The buoyancy of the integument of Atlantic bottlenose dolphins (*Tursiops truncatus*): Effects of growth, reproduction, and nutritional state

ROBIN C. DUNKIN

Department of Ecology and Evolutionary Biology,
University of California at Santa Cruz,
100 Shaffer Road,
Santa Cruz, California 95060, U.S.A.
and
Department of Biology and Marine Biology,
University of North Carolina Wilmington,
601 S. College Road,
Wilmington, North Carolina 28403, U.S.A.
E-mail: dunkin@biology.ucsc.edu

WILLIAM A. MCLELLAN

Department of Biology and Marine Biology,
University of North Carolina Wilmington,
601 S. College Road,
Wilmington, North Carolina 28403, U.S.A.

JAMES E. BLUM

Department of Mathematics and Statistics,
University of North Carolina Wilmington,
601 S. College Road,
Wilmington, North Carolina 28403, U.S.A.

D. ANN PABST

Department of Biology and Marine Biology,
University of North Carolina Wilmington,
601 S. College Road,
Wilmington, North Carolina 28403, U.S.A.

ABSTRACT

In Atlantic bottlenose dolphins (*Tursiops truncatus*) the thickness and lipid content of blubber (the integument's specialized hypodermis) varies across ontogeny and with reproductive and nutritional state. Because the integument comprises up to 25% of total body mass in this species, ontogenetic changes in its lipid content may influence whole body buoyancy. The density and volume of the integument were measured and its buoyancy calculated across an ontogenetic series of dolphins and in pregnant and emaciated adults (total $n = 45$). Regional differences between the metabolically labile trunk integument and the structural tailstock integument

were also investigated. Mean densities of both trunk and tailstock integument were similar across life history categories (trunk = 1,040.7 ± 14.1 kg/m^3; tailstock = 1,077.1 ± 21.2 kg/m^3) and were statistically similar to the density of seawater (1,026 kg/m^3). The mean buoyant force of integument from the trunk (−1.01 ± 1.74 N) and tailstock (−0.30 ± 0.21 N) did not vary significantly across ontogeny. In contrast, pregnancy and emaciation did influence the integument's buoyancy, which ranged between 9 N and −45 N in these categories. Although neutral during growth, the integument's contribution to whole body buoyancy can be influenced by an individual's reproductive and nutritional status.

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 Ling 1974, Costa and Williams 1999, Pabst *et al.* 1999*a*, McLellan *et al.* 2002). Blubber, the hypertrophied hypodermal layer, is composed primarily of adipocytes and structural fibers (Parry 1949, Ling 1974, Ackman *et al.* 1975, Lockyer *et al.* 1984, Koopman 1998, Pabst *et al.* 1999*a*, Struntz *et al.* 2004, Montie *et al.* 2008). Blubber is a multifunctional tissue that acts to store metabolic energy, streamline the body, and insulate the body core (*e.g.,* Parry 1949; Ling 1974; Worthy and Edwards 1990; Koopman 1998, 2007; Hamilton *et al.* 2004; Montie *et al.* 2008). Because blubber is composed primarily of low-density lipid, this tissue has also been hypothesized to contribute positively to whole body buoyant force (Struntz *et al.* 2004), however to date, few data exist to explicitly test this hypothesis (but see Kipps *et al.* 2002).

Buoyancy, the force exerted on an object by the fluid surrounding it, is calculated as

$$B = \left(\rho_f - \rho_0\right) V_0 g \qquad (1)$$

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. While many mammalian 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).

Blubber's contribution to whole body buoyancy is, thus, reliant upon its quantity and quality, which can both vary significantly across ontogeny in bottlenose dolphins. Blubber thickness, a measure of blubber quantity, can more than double between neonatal and adult animals and lipid content, or blubber quality, can vary by as much as 37% between life history categories (Struntz *et al.* 2004, Montie *et al.* 2008). Reproductive and nutritional status can also influence blubber's quality and quantity. For example, the blubber of pregnant bottlenose dolphins can contain on average 27% more lipid, while the blubber of emaciated adult dolphins can contain, on average, 48% less lipid, than that of robust adults (Struntz *et al.* 2004, Dunkin *et al.* 2005). These ontogenetic, reproductive, and nutritional differences in blubber's

lipid content have been demonstrated to influence the integument's thermal function. Ontogenetic differences in the thermal properties of bottlenose dolphin integument are as great as those reported across a phylogenetically diverse sample of cetaceans (Dunkin *et al.* 2005). Similarly, the thermal conductivities of the integument from pregnant and emaciated adults differ significantly from that of other adults (Dunkin *et al.* 2005).

Because blubber's density is dependent upon its lipid content, it was hypothesized that the buoyant force of the integument would also vary across ontogeny and between adults of different reproductive and nutritional states. To calculate the integument's buoyant force, its density and mass were measured and its volume was calculated for fetal, neonatal, juvenile, subadult, and adult bottlenose dolphins. In addition, pregnant and emaciated adults were segregated from the adult category to determine how the integument's contribution to buoyancy may change with reproductive and nutritional status. The lipid and water content of blubber, two indicators of blubber quality (Struntz *et al.* 2004, Dunkin *et al.* 2005), were also measured across these life history categories to investigate their relationship to the integument's buoyant force. Previous studies have demonstrated that postcranial blubber is regionally specialized; trunk blubber is more metabolically labile than the structural blubber of the caudal tailstock (Koopman *et al.* 1996; Koopman 1998; Pabst *et al.* 1999*a*, *b*; Hamilton *et al.* 2004). These functional differences rely, in part, upon blubber's regionally specific composition, which could influence tissue density. To investigate whether there were regional differences in buoyancy, the integument was divided into two postcranial regions, the trunk and the tailstock.

