**
Trunk Blubber Density (kg/m^3)	1037.5 ± 23.4 a	1084.8 ± 47.0 a	1003.6 ± 20.7 a	1033.3 ± 13.4 a	1038.9 ± 24.6 a	977.9 ± 9.1 a	1112.1 ± 49.2 a
Tailstock Blubber Density (kg/m^3)	1068.3 ± 42.1 a	1083.4 ± 73.6 a	1076.7 ± 57.3 a	1077.1 ± 9.3 NE	1087.1 ± 98.2 NE	1026.9 NE	1094.2 ± 27.6 a
Trunk Blubber Volume (dm^3)	0.852 ± 0.37 d	3.516 ± 0.31 c, d	9.338 ± 1.3 c	19.536 ± 1.7 b	34.158 ± 2.7 a	36.136 NE	20.4 ± 4.1 NE
Tailstock Blubber Volume (dm^3)	0.084 ± 0.02 a	0.617 ± 0.04 b	1.113 ± 0.08 c	1.29 ± 0.09 NE	4.69 ± 1.1 NE	NE	3.395 ± 0.8 NE
Trunk Blubber Buoyant Force (N)	-0.397 ± 0.17 a	-1.54 ± 1.44 a	2.56 ± 2.36 a	-2.62 ± 3.00 a	-2.34 ± 7.07 a	8.79 NE	-25.89 ± 19.6 NE
Tailstock Blubber Buoyant Force (N)	-0.0371 ± 0.05 a	-0.351 ± 0.44 a	-0.581 ± 0.58 a	-0.669 ± 0.16 NE	-1.769 ± 3.82 NE	NE	-2.642 ± 2.13 NE
Total Blubber Buoyant Force (N)	-0.268 ± 0.19 a	0.612 ± 2.46 a	1.79 ± 6.54 a	0.925 ± 1.57 NE	14.08 ± 10.50 NE	NE	-28.54 ± 21.73 NE
% Lipid/ Wet Weight (Trunk)	35.06 ± 6.09 b, c	55.82 ± 2.86 a, b	69.72 ± 4.08 a	62.59 ± 2.31 a	54.31 ± 4.88 a, b	69.20 ± 5.73 a	28.22 ± 9.14 c
% Water/ Wet Weight (Trunk)	50.3 ± 3.52 a	33.06 ± 1.97 b	29.9 ± 0.96 b	30.16 ± 2.35 b	32.78 ± 1.08 b	31.66 ± 1.57 b	59.34 ± 6.63 a
% Water/ Wet Weight (Tailstock)	60.3 ± 3.10 a	41.73 ± 2.24 b	37.84 ± 2.37 b	38.73 NE	50.39 ± 6.74 a, b	39.29 ± 0.39 NE	68.24 ± 4.5 a
% Blubber mass/Total Body Mass	16.42 ± 1.91 b	23.05 ± 1.41 a, b	25. 47 ± 1.18 a	22.08 NE	21.36 ± 2.69 a, b	14.5 NE	16.20 ± 1.40 NE

* Only categories with a sample size of 3 or more were tested for statistical differences. Categories not included in the statistical analyses are denoted by an NE (not examined). In categories where n = 2, the mean ± standard error is given. If n = 1, the value for that animal is given.

declined. Blubber lipid content of pregnant females increased 27% compared with adult animals and was similar to that of juveniles. Emaciated animals had significantly less lipid than all other life history categories except fetuses ($F = 9.33$; $p<0.0001$) (Table 3, Figure 2a).

In fetuses and emaciated animals, water content of trunk blubber was significantly higher compared to that of other life history categories ($F = 13.76$; $p<0.0001$) (Table 3, Figure 2b). The water content of tailstock blubber of fetuses and emaciated adults was significantly higher than that of neonates and juveniles ($F = 14.02$; $p<0.0001$) (Figure 2c). Overall, tailstock blubber had a significantly greater percentage of water (mean = 45.53 ± 2.53%) than that of the trunk blubber (mean = 34.83 ± 1.47%) ($F =15.31$; $p = 0.0003$) (Figure 2b,c).

