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Histomorphological stratification of blubber of three dolphin species from subtropical waters

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Abstract

Blubber is a highly specialized and dynamic tissue unique to marine mammals and presents a reflection of the individuals' nutrition, environment, and life history traits. Few studies have investigated the histomorphology of cetacean blubber in subtropical environments. The aim of this study was to investigate the blubber histomorphology of three different dolphin species off the subtropical KwaZulu-Natal coast, South Africa, using adipocyte cell size, number, and density. Blubber tissue samples from the saddle area of 43 incidentally bycaught animals (four *Sousa plumbea*, 36 *Tursiops aduncus*, and three *Delphinus delphis*) were used to compare cell parameters between blubber layers. Samples were divided into the upper third (corresponding to the superficial layer closest to the epidermis), middle third, and lower third (corresponding to the deep layer). For *T. aduncus*, factors potentially affecting blubber histomorphology, such as sex, age class, and season, were also assessed. Our results showed that no stratification was present in *S. plumbea*, which could be ascribed to the species' warmer inshore habitat, large body size, and apparent lower mobility. For *T. aduncus* and *D. capensis*, however, blubber stratification was determined, characterized by a gradual transition of cell size, number, and density between layers rather than clearly defined layers. Significant differences in adipocyte cell number and density were found for different sexes and age classes of *T. aduncus*. However, there were no significant differences between seasons, which was attributed to the small temperature differences between seasons. This study represents the first investigation of odontocete blubber histomorphology in subtropical waters. It is recommended that future studies investigate blubber lipid content, while also taking into consideration the reproductive status of the females and the temperature range of their study area. It is hoped that our results, in conjunction with histopathology and other health indicators, could assist in assessing health and body condition.

KEYWORDS

adipocytes, condition, *Delphinus delphis*, *Sousa plumbea*, *Tursiops aduncus*

[†]Deceased.

1 | INTRODUCTION

Blubber is a tissue unique to marine mammals, which aids in insulation, streamlining of the body, serves as a metabolic energy storage site, and contributes to positive buoyancy (Derous et al., 2020; Dunkin et al., 2005; Pabst, 1996; Parry, 1949; Schmidt-Nielsen, 1995; Smith & Worthy, 2006; Strandberg et al., 2008). In addition, some marine mammals display vertical stratification of the blubber layer, exhibited by both variations in the structure of cells and lipid composition with depth (Ball et al., 2015; Guerrero & Rogers, 2017; Montie et al., 2008; Samuel & Worthy, 2004; Strandberg et al., 2008; Struntz et al., 2004). When used as a proxy for body condition, blubber measurements/parameters furthermore offer insights into individual fitness, survival, and reproductive success (Castrillon & Nash, 2020). Histological and biochemical data collected from stranded bottlenose dolphins (*Tursiops truncatus)* from the south-eastern United States showed that the blubber is stratified into clearly defined layers (Montie et al., 2008). Similarly, Ball et al. (2015) quantified the bowhead whale (*Balaena mysticetus*) blubber, showing distinct characteristics for deep, middle, and superficial layers of blubber based on structural fiber density and adipocyte cell count and size. However, these differences appeared to be gradual rather than composed of clearly defined layers. Other studies have verified that some cetaceans, such as bottlenose dolphins, harbor porpoises, sei, and fin whales, display well-defined blubber layers (Ackman, Hingley, Eaton, et al., 1975; Aguilar & Borrell, 1990; Koopman et al., 1996, 2002; Lockyer et al., 1984; Samuel & Worthy, 2004), while other species, such as humpback whales, show a more gradual transition between blubber layers (Ackman, Hingley, Eaton, et al., 1975; Elfes, 2008; Waugh et al., 2014).

Histologically, blubber is a highly organized matrix of tissue comprised of adipocytes surrounded by collagen and elastin structural fibers (Struntz et al., 2004). Adipocytes are specialized lipid storage cells and thus adipose tissue can experience large changes in size, with most of the expansion or shrinkage of adipose depots being due to changes in adipocyte dimensions (Koopman et al., 2002). Little variation in adipocyte size and numbers were detected in external blubber layers, but greater variations in these traits were seen in the inner layers of the harbor porpoise (*Phocoena phocoena*) and *T. truncatus* blubber (Koopman et al., 1996; Struntz et al., 2004), indicating that it is more metabolically active. In addition, biochemical stratification in lipid composition between external and internal blubber layers has been shown in detail for harbor porpoises (Koopman et al., 1996) and beaked whales (Koopman, 2007). Rather than cell generation or loss of existing cells (apoptosis), adipocytes may undergo 10-fold changes in volume depending on the number of resources available and the energetic demands of the animal. For example, healthy porpoises displayed smaller adipocytes in the outer blubber layer compared to the larger adipocytes in the inner blubber layer. Starved porpoises, however, had fewer, smaller adipocytes in the inner blubber layer, suggesting a possible combination of adipocyte shrinkage and loss (Koopman et al., 2002). Thus, the internal blubber layers are closely associated with lipid utilization, which can be used as a measure of health and nutritional status in cetaceans, whereas the external layers are less dynamic and fulfill a primarily insulative and biomechanical function of keeping the body together (Koopman et al., 2002).

However, the majority of studies to date have been conducted on cetaceans inhabiting temperate waters (Kershaw et al., 2019; McClelland et al., 2012; Montie et al., 2008). A major hurdle is a difficulty in obtaining adequate samples. Furthermore, some collecting methods, such as biopsy sampling, are costly, so where possible, opportunities to obtain samples from bycaught or stranded animals are utilized. However, stranded animals are often sick and thus may not yield representative samples (Reiling et al., 2019) and the quality of samples may be compromised by decomposition (Plön, De Wet, et al., 2015). Thus, investigating wild dolphins incidentally caught in the bather protection nets (BPN) along the KwaZulu-Natal (KZN) coastline represents a unique opportunity to contribute to our understanding of cetacean blubber histomorphology in a subtropical region as it is assumed that animals get caught in the nets accidentally and are therefore representative of the wild population (Plön, Cockcroft, et al., 2015). Three dolphin species are commonly found in the BPN, all of which differ in habitat, sexual dimorphism, and natural history parameters (Best, 2007; Plön et al., 2012), that is, the Indian Ocean humpback dolphin (*Sousa plumbea*), the Indo-Pacific bottlenose dolphin (*Tursiops aduncus)* and the common dolphin (*Delphinus delphis*).