MATERIALS AND METHODS

Specimens

Integument samples were acquired from 40 robust Atlantic bottlenose dolphins and three emaciated dolphins that either stranded or were killed incidentally in fishing operations in North Carolina and Virginia. The data set also included one emaciated adult from Florida and one from New Jersey. The integument samples included the epidermis, dermis and hypertrophied hypodermis, or blubber. 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) and Dearolf *et al.* (2000). These categories include fetus ($n = 7$), neonate ($n = 8$), juvenile ($n = 7$), subadult ($n = 8$), adult ($n = 6$), pregnant female ($n = 4$), and emaciated adult ($n = 5$). Complete data sets (*i.e.,* trunk and tailstock blubber samples) were not always available for every animal, thus, for some measurements the sample size may be reduced and is so stated in the text.

Each animal was first weighed to the nearest kilogram (ED-2000, 2,000 kg capacity scale, Dillon, Fairmont, MN) and measured using a standard set of external morphometrics. The postcranial integument was divided into two regions, the trunk (defined here from the leading edge of the pectoral flippers to the anus) and the tailstock (from the anus to the fluke insertion), and the length of each of these regions was measured (Fig. 1A). At each body region, the integument was dissected cleanly from the underlying subdermal connective sheath (Pabst 1994) and weighed
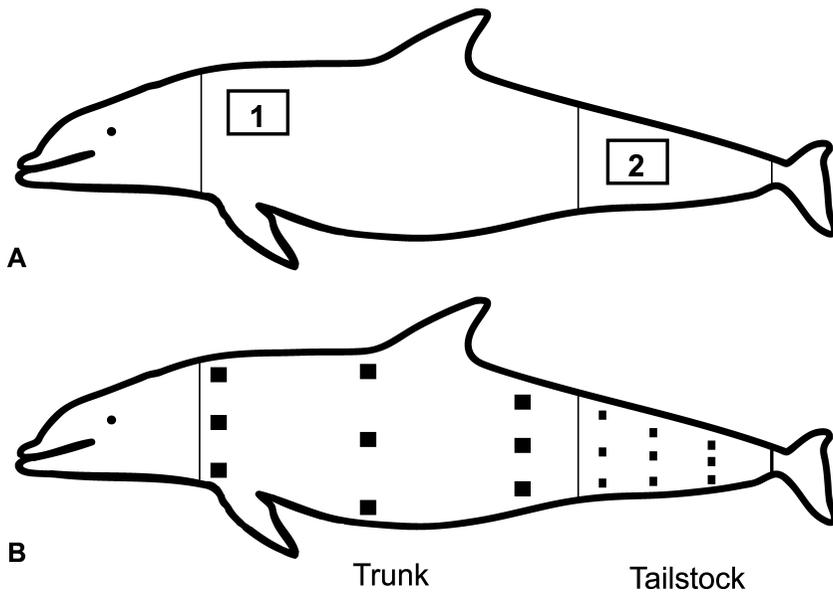
Figure 1. (A) The integument sample locations for the (1) trunk and (2) tailstock. (B) The sites for the intraregional density measurements.

separately to the nearest 0.1 g (I10, Ohaus, Pinebrook, NJ). Integument mass, as a percentage of total body mass, was calculated from these measurements and recorded. The integument and melon structures of the head were excluded from this analysis.

Trunk integument (total $n = 40$ robust and five emaciated animals) was sampled at a dorsolateral position, just caudal to the pectoral flipper, and tailstock integument (total $n = 17$ robust and three emaciated animals) was sampled at a lateral position just caudal to the anus (Fig. 1A). Samples between 5×5 cm and 15×15 cm were either vacuum sealed (EZPack, Koch Equipment, Kansas City, MO) 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 1 g full depth blubber sample (excluding the epidermis) was weighed to the nearest 0.001 g, macerated, and dried with approximately 30 g of sodium sulfate (Na_2SO_4). The lipid was then extracted using hexane and an accelerated solvent extractor (Dionex, Salt Lake City, UT). The excess solvent was evaporated (Turbo Vap II, Zymark, Hopkinton, MA) and the extracted lipid was then reweighed to the nearest 0.001 g.

For both trunk and tailstock blubber samples, water content was determined by excising an approximately 1×1 cm^2 sample and weighing it prior to and after freeze-drying (FreeZone 4.5, Labconco, Kansas City, MO). Samples were weighed each day until the mass of the sample was stable (within 0.001 g) for two consecutive days (total time $= 5$ d).

Integument Density and Volume Measurements

To calculate the integument'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 1 × 1 cm full depth subsamples were taken from each trunk and tailstock sample. Each subsample was weighed to the nearest 0.001 g (PT-6, Sartorius, Edgewood, NY) and then placed in room temperature distilled water, in a 25 mL graduated cylinder. To ensure that the entire sample was submerged, a paperclip was suspended from a thread and used to gently push the sample completely underwater. This procedure was used on all samples so that any small error associated with the displacement caused by the tip of the paperclip was negligible and uniform. The volume of the sample (to the nearest 0.1 mL) 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 integument 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 integument, additional density measurements were performed. On a subset of animals ($n = 3$) across several life history categories, integument density was measured at nine additional positions within the trunk and tailstock regions (Fig. 1B).