Mass, Density, Volume, and Buoyant Force

Blubber mass as a percentage of total body mass was significantly different between fetuses and juveniles but was similar between all other life history categories ($F = 4.28$; $p = 0.0128$). Blubber contributed between 14.5 and 25.47% of total body mass across life history categories and reached maximal values in juvenile animals (Table 3).

Within each body region, the density of blubber, sub-sampled at multiple body positions, was similar (trunk - $F = 0.32$; $p = 0.94$; tailstock - $F = 1.4$; $p = 0.25$). The overall mean blubber density of the of the trunk was 1043.1 ± 13.18 kg/m^3 and that of the tailstock was 1077.1 ± 24.17 kg/m^3 and these values were not significantly different ($F = 2.2$; $p = 0.14$) (Figure 3).

Across life history categories, blubber density was similar in both the trunk ($F = 1.74$; $p = 0.14$) and tailstock regions ($F = 0.04$; $p = 0.98$) (Table 3, Figure 4a, b).

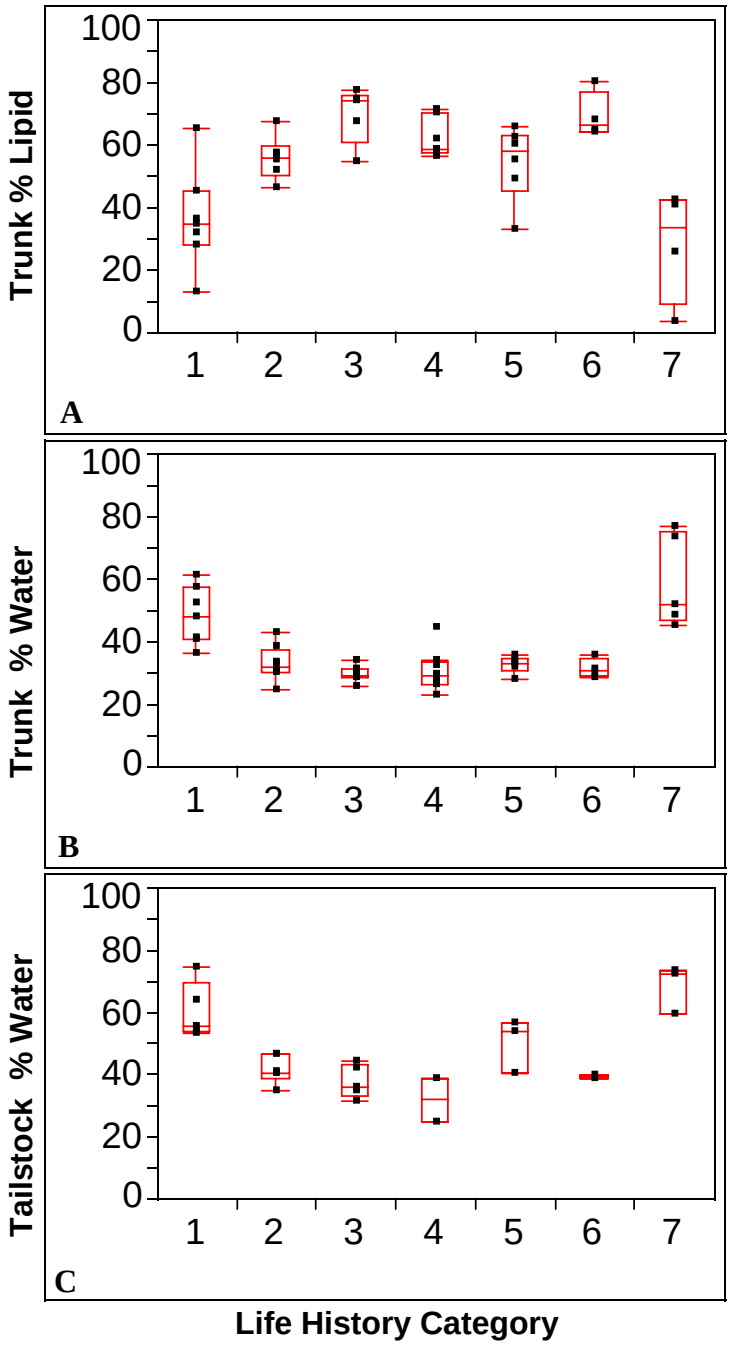

Figure 2– (A) Trunk lipid content and water content of (B) trunk and (C) tailstock. Life history categories are (1) fetus, (2) neonate, (3) juvenile, (4) sub-adult, (5) adult, (6) pregnant female, and (7) emaciated animal.

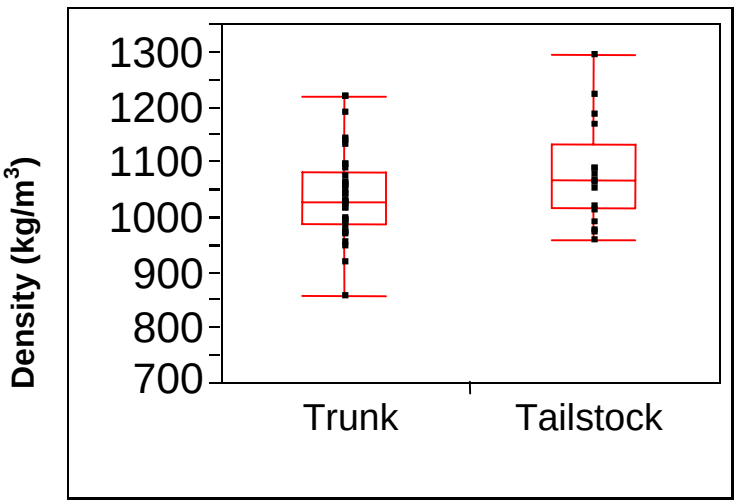

Figure 3 – Comparison of blubber density of the trunk and tailstock. Includes fetal through adult life history categories.