S. plumbea ranges geographically from False Bay in South Africa to the Bay of Bengal in India (Best, 2007). *S. plumbea* are usually found in shallow coastal waters less than 25 m in depth and are found predominantly within 500 m from shore (Plön, Cockcroft, et al., 2015; Plön et al., 2012). Off South Africa, sexual dimorphism is apparent, with fully grown males recorded at 260 kg and 2.7 m in comparison to females of 170 kg and 2.4 m (Parra & Ross, 2009). Sexual maturity is reached at 10 years in females and 13 years in males (Plön, Cockcroft, et al., 2015). *T. aduncus* occupies a wide geographical distribution, including the waters off South Africa to India, the coast of Australia, and surrounding waters and into the Pacific Ocean. The species prefers inshore habitats where the water is less than 50 m deep (Cockcroft & Ross, 1990; Plön et al., 2012). In South African waters, females reach sexual maturity between 9.5 and 11 years of age (corresponding to a total length of between 2.1 and 2.3 m), while males only reach sexual maturity at 14.5 years (corresponding to a total length of about 2.4 m; Cockcroft & Ross, 1990). *D. delphis* can be found in offshore warm temperate waters, and tropical waters worldwide from about 40–60°N to about 50°S, preferring waters less than 500 m in depth (Best, 2007). Attainment of sexual maturity varies by region and occurs between 7 and 12 years in males and 6–8 years in females in South African waters (Mendolia, 1989).

Since blubber is a dynamic tissue that can reflect the nutrition, environment, and life history traits of an individual, such as age, sex, and reproductive status, it is likely to have evolved to suit the individual species (Adamczak et al., 2021; Iverson, 2009; Raverty et al., 2020). To investigate this further, we chose a comparative

approach of examining the histomorphology of blubber from three dolphin species found in subtropical waters off the KZN coastline, South Africa. We hypothesized that the species would exhibit clearly defined stratification of blubber as seen in other dolphin species, such as *T. truncatus* (Montie et al., 2008; Struntz et al., 2004). Previous studies showed an influence of seasonal differences, geographical area, ontogeny, and reproductive state on histomorphological parameters in the blubber, such as structural fiber density and adipocyte number and size (Ball et al., 2015; Montie et al., 2008; Struntz et al., 2004) and we, therefore, expected significant differences between blubber layers when comparing sex, age, life history categories, and season. Thus, the aim of our study was to compare the blubber of three different dolphin species using histological analyses of adipocyte cell size, number, and density. Where possible, comparisons based on sex, age class, and season were also conducted.

2 | MATERIALS AND METHODS

The BPN has been deployed at the most popular bathing beaches of the central and south coast of KZN since 1952 (Figure 1). The KwaZulu-Natal Sharks Board (KZNSB) maintains these nets, which are checked every weekday morning (weather permitting). All animals found alive are released, while carcasses are removed and taken back to the laboratory at the KZNSB headquarters for research purposes. This study was conducted under ongoing research permits issued by the Department of Forestry, Fisheries and the Environment to the KZNSB for scientific investigations. Under a long-standing agreement between the KZNSB and the Port Elizabeth Museum (PEM), detailed measurements and samples are taken from the carcasses for accession to the Graham Ross Marine Mammal Collection at the PEM (Cockcroft, 1990; Plön et al., 2012).

Blubber tissue samples from the saddle area, approximately 4 × 2 cm in size, were removed from incidentally caught dolphins before the carcass was frozen to ensure intact histology of the tissue (Figure 2). Care was taken to sample the entire depth of the blubber tissue, which was then stored in 10% buffered formalin. The saddle area is the standard biopsy/surgical site for dolphin health assessments; in addition, dolphin blubber in this location is dynamic with regard to lipid deposition and mobilization (Koopman et al., 1996, 2002). In total, 43 samples were available for analysis, including four *S. plumbea*, 36 *T. aduncus*, and three *D. delphis* specimens dating from 2006 to 2014 (see Table 1). Individuals were classified as juveniles (including neonates and calves) or adults according to total body length following Plön, Cockcroft, et al. (2015), Cockcroft and Ross (1990), and Mendolia (1989) for *S. plumbea*, *T. aduncus*, and *D. delphis*, respectively.

2.1 | Histological analysis

Histological analysis followed a modified standard protocol based on previous recommendations by Montie et al. (2008). Samples were processed using a standard histology protocol, embedded in paraffin, sectioned using a rotary microtome (Kedee KD2258), stained with hematoxylin and eosin, and mounted on glass slides.

Slides were viewed using a Motic binocular microscope and camera system using Stream Basic V 1.9.3 2014 (Olympus Soft Imaging Solutions) and color images were captured for each sample. Images were taken from (a) the upper third (corresponding to the superficial layer) of the blubber closest to the epidermis, (b) the middle third (corresponding to the middle layer of the entire blubber depth), and (c) the lower third (corresponding to the deep layer closest to the muscle) at ×100 magnification (Figure 3).

2.2 | Cellular measurements

Within each blubber layer, the area of images captured was a 684 × 684 µm box (468 065.1 µm^2) and analyzed using Stream Basic V 1.9.3 2014 by Olympus Soft Imaging Solutions. The cellular measurements (i.e., adipocyte cell size, adipocyte number, and adipocyte density) were calculated for the superficial, middle, and deep layers, respectively (Figure 3), as follows:

2.2.1 | Adipocyte cell size (area)

Within each layer, a minimum of 10 adipocytes were measured using the polygon area calculator. Only cells that were intact and had clearly defined cell membranes were measured.

2.2.2 | Adipocyte cell numbers

For each image, all intact adipocytes were counted using the cell counter tool in *Image J* V 1.41o software (National Institutes of Health).

2.2.3 | Adipocyte cell density (%)

To calculate the percentage (%) of adipocytes and structural fibers, each image of blubber depth was converted to greyscale and the threshold function in *Image J* was used to calculate adipocyte area per image by analyzing particles.

2.3 | Sex, age class, and seasonality in *T. aduncus*

Only samples collected from *T. aduncus* were analyzed for differences due to sex, age class, and seasonality (see Table 1, Figure 4) as sample sizes for the other species were insufficient. More samples were available for females (n = 19) than for males (n = 17) and the majority of samples originated from adults (n = 22; Table 1).