Total postcranial integument volume was calculated by dividing its mass from each region (trunk and tailstock) by the mean integument density at each region. For a subset of animals, trunk integument mass ($n = 16$) or tailstock integument mass ($n = 1$) was unavailable. For these animals an interpolated integument mass was determined based on a linear regression of total body length and blubber mass of all animals for which those data were 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 of small sample size. The buoyant force of the trunk and tailstock integument was calculated separately using Equation 1, and these values were then summed to obtain the total buoyant force of the integument. It should be noted that both trunk and tailstock buoyant force could not be calculated for every individual, thus, sample sizes are reduced in some categories and are so noted in the text.

Statistics

One-way ANOVAs were performed to determine if there were significant differences in the variables between life history categories at either the trunk or tailstock body sites and the residuals from these analyses were checked for normality using a Shapiro-Wilk test (JMP 5.1, SAS Inc., Cary, NC) (alpha = 0.05). When an ANOVA determined that a significant result was present, a Tukey-Kramer HSD test was performed to determine which groups were significantly different from one another. For each body region, Student's *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. Multiple linear regression was used to determine how well blubber lipid and water content predicted trunk integument buoyant force. Data are presented as the mean ± the standard error. The standard error was calculated using the pooled variance from the one-way ANOVAs unless a particular life history category was

excluded from an ANOVA in which case the standard error was calculated using the standard deviation for that life history category. Life history categories with less than three samples for a particular analysis were excluded from the statistical tests.

RESULTS

Blubber Lipid and Water Content

Trunk blubber (see also Dunkin *et al*. 2005 and Struntz *et al*. 2004) lipid content increased consistently from fetal through juvenile life history categories (Table 1). Blubber lipid content of pregnant females was on average 27% higher than that of adult animals and was similar to that of juveniles. Emaciated animals had significantly less lipid than all other life history categories except fetuses ($F = 9.33; P < 0.0001$; df $= 6, 32$) (Table 1).

In fetuses and emaciated animals, the water content of trunk blubber was significantly higher than that of other life history categories ($F = 13.76; P < 0.0001$; df $= 6, 38$) (Table 1). The water content of the tailstock blubber of fetuses and emaciated adults was significantly higher than that of neonates and juveniles ($F = 11.96; P < 0.0001$; df $= 6, 19$). Overall, tailstock blubber had a significantly greater water content (mean $= 45.5 \pm 2.2\%$) than trunk blubber (mean $= 34.8\% \pm 1.7\%$) ($F = 15.32; P = 0.0003$; df $= 1, 55$).

Integument Mass, Density, Volume, and Buoyant Force

Integument mass, as a percentage of total body mass, differed significantly between fetuses and juveniles but was similar between all other robust, nonpregnant life history categories ($F = 10.42; P = 0.0002$; df $= 3, 23$, note sample size precluded statistical analysis for subadults). The integument contributed between 14.5% and 25.1% of total body mass across life history categories and reached maximal values in juvenile animals (Table 1).

Within each body region, the density of the integument, subsampled at multiple body positions, was similar (trunk $F = 0.02; P = 0.98$; df $= 2, 23$; tailstock $F = 1.4; P = 0.26$, df $= 2, 24$). The overall mean density of the trunk integument for nonpregnant, nonemaciated animals was $1,040.7 \pm 14.1 \text{ kg/m}^3$ and that of the tailstock was $1,077.1 \pm 21.2 \text{ kg/m}^3$; the densities of trunk and tailstock integument were not significantly different from each other ($F = 2.04; P = 0.16$; df $= 1, 50$).

When compared across life history categories, integument density was similar in both the trunk ($F = 1.74; P = 0.13$; df $= 6, 38$) and tailstock regions ($F = 0.06; P = 0.99$; df $= 6, 13$) (Table 1). With the exception of the trunk integument of emaciated adults, the mean densities of both the trunk and tailstock integument were similar to the density of seawater (all P values > 0.05) across all life history categories. The trunk integument of emaciated adults was significantly more dense than seawater ($P = 0.021$).

As expected, both trunk and tailstock volume increased steadily and significantly with ontogeny (trunk-fetus to adult, $F = 54.49; P < 0.0001$; df $= 6, 31$; tailstock-fetus to juvenile, $F = 103.32; P < 0.0001$; df $= 2, 18$; note that subadult and adult tailstock data excluded due to low sample size) (Table 1). The trunk integument volume of the single pregnant female was similar to adult animals, while that of emaciated animals ($n = 2$) was similar to subadults.

Table 1. Summary of integument's density, buoyancy, composition, and contribution to total body mass across life history categories in Atlantic bottlenose dolphins reported as the mean ± standard error. Only categories with a sample size of three or more were tested for statistical differences. Different letters indicate categories that are statistically different from one another. 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. Sample sizes for the trunk lipid content (total $n = 39$) were reduced in some categories from the sample size for the water content analysis (total $n = 45$) and thus, the percentage lipid and water will not necessarily add up to a constant total fraction of the tissue in all categories.