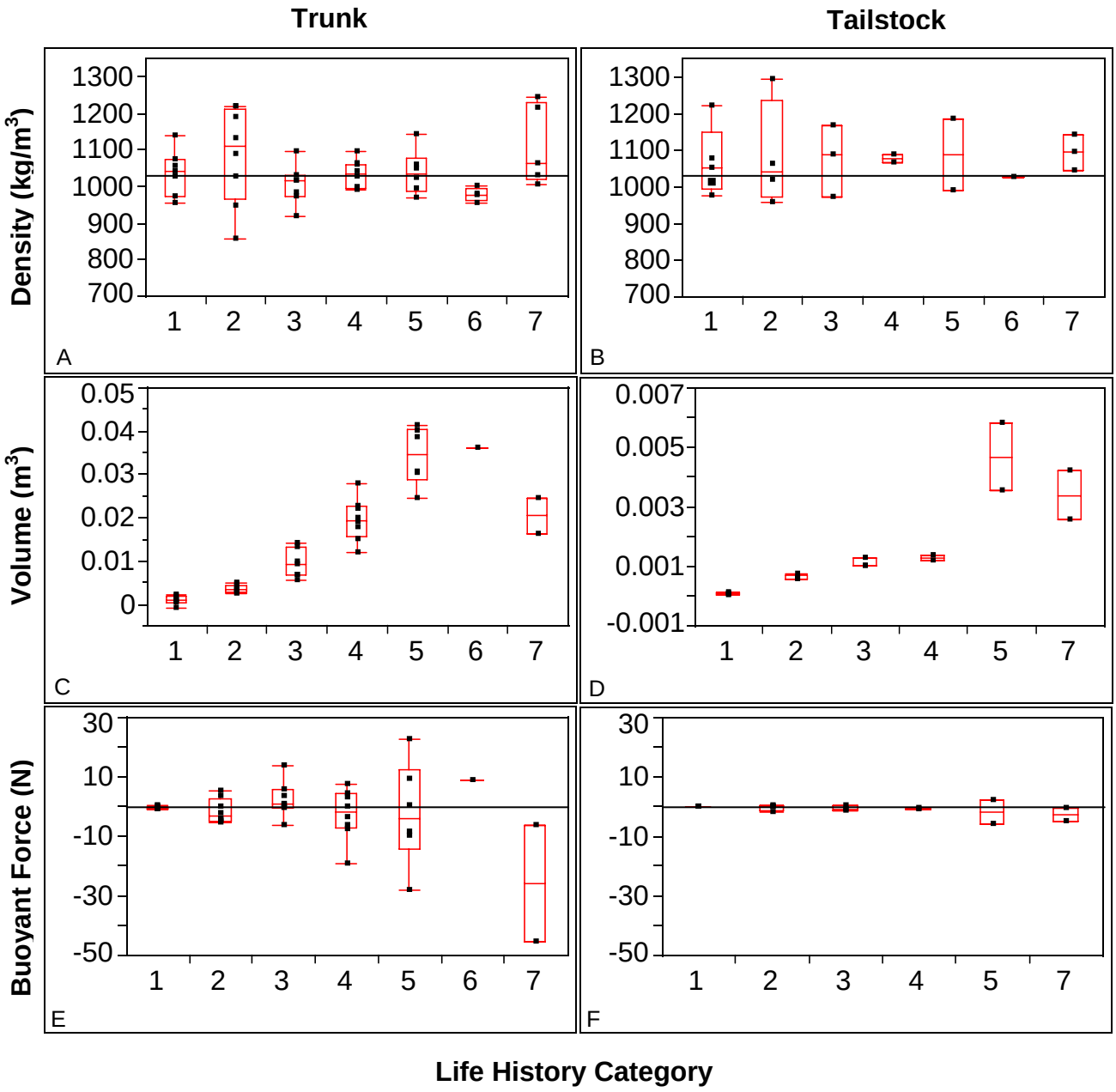

Figure 4 – (A, B) Blubber density, (C, D) volume, and (E, F) buoyant force for the trunk and tailstock. Life history categories are (1) fetus, (2) neonate, (3) juvenile, (4) sub-adult, (5) adult, (6) pregnant female, (7) emaciated adult. Due to sample availability, a single pregnant female is included in C and E; no pregnant females are included in D and F. The horizontal line in A and B denotes the density of sea water. In E and F the horizontal line denotes 0N.

Although, not significantly different, juveniles and pregnant females had the lowest, and emaciated adults had the highest, blubber densities of all life history categories (Table 3). Mean blubber densities of each life history category for both the trunk and tailstock regions (pregnant females excluded from tailstock analysis) were not significantly different from the density of seawater (all p values > 0.05).

Trunk blubber volume increased steadily between fetal and adult life history categories ($F = 75.21$; $p<0.0001$) (Table 3, Figure 4c). The trunk blubber volume of the single pregnant female was similar to adult animals, while that of emaciated animals (n=2) was similar to sub-adults. Tailstock blubber volume significantly increased across fetal, neonatal, and juvenile life history categories ($F = 105.5$; $p<0.001$) (Table 3, Figure 4d). Tailstock blubber volume increased markedly between sub-adult and adult life history categories (Figure 4d).

The buoyant force of trunk blubber was similar for fetal through adult life history categories (mean = –0.91 ± 8.85) ($F = 0.39$; $p = 0.81$) (Table 3, Figure 4e). The pregnant female possessed trunk blubber with the highest buoyant force. Trunk blubber of emaciated animals was twelve times more negatively buoyant than of adults (Table 3, Figure 4e). The buoyant force of tailstock blubber was also similar between all life history categories (mean = –0.30 ± 1.83) ($F = 0.46$; $p = 0.65$). Mean total blubber buoyant force (i.e. trunk and tailstock combined) across fetal, neonatal, and juvenile life history categories was 0.61 ± 7.45N and was not significantly different between these groups ($F = 0.08$; $p = 0.91$) (Figure 5). The mean buoyant force of trunk and tailstock