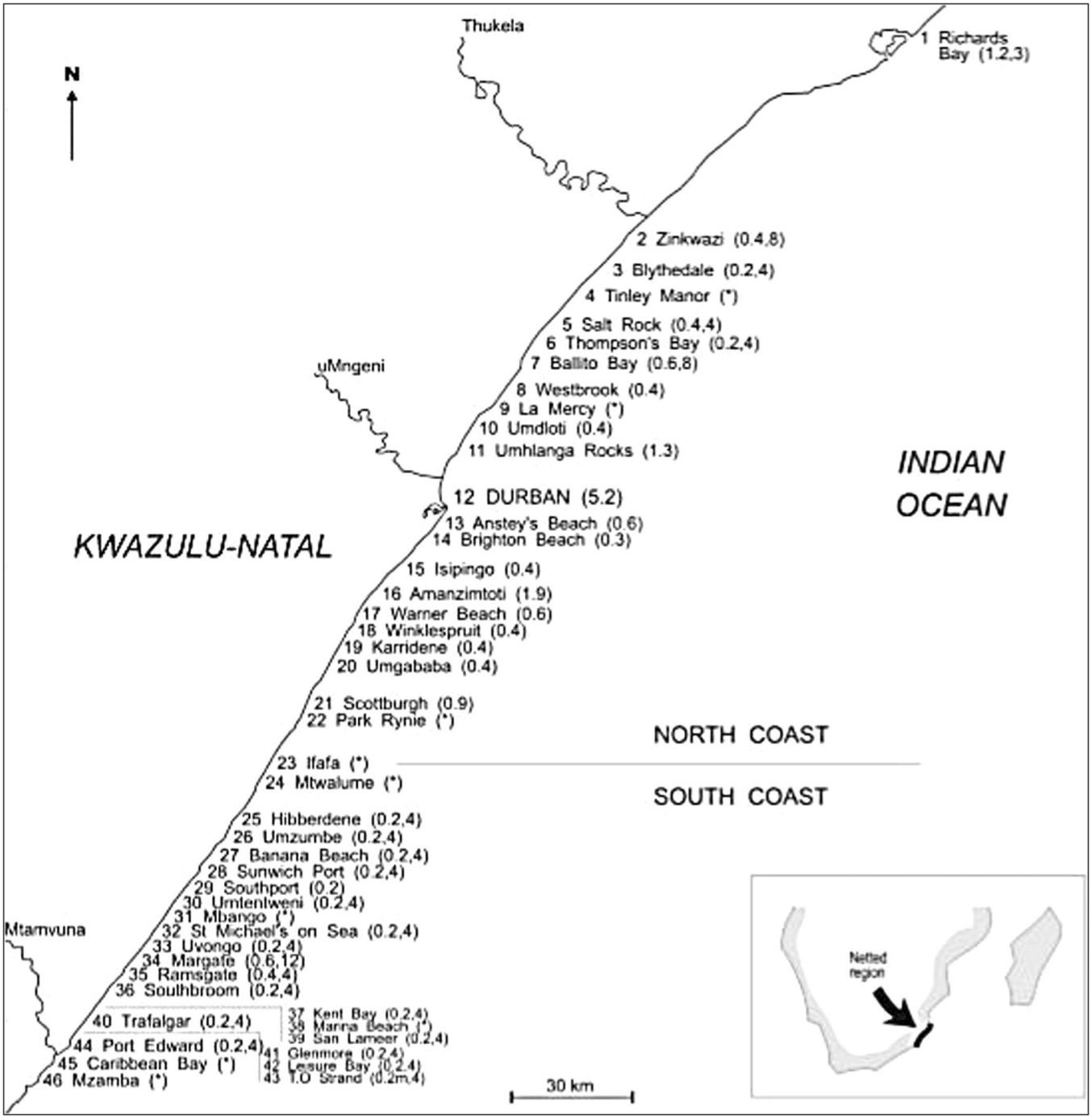

FIGURE 1 The location of individual bather protection nets along the KZN coastline (numbers in parentheses indicate the length of nets in kilometer at respective beaches and the number of drumlines as of December 2009). KZN, KwaZulu-Natal.

To analyze potential effects of seasonality on the stratification of *T. aduncus* blubber, temperature data were obtained from the KZNSB. Since KZN is considered a subtropical climate, seasons are not pronounced, and thus only two seasons are described: summer and winter. The mean sea surface temperature along the KZN coastline ranges from 16°C to 22°C in winter and from 18°C to 27°C in summer (Smit et al., 2013). Thus, a temperature of 22°C was chosen for the delineation of samples into two seasons: winter (range: 19–21°C) and summer (range: 22–26°C). These temperature ranges were also checked to confirm that they provided for a representative number of animals per season (Figure 4).

2.4 | Statistical analyses

To test for differences in cellular measurements between blubber layers, both between species as well as within species, a one-way permutational MANOVA (PERMANOVA) design was used. This

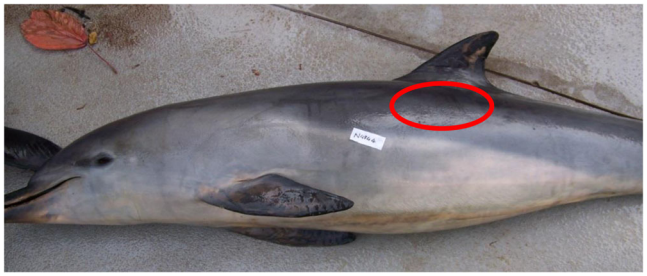

FIGURE 2 Location (circled in red) where blubber samples were taken.

TABLE 1 Breakdown of blubber samples for three dolphin species by sex and age class.

	Sousa plumbea (*n* = 4)	*Tursiops aduncus* (*n* = 36)	*Delphinus delphis* (*n* = 3)
Sex			
Male	2	17	1
Female	2	19	2
Age class			
Adult	1	22	2
Juvenile	3	14	1

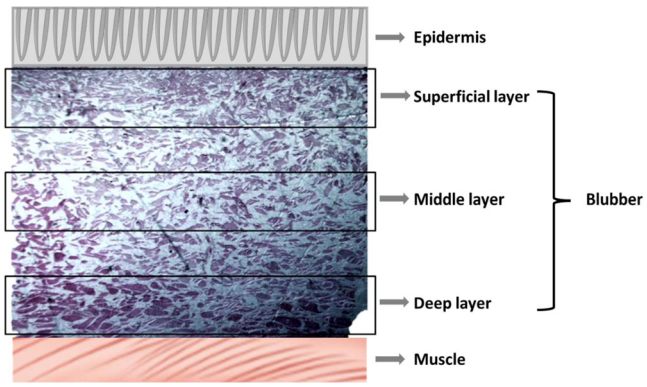

FIGURE 3 Histograph of a blubber section divided into superficial, middle, and deep layers. Images for analysis were taken immediately below the epidermis (superficial layer), at the midpoint of the blubber section (middle layer), and immediately above the muscle (deep layer). Blubber was stained with hematoxylin and eosin.

analysis was also carried out to determine any differences between the sexes, age classes, and seasons of *T. aduncus*. For all PERMANOVA tests, the cellular measurements were first converted to a Bray–Curtis similarity matrix. If a given test result showed an overall significant difference, post hoc pairwise comparisons of the mean ranks for all groups (i.e., blubber layers between species, within each species, and between sex, age classes, and season) were performed to determine which parameters were driving the overall difference.