	Fetus	Neonate	Juvenile	Subadult	Adult	Pregnant female	Emaciated adult
Trunk integument density (kg/m³)	1,037.5 ± 30.2 a	1,084.8 ± 28.2 a	1,003.6 ± 30.2 a	1,033.3 ± 28.2 A	1,038.9 ± 32.6 a	977.9 ± 39.9 a	1,112.1 ± 35.7 A
Tailstock integument density (kg/m³)	1,068.3 ± 47.5 a	1,083.4 ± 53.2 a	1,076.7 ± 61.4 a	1,077.1 ± 9.3 NE	1,087.1 ± 98.2 NE	1,026.9 NE	1,094.2 ± 61.4 A
Trunk integument volume (dm³)	1.14 ± 1.61 a	3.52 ± 1.39 a, b	9.34 ± 1.49 b	19.54 ± 1.39 C	34.16 ± 1.61 d	36.14 NE	20.4 ± 4.08 NE
Tailstock integument volume (dm³)	0.09 ± 0.05 a	0.62 ± 0.05 b	1.11 ± 0.05 c	1.29 ± 0.09 NE	4.69 ± 1.13 NE	NE	3.39 ± 0.81 NE
Trunk integument buoyant force (N)	−0.39 ± 0.21 NE	−1.54 ± 3.43 a	2.55 ± 3.66 a	−2.62 ± 3.43 a	−2.33 ± 3.96 a	8.8 NE	−25.89 ± 19.60 NE
Tailstock integument buoyant force (N)	−0.05 ± 0.37 a	−0.35 ± 0.37 a	−0.58 ± 0.43 a	−0.67 ± 0.16 NE	−1.77 ± 3.82 a	NE	−2.64 ± 2.13 NE
Total integument buoyant force (N)*	−0.27 ± 3.21 a	0.61 ± 3.21 a	1.8 ± 3.71 a	0.93 ± 1.56 NE	14.1 ± 10.5 NE	NE	−28.5 ± 21.7 NE
% Lipid/ wet weight (trunk)	36.5 ± 4.35 b, c	55.8 ± 4.70 a	69.7 ± 5.14 a	62.03 ± 4.35 a	54.3 ± 4.70 a, b	69.2 ± 5.76 a	28.2 ± 5.76 C
% Water/ wet weight (trunk)	48.3 ± 2.76 a	33.1 ± 2.58 b	29.9 ± 2.76 b	30.6 ± 2.58 b	32.8 ± 2.98 b	31.7 ± 3.65 b	59.3 ± 3.27 A
% Water/ wet weight (tailstock)	60.3 ± 3.09 a	41.7 ± 2.82 b	37.8 ± 3.09 b	31.9 ± 6.83 NE	50.4 ± 3.99 a, b	39.3 ± 0.39 NE	68.2 ± 3.99 A
% Integument mass/total body mass†	16.4 ± 1.23 b	23.6 ± 1.07 a, b	25.1 ± 1.14 a	22.1 NE	20.7 ± 1.23 a, b	14.5 NE	16.2 ± 1.39 NE

*Total buoyant force could only be calculated for a subset of animals for which both trunk and tailstock buoyant force was measured, thus, this row will not be the sum of the trunk and tailstock buoyant force values in this table.

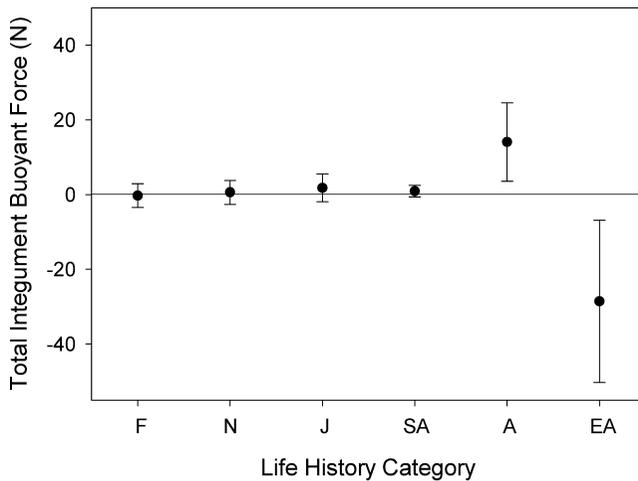†These values were calculated using only measured blubber mass values.

Figure 2. Total integument buoyant force across life history categories. Due to limited sample availability, pregnant females were excluded. Life history categories are fetus (F), neonate (N), juvenile (J), subadult (SA), adult (A), and emaciated adult (EA). The horizontal line denotes neutral buoyancy (0N). Data are shown as the mean ± standard error. Note that for adult values for total buoyant force, only a subset of animals ($n = 2$) had both trunk and tailstock blubber buoyant force values available and thus, this value will not be the total of trunk and tailstock blubber as so noted in Table 1.

The buoyant force of trunk integument was similar for neonatal through adult life history categories (mean $= -1.01 \pm 1.75$ N) ($F = 0.43$; $P = 0.73$; df $= 3, 25$; fetuses were excluded due to low variance, pregnant and emaciated animals were excluded due to low sample size) (Table 1), and the mean value for each life history category was not significantly different from neutral buoyancy (0 N) (all $P > 0.05$). The trunk integument of pregnant females had a large positive buoyant force (9 N), while that of emaciated animals had the largest negative buoyant force (-25.9 ± 19.6 N) (Table 1). The buoyant force of tailstock integument was also similar between fetal, neonatal, and juvenile life history categories (mean $= -0.30 \pm 0.21$ N) ($F = 0.46$; $P = 0.65$; df $= 2, 8$), and the mean value for each life history category was also not significantly different from neutral buoyancy (0 N) (all $P > 0.05$).