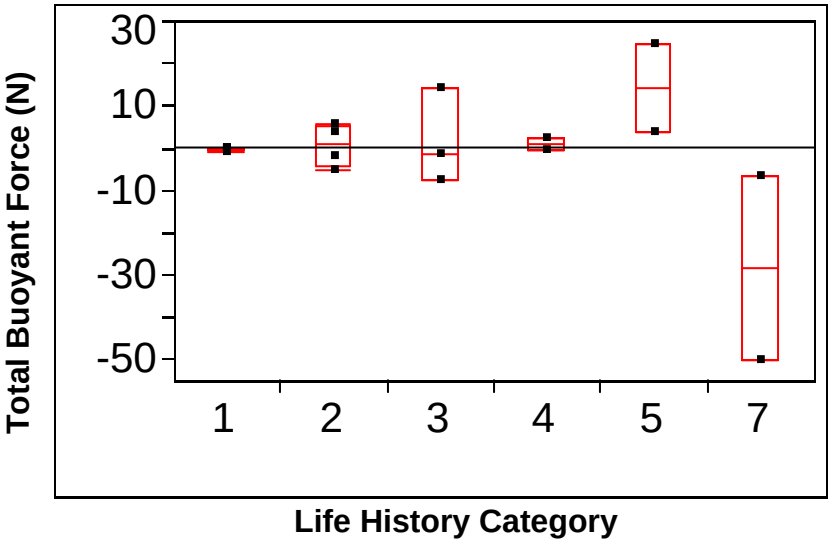

Figure 5 – Total blubber buoyant force across life history categories. Due to sample availability, pregnant females were not included. Life history categories are (1) fetus, (2) neonate, (3) juvenile, (4) sub-adult, (5) adult, and (7) emaciated adult. The horizontal line indicates neutral buoyancy.

blubber for fetal through adult life history categories, as well as emaciated animals, was not significantly different from neutral buoyancy (0N) (all p values >0.05).

DISCUSSION

Blubber's Contribution to Buoyancy Across Ontogeny

Based on known ontogenetic differences in blubber thickness and lipid content (Struntz et al., 2004) it was hypothesized that blubber's contribution to buoyancy may vary across developmental stages in bottlenose dolphins. Specifically, it was predicted that neonatal blubber may be specialized to contribute relatively more to positive buoyancy than in adult dolphins. Instead, blubber contributed to neutral buoyancy across all non-emaciated life history categories. In fetal animals, neutral buoyancy may be important to prevent a large influence on the mother's overall buoyancy. In neonatal animals, blubber is still growing and doesn't achieve maximal lipid content or adult thickness until later developmental stages (Dunkin, 2004; Struntz et al., 2004). Neonatal blubber may, thus, be neutrally buoyant simply as a result of an insufficient amount of time since birth to increase blubber volume and lipid. Additionally, because a neonatal animal must spend a significant amount of time nursing and swimming along side its mother (Gubbins et al., 1999; Weihs, 2003), neutral buoyancy may reduce the energetic cost of maintaining this position underwater. The observation that a young animal was unable to control its position in the water column until six months of age (Cockroft and Ross, 1990) may, thus, be the result of changes in the developing lung or the animal's ability to finely control its lung volume, rather than changes in blubbers density.