All tests were performed using an a priori significance level of $\alpha = .05$. A PERMANOVA was performed using the PERMANOVA routine of the PERMANOVA + add-on package (Anderson et al., 2008) to PRIMER V6. *p* Values for PERMANOVA models were tested using unrestricted permutation of the residuals.

3 | RESULTS

3.1 | Blubber stratification

The average adipocyte cell size, number, and density varied between the blubber layers of the three species (Figures 5 and 6). However, the PERMANOVA analyses examining differences in cellular measurements between the three species indicated nonsignificant differences for adipocyte cell size ($df = 2$; $p = .7$), numbers ($df = 2$; $p = .07$), and density ($df = 2$; $p = .2$; Table 2). In contrast, PERMANOVA results examining differences between layers within each of the species varied (Table 2, Figure 6): while the results for *S. plumbea* showed no significant differences between layers for all three cellular measurements (adipocyte cell size ($df = 2$; $p = .83$), number ($df = 2$; $p = .45$), and density ($df = 2$; $p = .32$; Table 2, Figure 6), the results for *T. aduncus* indicated a statistical significance between blubber layers for adipose cell size ($df = 2$; $p = .003$). Post hoc pairwise tests showed this could be attributed to differences between the middle layer and other layers (Table 2, Figure 6), with the middle layer having significantly larger cells (5571.73 μm^2/cell) than the superficial (3958 μm^2/cell) and deep layers (4218.31 μm^2/cell). Adipocyte cell numbers showed no significant difference between the three layers ($df = 2$; $p = .98$). Adipocyte cell densities showed a significant difference between blubber layers ($df = 2$; $p = .024$), with the superficial layer having significantly fewer cells (68.21%/468 065.1 μm^2) than the middle (75.59%/468 065.1 μm^2) and deep (73.42%/468 065.1 μm^2) layers (Table 2, Figure 6).

PERMANOVA results for *D. delphis* showed no significant difference in adipocyte cell size ($df = 2$; $p = .47$) between the three layers (Table 2, Figure 6). Adipocyte cell numbers did show a significant difference ($df = 2$; $p = .008$); however, post hoc pairwise tests did not indicate which layer this significance could be ascribed to. No significant differences were observed between blubber layers for cell density ($df = 2$; $p = .61$; Table 2; Figure 6).

3.2 | Cellular measurements in *T. aduncus* by sex, age class, and season

3.2.1 | Sex

The average adipocyte cell size, number, and densities were compared for differences in blubber layers between sexes of *T. aduncus* (Figure 7). Average adipocyte cell numbers differed significantly in the deep layer ($df = 1$; $p = .004$; Figure 7), with females having more cells (78 cells/468 065.1 μm^2) than males (60 cells/468 065.1 μm^2). However, no other significant differences were

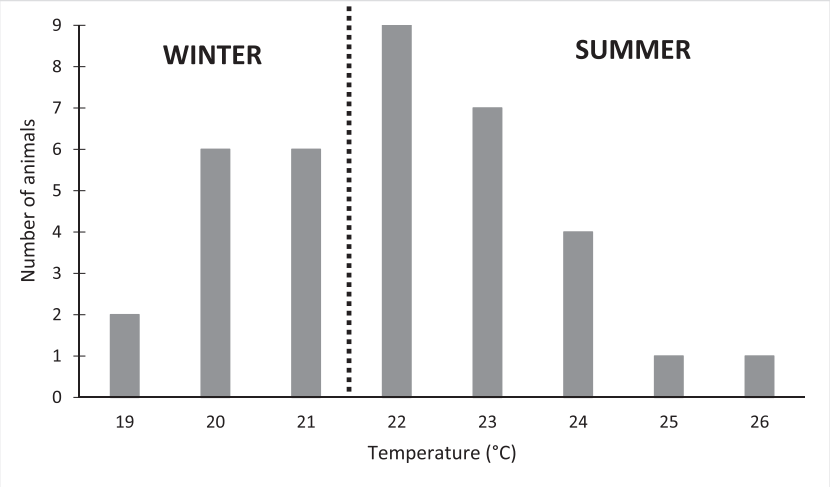

FIGURE 4 Number of bycaught *Tursiops aduncus* per temperature interval (°C). The dashed line indicates the temperature delineation between winter and summer.