The mean total integument buoyant force (*i.e.*, trunk and tailstock combined) across fetal, neonatal, and juvenile life history categories was 0.61 ± 1.75 N (note that total buoyant force was only measured in a subset of animals, $n = 11$) and was not significantly different between these groups ($F = 0.08$; $P = 0.91$; df $= 2, 8$) (Fig. 2). The mean total integument buoyant force was also not significantly different from neutral buoyancy (0 N) ($P = 0.73$). The mean total buoyant force of integument from emaciated animals ($n = 2$) was -28.5 ± 21.7 N (Table 1).

Trunk Blubber Composition and Integument Buoyancy

A multiple linear regression with lipid and water content as the effect variables and buoyant force as the response variable was used to determine the influence of trunk blubber composition on integument buoyancy for postpartum, robust animals.

The best fit model was

$$B_{ti} = 27.4 - (0.09)\,LC_{tb} - (0.67)\,WC_{tb}$$

where B_{ti} is the buoyant force of trunk integument, LC_{tb} is the lipid content and WC_{tb} is the water content of trunk blubber ($P = 0.0024$, $F = 7.40$, $r^2 = 0.33$) (Fig. 3A, B). While lipid content was not significantly correlated with trunk integument buoyant force ($P = 0.49$) there was a significant negative correlation between blubber water content and the buoyant force of trunk integument ($P = 0.003$). Despite large differences in blubber lipid and water content, more than 95% of the trunk integument buoyant force values fell between −10 N and 10 N (Fig. 3A, B).

DISCUSSION

The Integument's Contribution to Buoyancy across Ontogeny

The integument's contribution to whole body buoyancy was hypothesized to vary across ontogeny and across body regions in bottlenose dolphins, based upon known ontogenetic differences in blubber thickness and lipid content (Struntz *et al.* 2004, Dunkin *et al.* 2005) as well as regional differences in blubber's structure and function (Hamilton *et al.* 2004, Koopman *et al.* 2002). The results of this study do not support this hypothesis. Instead, the integument's contribution to whole body buoyancy remained effectively neutral despite an over three-fold increase in volume, across all robust, nonpregnant life history categories as well as between body regions. These unexpected results suggest new hypotheses regarding the influence of this lipid-rich tissue on whole body buoyancy.

For example, a neutrally buoyant integument may reduce the energetic cost of submerged swimming in newborn bottlenose dolphins. A neonatal bottlenose dolphin spends considerable time at depth nursing and swimming alongside its mother (Gubbins *et al.* 1999, Weihs 2004). Dolphins in their first year of life spend between 23% and 67% of their time in the submerged echelon position, a behavior that has been suggested to reduce the locomotor cost of swimming in these young animals (Norris and Prescott 1961, Gubbins *et al.* 1999, Weihs 2004, Noren *et al.* 2006). Similarly, during submerged horizontal swimming, a neutrally buoyant integument would obviate the locomotor costs that would otherwise be incurred to overcome additional vertical positive or negative buoyant force across all age classes (Lovvorn and Jones 1991). During the descent phase of vertical diving, dolphins can utilize the progressively decreasing buoyant force associated with decreasing lung air volume to periodically glide, rather than actively swim, and, therefore reduce their locomotor cost (Skrovan *et al.* 1999, Williams 2001). During a dive, the integument, unlike air in the lungs, maintains a constant volume and therefore, an essentially static (and neutral) contribution to buoyancy regardless of depth. If the integument was positively buoyant, the depth at which the animal achieved neutral buoyancy would increase (Taylor 1994). Although neutral buoyancy may or may not be the driving force behind the observed patterns, the results of this study strongly suggest that Atlantic bottlenose dolphins grow their integument, and most importantly their blubber, in a way that neutralizes the influence of this lipid-rich body compartment on whole body buoyancy.
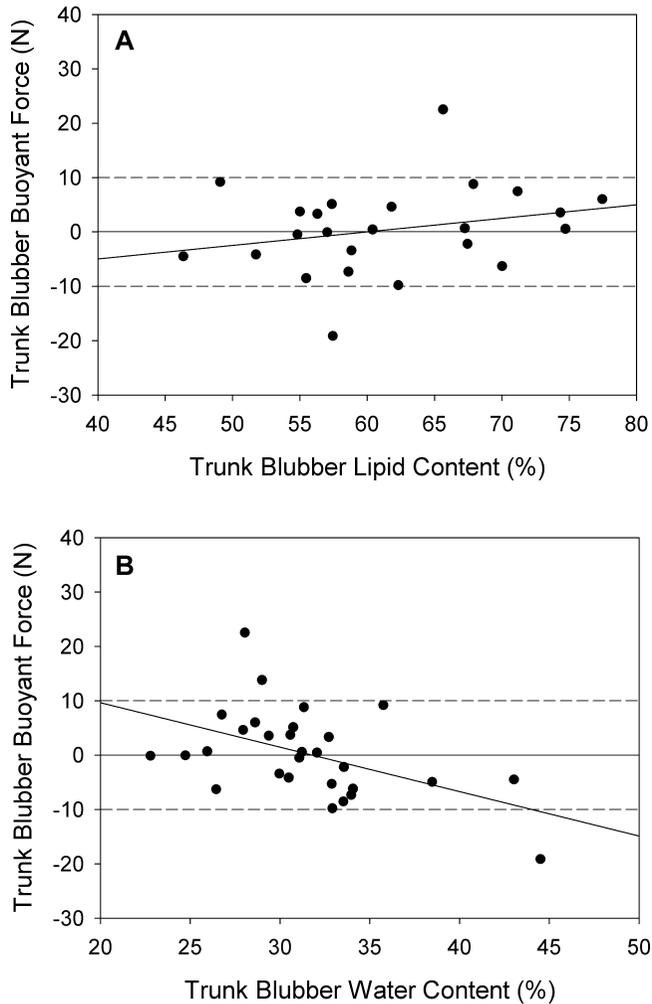
Figure 3. Linear regression of trunk integument lipid content (A) and water content (B) against the buoyant force of trunk integument. Fetal and emaciated life history categories are omitted from this analysis. The solid lines represent neutral buoyancy and the dashed lines represent the window between −10 N and 10 N where a majority of animals are represented. The multiple linear regression for trunk integument buoyant force *vs.* lipid and water content is $B_{ti} = 27.4 − (0.09)LC_{tb} − (0.67)WC_{tb}$ where *B* is buoyant force of the trunk integument, *LC* is lipid content, and *WC* is water content of trunk blubber.