Between the juvenile, sub-adult, and adult life history categories, there was a non-significant decline in blubber lipid content which was correlated with a non-significant increase in blubber density. Despite an over three-fold increase in blubber volume between juveniles and adults, blubber's contribution to buoyancy remained neutral. Indeed, neutral buoyancy appeared to be tightly constrained across all non-pregnant, non-emaciated life history categories. Neutral buoyancy may be especially important once nutritional independence is achieved. At this stage in development, bottlenose dolphins achieve the aerobic dive capacity of adults, and, thus, are likely to spend more time at depth (Noren et al., 2002). During horizontal swimming, neutral blubber buoyancy avoids the locomotor cost that would otherwise be incurred overcoming a vertical positive or negative buoyant force (Lovvern and Jones, 1991). During diving, dolphins utilize the progressive decrease in buoyant force associated with decreasing lung volume to periodically glide, rather than actively swim, and, thus reduce their locomotor cost (Skrovan et al., 1999; Williams, 2001; Williams et al., 2000). Blubber, unlike air in the lungs, maintains a constant volume and, thus, its contribution to buoyancy will remain static regardless of depth. If blubber, for example, contributed to positive buoyancy, the depth at which the animal achieved neutral buoyancy would increase and, thus, opportunities to glide would diminish. In sub-adult and adult dolphins, blubber's neutral buoyant force may represent an adaptation for maximizing locomotor efficiency during both horizontal swimming and vertical diving.

Although blubber volume remained similar between adults and pregnant females, blubber lipid content was higher and density lower, in pregnant females. The positive buoyant force of trunk blubber of the pregnant female was also more than four times

higher than that of adult animals. In a pregnant animal, blubber's contribution to positive buoyancy may incur an additional locomotor cost during both swimming and diving.

Emaciation had the most profound effect on blubber's lipid content, density, volume, and, thus, buoyant force. The trunk blubber of emaciated animals was twelve times more negatively buoyant than that of adults. A wide degree of variation was observed in blubber's contribution to buoyancy, though, in emaciated animals. For example, trunk blubber density varied from near normal adult values to as high as 1245 kg/m^3. Similarly, the two animals for which trunk blubber buoyant force was calculated ranged from –6 to –45 N. This last value is similar to the buoyant force recorded for the integument of a manatee (*Trichechus manatus latirostris*), which is hypothesized to rely on negative buoyancy to maintain its position on the sea floor at shallow depths (Kipps et al., 2002; Taylor, 1994). Such a decrease from neutral buoyancy may substantially increase the cost of locomotion (Lovvorn and Jones, 1991) in a tetrapod that controls its position in the water hydrodynamically. Thus, there is the potential for a positive feedback loop in animals of poor nutritional status, in which increasingly more energy must be expended to compensate for decreased buoyancy. This increased energy expenditure may deplete lipid stores, which may further decrease blubber's buoyant force.

Comparisons Across Body Sites

The densities of blubber from the trunk and tailstock body regions were statistically similar. There were however, significant differences in the pattern of blubber volume growth between these body regions. Blubber volume in the trunk region, which has been suggested to be a more metabolically active site of blubber deposition and

mobilization (Koopman, 1998; Koopman et al., 1996), increased steadily and significantly between fetal and adult life history categories. The volume of tailstock blubber, which has been described as primarily structural and less metabolically active (Hamilton et al., *in press;* Koopman, 1998; Koopman et al., 1996; Pabst et al., 1999a), also increased across fetal through sub-adult animals, but the magnitude of this increase was small in comparison to that observed between sub-adults and adults, a phase in which bottlenose dolphins are known to undergo a period of rapid growth (Read et al., 1993). Read et al. (1993) commented that the growth spurt observed in sub-adult animals is primarily the result of increases in girth and body mass. The results of this study indicate that these increases in girth and body mass may largely be a result of rapid blubber growth in the tailstock region.

The buoyant force of blubber from the tailstock was similar across all life history categories, including emaciated animals. There was also less variation within life history categories in the buoyant force of tailstock blubber. The absence of a significant decrease in blubber density, or large changes in volume or buoyant force of tailstock blubber in emaciated animals, further supports the characterization of this blubber as a primarily structural tissue.

Summary

Despite significant differences in lipid content and blubber volume, across life history categories of bottlenose dolphins, blubber's contribution to buoyancy remains neutral. Thus, neonatal animals do not possess blubber that is specialized to contribute to positive buoyancy. Rather, these young animals appear to utilize the same strategy as adults, controlling their position in the water column with hydrodynamic, rather than

hydrostatic mechanisms of buoyancy control. Because this body compartment makes up nearly a quarter of total body mass, it may be essential for this tissue to be neutrally buoyant.

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