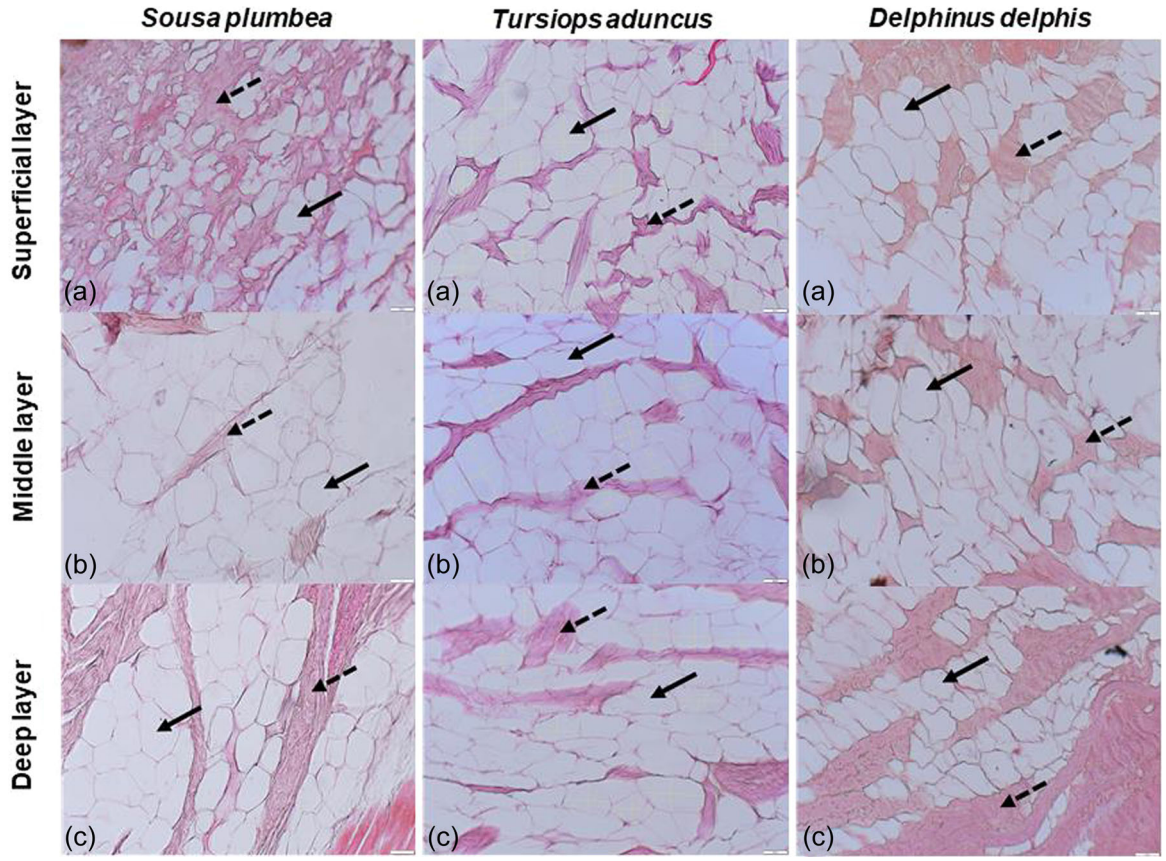

FIGURE 5 Light micrographs of blubber with hematoxylin and eosin staining for three different dolphin species: *Sousa plumbea*, *Tursiops aduncus*, and *Delphinus delphis*; where (a) represents the superficial blubber layer closest to the skin, (b) represents the middle blubber layer, and (c) represents the deep blubber layer. Solid arrows indicate the adipocytes, while broken arrows indicate structural fibers (×100).

recorded between males and females in different blubber layers for the three cellular measurements ($p > .05$).

3.2.2 | Age

The average adipocyte cell sizes, numbers, and densities between blubber layers for *T. aduncus* adults and juveniles were compared (Figure 8). No significant differences were found in adipocyte cell sizes between juveniles and adults ($p > .05$; Figure 8). PERMANOVA results for average adipocyte cell numbers were only significant for the middle ($df = 1$; $p = .003$) and superficial ($df = 1$; $p = .001$) layers, with juveniles having significantly more cells than adults in both the middle (78 and 60 cells/468065.1 μm^2, respectively) and superficial (88 and 58 cells/468065.1 μm^2, respectively) layers (Figure 8). Adipocyte cell densities were significantly different in the deep

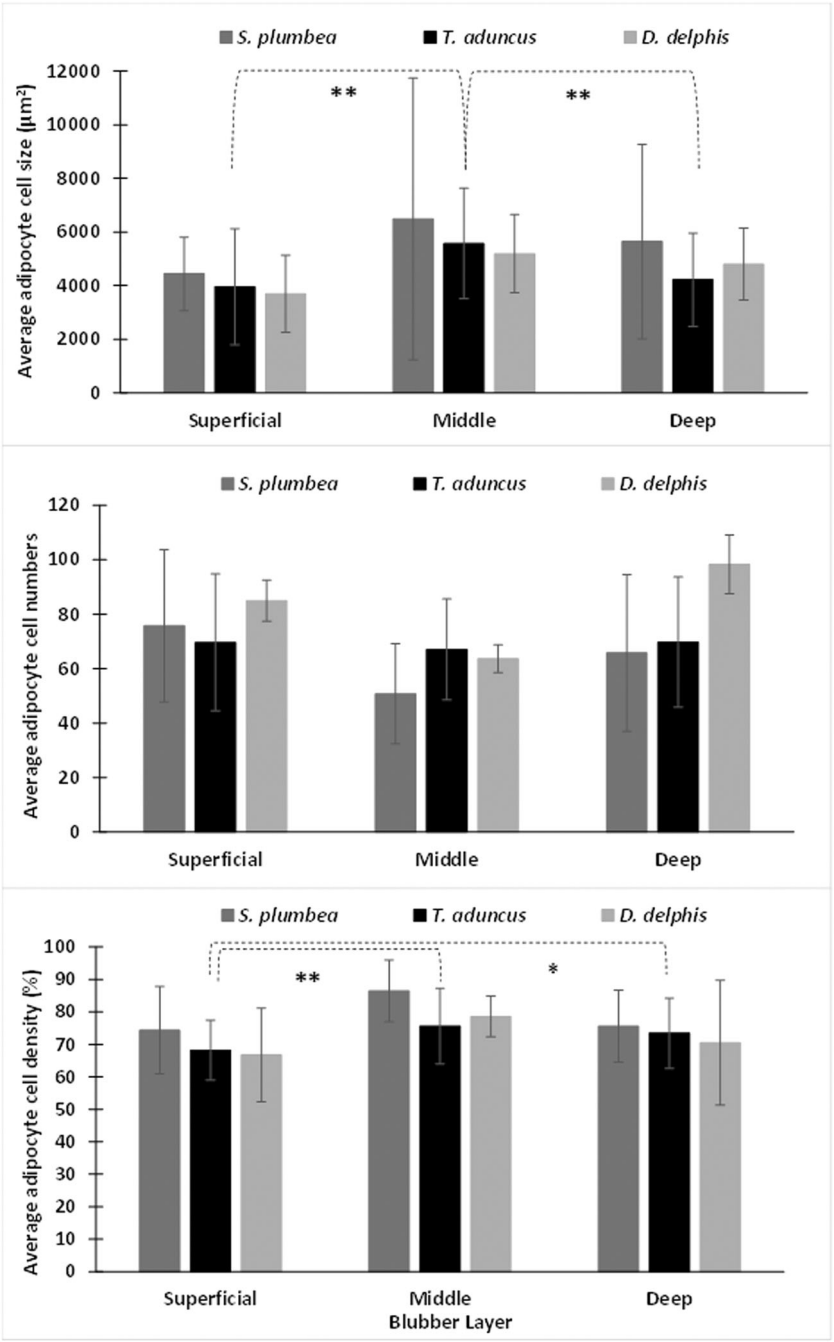

FIGURE 6 Average adipocyte cell size, number, and density of different blubber layers for *Sousa plumbea*, *Tursiops aduncus*, and *Delphinus delphis*, respectively. Error bars represent standard deviation (SD). Significance: $^{*}p \leq .05$; $^{**}p \leq .01$.

(*df* = 1; *p* = .02) and middle (*df* = 1; *p* = .04) layers only; with juveniles once again having higher cell densities (deep: 79%/468065.1 µm^2; middle: 81%/468065.1 µm^2) when compared to adults (deep: 70%/468065.1 µm^2; middle: 72%/468065.1 µm^2) in both layers. All other comparisons showed no significant differences between the two age classes (*p* > .05; Figure 8).

3.2.3 | Season

Lastly, seasonal differences on average adipocyte cell size, number, and density between blubber layers were investigated (Figure 9). However, no significant differences were recorded between winter and summer for the three cellular measurements in the different blubber layers (*p* > .05).

4 | DISCUSSION

4.1 | Blubber stratification in *S. plumbea*, *T. aduncus*, and *D. delphis*

Most marine mammals have been reported to have stratified blubber (Guerrero et al., 2016; Quérouil et al., 2013), which implies that the

TABLE 2 PERMANOVA results for tests of differences in cellular measurements between the three blubber layers.