The Influence of Reproductive Status and Emaciation

While the integument's buoyancy remained effectively neutral across ontogeny, pregnancy and emaciation could alter this force. Although the samples sizes for these categories were small, the positive buoyant force of trunk integument of the pregnant female was over four times greater than that of other adult animals. Thus, a pregnant animal may incur an additional locomotor cost, relative to other adult

dolphins, during both horizontal swimming and diving due to the integument's positive buoyancy.

Emaciation profoundly altered the integument's buoyancy. This effect however, was only noted in the trunk integument; the buoyant force of the tailstock integument was similar to that of other adults. This result is consistent with those of Koopman *et al.* (2002). These authors demonstrated that in emaciated harbor porpoises (*Phocoena phocoena*) trunk blubber was significantly thinner than in robust animals, but no such differences were observed in the structural tailstock blubber. The trunk integument of emaciated animals was, on average, 12 times more negatively buoyant than that of other adults. The trunk integument's buoyancy was, though, highly variable in emaciated animals because its density varied from near normal adult values to as high as 1,245 kg/m^3. The two animals for which trunk integument buoyant force was calculated ranged from -6 N to -45 N. This last value is similar to the buoyant force recorded for the integument of a manatee (*Trichechus manatus latirostris*) (Kipps *et al.* 2002), an animal that is hypothesized to rely upon negative buoyancy to maintain its position on the sea floor at shallow depths (Taylor 1994). Thus, an emaciated bottlenose dolphin may experience an increase in the cost of locomotion to overcome its negatively buoyant integument, which may potentially exacerbate nutritional stress.

Tissue Properties Contributing to the Integument's Neutral Buoyancy

Although blubber's lipid content varies over 50% across ontogeny, and blubber can be composed of up to 80% of this relatively low-density material, the mean buoyant force of the integument was essentially neutral across ontogeny. The variation around the calculated mean buoyant force of the integument was also low; nearly all calculated buoyant force values of trunk integument fell between 10 N and -10 N (Fig. 3A, B). Given that lipid content can fluctuate with life history stage and reproductive status and, as recently documented for this species, can also vary with season (Meagher *et al.* 2008, Montie *et al.* 2008), why are there not larger fluctuations in buoyancy in this tissue?

The two main constituents of the integument are connective tissue structural fibers, formed of collagen and elastin (Hamilton *et al.* 2004), and lipid. The densities of proteins, such as collagen and elastin, range between 1,220 kg/m^3 and 1,430 kg/m^3 (reviewed in Fischer *et al.* 2004), making them more dense than seawater (1,026 kg/m^3). Lipid, which constitutes between 30% and 80% of the total blubber layer in robust individuals (Struntz *et al.* 2004, Dunkin *et al.* 2005, Montie *et al.* 2008), is usually less dense than seawater, however, lipid density can vary depending upon the lipid type. For example, the density of lipids found in organisms range between 860 kg/m^3 for wax esters and 1,070 kg/m^3 for cholesterol (Phleger 1998). Triacylglycerides, the major lipid type found in dolphin blubber (Samuel and Worthy 2004, Koopman 2007), have an intermediate density of 930 kg/m^3 (Phleger 1998). Blubber lipid content, though, varies inversely with water content (Dunkin *et al.* 2005). Replacement of relatively low-density lipid with body water, a substance with an intermediate density (1,009 kg/m^3 for lactated Ringer's solution, MSDS no.:159–00) between triacylglycerides and seawater, may buffer the impact of changes in lipid content. It is likely that individuals do experience both short- and long-term fluctuations in the buoyant force of their integument. However, the results of this study suggest that these fluctuations, except in cases of severe emaciation,

are likely to be bounded in a range between 10 N and −10 N by the densities of the major constituents of blubber. It is important to note that this study did not explicitly investigate the contribution of the epidermis to the integument's overall density, and it is possible that this layer may also contribute to the relatively narrow range of integument buoyancy observed here.