	PERMANOVA					Post hoc pairwise comparisons		
Cellular measurements	***df***	**SS**	**MS**	***F***	***p***	**Groups**	***T***	***p***
Between species								
Adipocyte cell size	2	498.71	249.35	0.47	.69			
Adipocyte cell numbers	2	1169	584.49	2.47	.07			
Adipocyte cell densities	2	197.83	98.91	1.54	.23			
Sousa plumbea								
Adipocyte cell size	2	664.49	332.24	0.39	.83			
Adipocyte cell numbers	2	701.05	350.52	0.77	.45			
Adipocyte cell densities	2	143.46	71.73	1.33	.32			
Tursiops aduncus								
Adipocyte cell size	2	5834	2917	6.04	.003	Deep, middle	2.62	.008
						Middle, superficial	3.22	.003
Adipocyte cell numbers	2	33.13	16.57	0.07	.98			
Adipocyte cell densities	2	464.24	232.12	3.93	.024	Deep, superficial	2.04	.035
						Middle, superficial	2.76	.009
Delphinus delphis								
Adipocyte cell size	2	474.17	237.09	0.89	.47			
Adipocyte cell numbers	2	703.02	351.51	15.49	.008			
Adipocyte cell densities	2	124.15	62.07	0.56	.61			

Note: Tests were performed both between species and within species. Only the *p* values that represent significant differences (α = .05) in post hoc pairwise comparisons are shown.

Abbreviations: *df*, degrees of freedom; *F*, *F*-statistic (ratio of two variances); MS, mean squares; *p*, probability value; SS, sum of squares; *T*, *T*-statistic (calculated difference represented in units of standard error).

superficial blubber layer has a different composition compared to the deep layer. Our histomorphological analyses revealed that the blubber was stratified in two of the three dolphin species we examined (i.e., *T. aduncus* and *D. capensis*). However, the stratification did not present clearly defined layers, but rather the differences between layers were gradual. A similar gradual transition of blubber layers was found in *B. mysticetus* from Barrow, Alaska (Ball et al., 2015); however, clearly defined blubber layers were reported in *T. truncatus* off the southeastern United States (South Carolina and Florida, Montie et al., 2008). For the three dolphin species investigated in our study, the superficial layer had the smallest cells and lowest cell density, the middle layer had the largest and most dense cells, and the deep layer had an intermediate cell size and density (Figures 5 and 6). Cell size follows a similar trend described by Montie et al. (2008) and Ball et al. (2015), while cell density follows the opposite trend to structural fiber density, namely low cell density, but higher structural fiber density in the superficial layer and highest cell density, but fewer structural fibers in the middle layer. In contrast, our study showed varying cell numbers, with *T. aduncus* and *D. delphis* having the highest cell counts in the deep layer and *S. plumbea* having the highest cell count in the superficial layer. All species had the least number of cells recorded in the middle layer (Figures 5 and 6). Thus, cell numbers per layer would appear to vary between species as has previously been reported for *T. truncatus* and *B. mysticetus* (Ball et al., 2015; Montie et al., 2008). For these species, the lowest cell count was in the superficial and deep layers, but both reported the highest cell numbers in the middle layer. Histological analysis of adipocytes and structural fibers suggests that the deep layers of blubber of several cetacean species are metabolically active, but that the superficial layer of blubber is structural (Koopman et al., 2002; Montie et al., 2008; Struntz et al., 2004). In the metabolic blubber layer of odontocetes in good body condition, the adipocytes are larger than those of the metabolically inert stores (Koopman et al., 2002; Montie et al., 2008; Struntz et al., 2004). During starvation, the metabolically active adipose tissue shrinks significantly, but the metabolically inert ("structural") blubber shows no change from that of a healthy cetacean (Koopman et al., 2002; Struntz et al., 2004).

The stratification of blubber in *T. aduncus* and *D. delphis* is further supported by the PERMANOVA results. For *T. aduncus*, significant differences between the three blubber layers were observed for adipocyte cell size and densities, while results for *D. delphis* indicated a significant difference in adipocyte cell numbers between the three blubber layers. *S. plumbea* does not appear to have any stratification of the blubber, and also had the largest adipocyte cell size and highest densities of cells in all blubber layers when compared to the other