Few other studies have calculated the buoyant force of the integument of a marine mammal using empirically measured blubber density values (but see Kipps *et al.* 2002). Most studies that have examined buoyancy in both phocid seals and cetaceans have calculated whole body buoyant force by estimating adipose tissue composition using tritiated water and an assumed mass-specific lipid buoyant force of 0.8871 N/kg (Webb *et al.* 1998, Beck *et al.* 2000) or have calculated buoyancy from passive drift ascent or descent rates in tagged animals (Skrovan *et al.* 1999, Williams *et al.* 2000, Nowacek *et al.* 2001, Biuw *et al.* 2003). The results of this study indicate that despite high-lipid content, the integument may not be positively buoyant across all species and may vary significantly with nutritional and reproductive status. Additionally, empirical measurement of the buoyant properties of the integument from other species, particularly those with higher proportions of low-density wax esters, may prove fruitful for testing whether the apparent selection to maintain the integument near neutrality is simply the result of the material properties and proportion of blubber's constituent components in the bottlenose dolphin, or represents a wider characteristic of cetacean integument.

The Integument's Contribution to Whole Body Buoyant Force

Although this study was specifically concerned with measuring how ontogeny, reproduction, and nutritional status might influence the buoyant force of one body compartment, the integument, ultimately it is the whole body buoyant force that is biologically relevant. Changes in whole body buoyancy associated with seasonal fluctuations in adipose tissue have been documented in phocid seals (Webb *et al.* 1998, Beck *et al.* 2000, Biuw *et al.* 2003) and in the North Atlantic Right whale (Nowacek *et al.* 2001). These studies have indicated that changes in on-board lipid stores can have a measurable influence on swimming and diving patterns of these animals.

In bottlenose dolphins, Skrovan *et al.* (1999) found that changes in buoyancy at depth, associated with changes in lung air volume, significantly influenced swimming and gliding patterns in bottlenose dolphins. These authors calculated that the whole body buoyant force of an adult bottlenose dolphin without air in the lungs was −33.2 N (Skrovan *et al.* 1999). With a full lung of air, the net buoyancy of the adult dolphin was 24.3 N near the surface and −25.7 N at a depth of 67.5 m. Thus, an adult bottlenose dolphin can experience a change in whole body buoyancy of 50 N during a single dive (Skrovan *et al.* 1999). This study found that the postcranial integument's mean buoyancy generally varied between −10 N and 10 N. Thus, while air in the lung will have the most influence on whole body buoyancy on the timescale of a single dive, the variability in the integument's buoyancy measured here could clearly influence whole body buoyant force.

This effect is likely to be particularly pronounced in pregnant and emaciated individuals. For example, if the integument of the captive animal used by Skrovan *et al.* (1999) was neutrally buoyant, as was observed in normal adults in this study, then the net positive buoyancy of the dolphin at the surface (24.3 N) was likely

predominantly due to the low-density air in the inflated lung. If this same animal were to become severely emaciated, it could experience a negative buoyant force, due to its integument, of as high as −45 N. Such an animal would not be able to achieve positive buoyancy, even at the surface.

In summary, despite significant differences in blubber lipid content and integument volume during development in bottlenose dolphins, the integument's contribution to buoyancy remains essentially neutral. However, pregnancy and emaciation can strongly influence the integument's contribution to whole body buoyancy, which may alter locomotor costs associated with swimming and diving. An individual dolphin is likely to experience fluctuations in blubber lipid content across its life, however, the impact of these fluctuations on the integument's contribution to whole body buoyant force may be minimized by the relatively small differences in densities between blubber's constituents and that of seawater. The integument's neutral buoyancy across ontogeny in Atlantic bottlenose dolphins is a surprising result and indicates that generalizations about blubber's contribution to whole body buoyancy across species should be viewed with caution.

Literature Cited

Ackman, R. G., J. H. Hingley, C. A. Eaton and J. C. Sipos. 1975. Blubber fat deposition in mysticete whales. Canadian Journal of Zoology 53:1332–1339.

Beck, C. A., W. D. Bowen and S. J. Iverson. 2000. Seasonal changes in buoyancy and diving behavior of adult grey seals. The Journal of Experimental Biology 203:2323–2330.

Biuw, M., B. McConnell, C. J. A. Bradshaw, H. Burton and M. Fedak. 2003. Blubber and buoyancy: Monitoring the body condition of free-ranging seals using simple dive characteristics. Journal of Experimental Biology 206:3405–3423.

Costa, D. P., and T. M. Williams. 1999. Marine mammal energetics. Pages 176–217 *in* J. E. Reynolds III and S. A. Rommel, eds. Biology of marine mammals. Smithsonian Institution Press, Washington, DC.

Dearolf, J. L., W. A. McLellan, R. M. Dillaman, D. Frierson Jr. and D. A. Pabst. 2000. Precocial development of axial locomotor muscle in bottlenose dolphins (*Tursiops truncatus*). Journal of Morphology 244:203–215.

Denny, M. 1993. Air and water: The biology and physics of life's media. Princeton University Press, Chichester, West Sussex, U.K.

Dunkin, R. C., W. A. McLellan, J. E. Blum and D. A. Pabst. 2005. The ontogenetic changes in the thermal properties of Atlantic bottlenose dolphin blubber *Tursiops truncatus*. Journal of Experimental Biology 208:1469–1480.

Fischer, H., I. Polikarpov and A. F. Craievich. 2004. Average protein density is a molecular-weight-dependent function. Protein Science 13:2825–2828.

Geraci, J. R., and V. J. Lounsbury. 1993. Marine mammals ashore: A field guide to strandings. Texas A&M University Sea Grant Program, College Station, TX.

Gubbins, C., B. McCowan, S. K. Lynn, S. Hooper and D. Reiss. 1999. Mother infant spatial relationships in captive bottlenose dolphins, *Tursiops truncatus*. Marine Mammal Science 15:751–765.