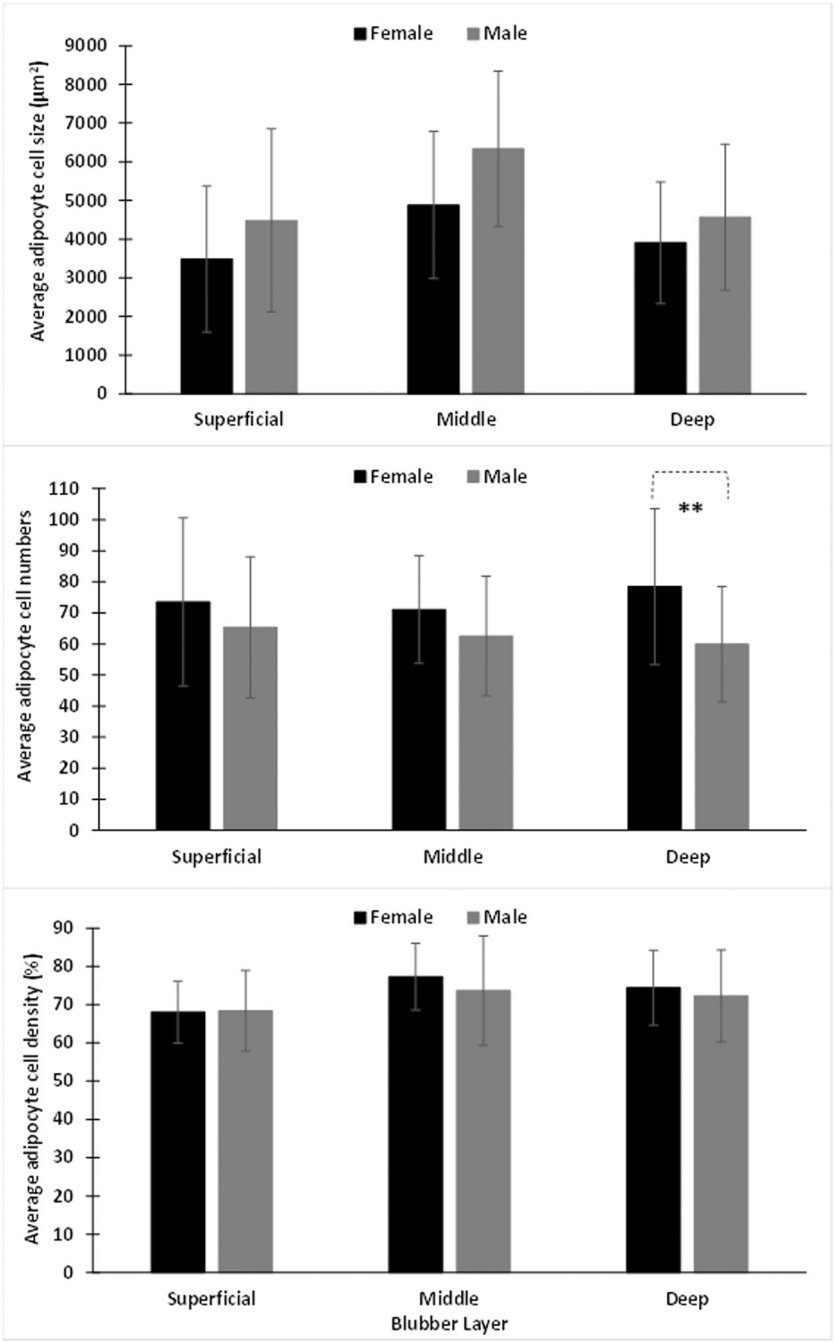

FIGURE 7 Average adipocyte cell size, number, and density of different blubber layers for male and female *Tursiops aduncus*. Error bars represent standard deviation. $^{**}p \leq .01$.

two species (Figures 5 and 6). This may be due to the fact that *S. plumbea* is an inshore subtropical species, whereas *T. aduncus* and *D. delphis* are found further offshore. Although only four animals were investigated in this study, these preliminary results are important, because of the endangered status of the species (Plön et al., 2016) and thus we discuss some possible explanations below.

Blubber has several fundamental adaptive roles in marine mammals. However, the two main functions of blubber are as a storage site for lipids that serve as an energy reserve and as the primary means of maintaining thermal balance in an environment that is usually colder than the body temperature of mammals (Koopman et al., 2002; Strandberg et al., 2008). However, subtropical inshore waters are substantially warmer than offshore waters (Smit et al., 2013). This suggests that *S. plumbea* may not require stratified blubber but rather relies on larger, more densely packed cells to regulate body temperature. In addition, *S. plumbea* also exhibits moderate sexual dimorphism—males are larger, with a maximum length of up to 279 cm, compared to females, which have been recorded of reaching up to 249 cm (Plön, Cockcroft, et al., 2015). This species is thus also the largest of the cetaceans investigated in our study and is reported to be a relatively slow swimmer (Plön et al., 2012). This could have implications for energy reserves, that is, *S. plumbea* may require less energy due to its lower mobility and warmer inshore waters when compared *to T. aduncus* or *D. delphis*.

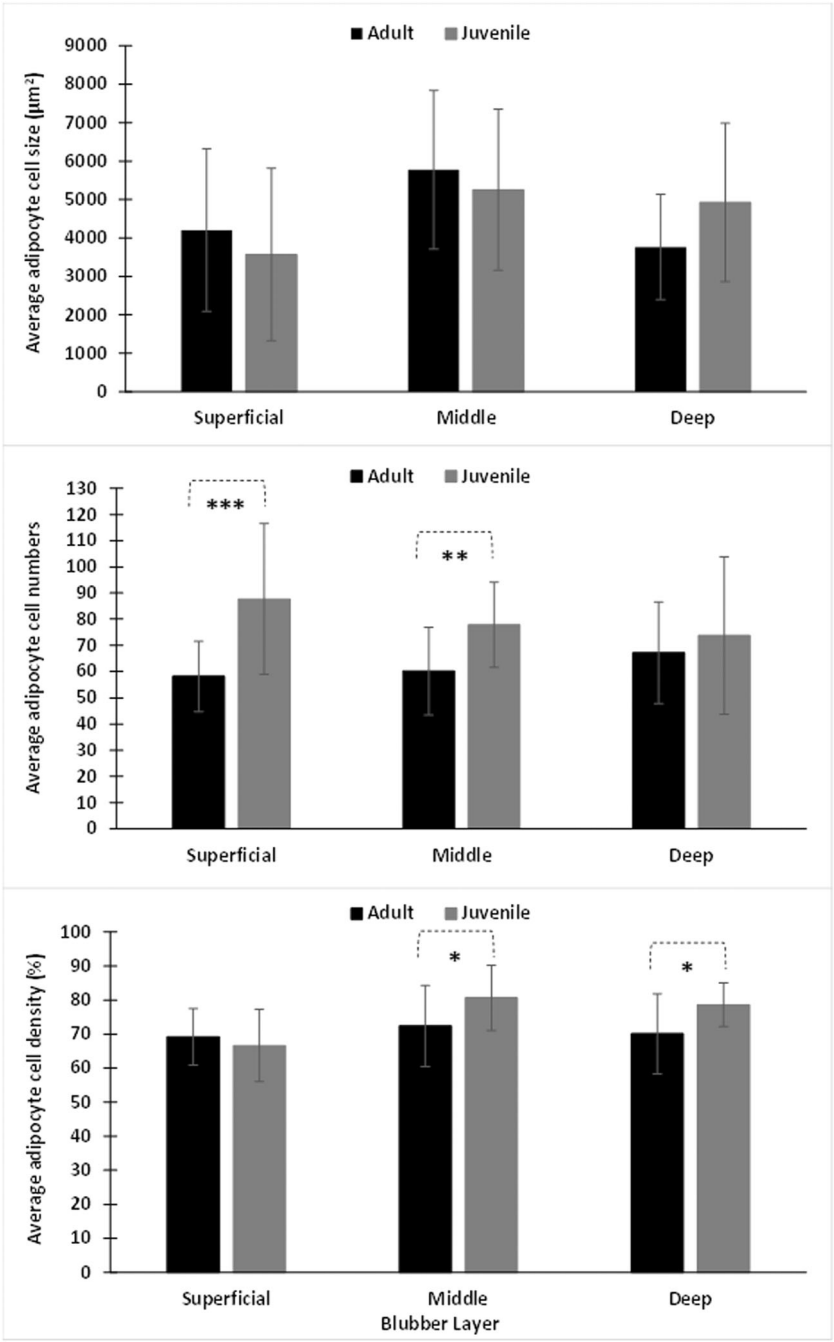

FIGURE 8 Average adipocyte cell size, number, and density of different blubber layers for different age classes of *Tursiops aduncus*. Error bars represent standard deviation. Significance: $^{*}p \leq .05$; $^{**}p \leq .01$; $^{***}p \leq .001$.

However, as this study represents the first histological investigation of blubber for an inshore subtropical species, a larger data set of *S. plumbea* samples and investigations into lipid storage in the blubber would be required to confirm these ideas.