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

Kipps, E. K., W. A. McLellan, S. A. Rommel and D. A. Pabst. 2002. Skin density and its influence on buoyancy in the manatee (*Trichechus manatus latirostris*), harbor porpoise (*Phocoena phocoena*), and bottlenose dolphin (*Tursiops truncatus*). Marine Mammal Science 18:765–778.

Koopman, H. N. 1998. Topographical distribution of the blubber of harbor porpoises (*Phocoena phocoena*). Journal of Mammalogy 79:260–270.

Koopman, H. N. 2007. Phylogenetic, ecological, and ontogenetic factors influencing the biochemical structure of the blubber of odontocetes. Marine Biology 151:277–291.

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

Koopman, H. N., D. A. Pabst, W. A. McLellan, R. M. Dillaman and A. J. Read. 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. Physiological and Biochemical Zoology 75:498–512.

Ling, J. K. 1974. The integument of marine mammals. Pages 1–44 *in* R. J. Harrison, ed. Functional anatomy of marine mammals. Academic Press, New York, NY.

Lockyer, C. H., L. C. McConnell and T. D. Waters. 1984. The biochemical composition of fin whale blubber. Canadian Journal of Zoology 62:2553–2562.

Lovvorn, J. R., and D. R. Jones. 1991. Effects of body size, body fat, and change in pressure with depth on buoyancy and costs of diving in ducks (*Aythya* spp.). Canadian Journal of Zoology 69:2879–2887.

McLellan, W. A., H. N. Koopman, S. A. Rommel, A. J. Read, C. W. Potter, J. R. Nicolas, A. J. Westgate and D. A. Pabst. 2002. Ontogenetic allometry and body composition of the harbour porpoises (*Phocoena phocoena*) from the western North Atlantic. The Journal of Zoology London 257:457–471.

Meagher, E., W. McLellan, A. Westgate, R. Wells, J. Blum and D. Pabst. 2008. Seasonal patterns of heat loss in wild bottlenose dolphins (*Tursiops truncatus*). Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology 178:529–543.

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

Noren, S. R., G. Biedenbach and E. F. Edwards. 2006. Ontogeny of swim performance and mechanics in bottlenose dolphins (*Tursiops truncatus*). Journal of Experimental Biology 209:4724–4731.

Norris, K. S., and J. H. Prescott. 1961. Observations on Pacific cetaceans in Californian and Mexican waters. University of California Publishing in Zoology 63:291–402.

Nowacek, D. P., M. P. Johnson, P. L. Tyack, K. A. Shorter, W. A. McLellan and D. A. Pabst. 2001. Buoyant balaenids: The ups and downs of buoyancy in right whales. Proceedings of the Royal Society B: Biological Sciences 268:1811–1816.

Pabst, D. A. 1994. Morphology of the sub-dermal connective tissue sheath of dolphins: A new fibre-wound, thin-walled, pressurized cylinder model for swimming vertebrates. Journal of Zoology London 238:35–52.

Pabst, D. A., J. L. Hamilton, W. A. McLellan, T. M. Williams and J. L. Grosline. 1999*a*. Streamlining dolphins: Designing soft-tissue keels. Eleventh international symposium on unmanned, untethered submersible technology. Autonomous Undersea Systems Institute, Lee NH 477–486.

Pabst, D. A., S. A. Rommel and W. A. McLellan. 1999*b*. The functional morphology of marine mammals. Pages 15–72 *in* J. E. Reynolds III and S. A. Rommel, eds. Biology of marine mammals. Smithsonian Institution Press, Washington, DC.

Parry, D. A. 1949. The structure of whale blubber, and a discussion of its thermal properties. Journal of Microscopical Sciences 90:13–25.

Phleger, C. F. 1998. Buoyancy in marine fishes: Direct and indirect role of lipids. American Zoologist 38:321–330.

Samuel, A. M., and G. A. J. Worthy. 2004. Variability in fatty acid composition of bottlenose dolphin (*Tursiops truncatus*) blubber as a function of body site, season, and reproductive state. Canadian Journal of Zoology 82:1933–1942.

Skrovan, R. C., T. M. Williams, P. S. Berry, P. W. Moore and R. W. Davis. 1999. The diving physiology of bottlenose dolphins (*Tursiops truncatus*). II. Biomechanics and changes in buoyancy at depth. Journal of Experimental Biology 202:2749–2761.

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

Taylor, M. 1994. Mechanics and physiology of animal swimming. Cambridge University Press, New York, NY.

Webb, P. M., D. E. Crocker, S. B. Blackwell, D. P. Costa and B. J. Le Boeuf. 1998. Effects of buoyancy on the diving behaviour of northern elephant seals. Journal of Experimental Biology 201:2349–2358.

Weihs, D. 2004. The hydrodynamics of dolphin drafting. Journal of Biology 3:8.

Williams, T. M. 2001. Intermittent swimming by mammals: A strategy for increasing energetic efficiency during diving. American Zoologist 41:166–176.

Williams, T. M., R. W. Davis, L. A. Fuiman, J. Francis, B. J. Le Boeuf, M. Horning, J. Calambokidis and D. A. Croll. 2000. Sink or swim: Strategies for cost-efficient diving by marine mammals. Science 288:133–136.

Worthy, G., and E. Edwards. 1990. Morphometric and biochemical factors affecting heat loss in a small temperate cetacean (*Phocoena phocoena*) and a small tropical cetacean (*Stenella attenuata*). Physiological Zoology 63:432–442.