4.2 | Cellular measurements in *T. aduncus* by sex, age class, and season

Correlations with sex, age class, and season in *T. aduncus* were also investigated. Our study found that males and females differed significantly in adipocyte cell numbers within the deep layer, with females having more cells than males. For age class, juveniles displayed significantly higher cell densities than adults in the deep and middle layers, while also having significantly more adipocyte cells in the middle and superficial layer compared to adults. Montie et al. (2008) found no significant differences in blubber morphology between sexes of *T. truncatus* off the south-eastern United States, but reproductively immature subadults had significantly more adipocytes in the middle layer when compared to the adults. Similarly, Ball et al. (2015) investigated differences between reproductively immature, juvenile *B. mysticetus* and adults, finding higher numbers of larger adipocytes at each layer in juveniles. It has been hypothesized that adults display significantly lower total

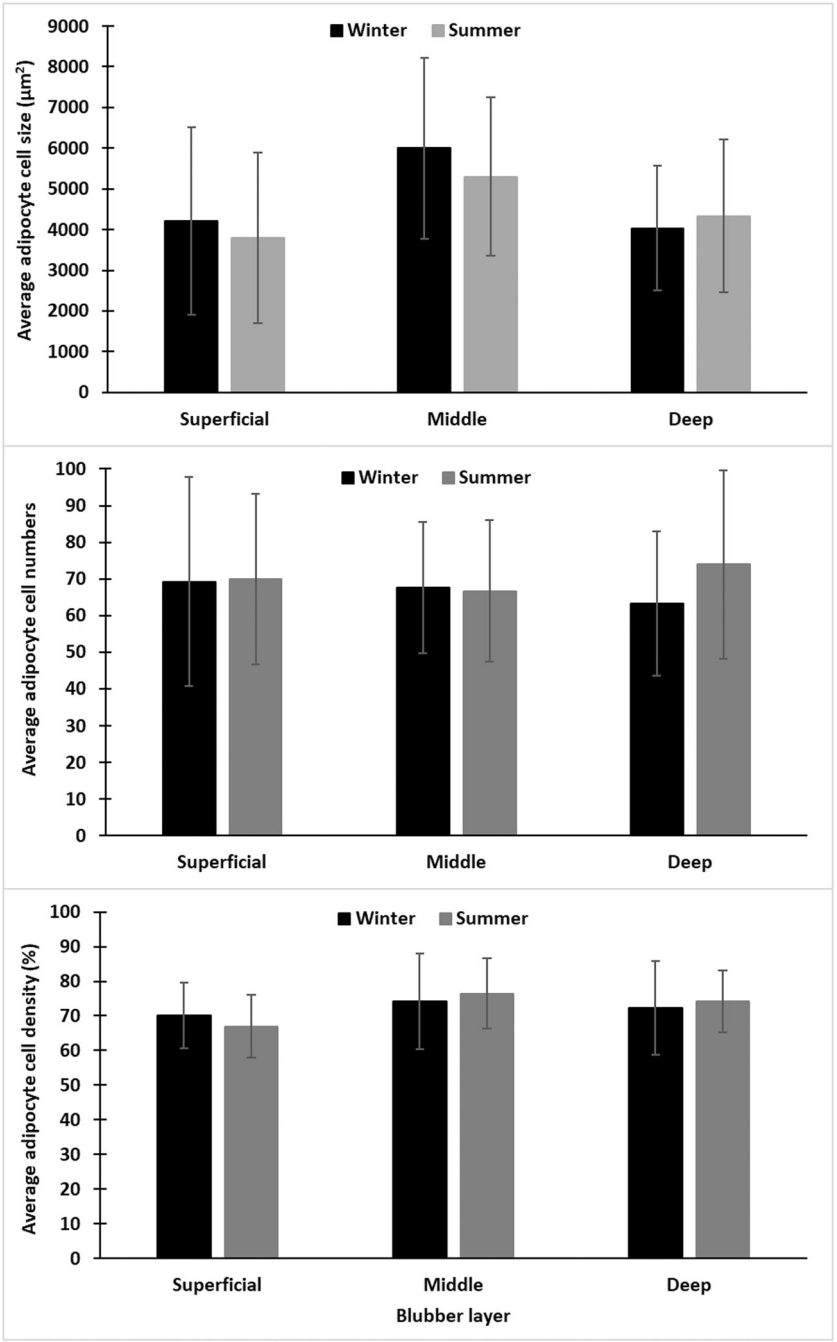

FIGURE 9 Average adipocyte cell size, number, and density of different blubber layers in *Tursiops aduncus* for summer and winter. Error bars represent standard deviation.

blubber lipid content, higher structural fiber areas (i.e., lower cell densities), and lower adipocyte numbers compared to subadults because as the dolphins' surface-area-to-volume ratio decreases with growth, there is less demand for insulation, but greater demand for energy to support growth (Dunkin et al., 2005; Noren & Wells, 2009; Struntz et al., 2004). This hypothesis corresponds to the results of our study. These results differ from those of Struntz et al. (2004) who showed that mean adipocyte sizes increased from *T. truncatus* fetus to adult, while mean adipocyte numbers were not significantly different among life history categories. However, Struntz et al. (2004) included pregnant females in the adult age class (5 of the 6 adults were pregnant females), which may have increased the blubber lipid content and adipocyte areas of adults, since it has been shown that pregnant cetaceans have the highest blubber mass, thickness, and lipid content of all life history categories (Lockyer, 1986; Montie et al., 2008). Our study consisted of 14 adult and 5 juvenile females, with at least 2 lactating females, 1 pregnant female, and 2 showing signs of recent pregnancies (but not lactating, indicating possible abortions) which could have influenced the results obtained in our study. Thus, the reproductive state of females should be taken into consideration in future studies.

Our results showed no significant seasonal differences in adipocyte cell size, number, and density in the blubber layers. In contrast, Ball et al. (2015) found significant seasonal variation in

B. mysticetus blubber between autumn and spring. Larger adipocyte cell sizes were recorded in autumn *B. mysticetus* in all three blubber layers when compared to those examined in spring. However, *B. mysticetus* were sampled in Barrow, Alaska, where temperatures are cold year-round (Ball et al., 2015). Because of this, *B. mysticetus* experience pronounced seasonal fluctuations in feeding patterns, feeding heavily in summer and only intermittently in winter, and hence whales would rely on stored lipid resources to meet energetic demands (Ball et al., 2015). This variation in nutrient availability affects mammalian adipocytes through the changes in cell size, but not the number of cells (Singh et al., 2012), which explains why no other significant differences were found (Ball et al., 2015). Thus, the seasonal nonsignificant difference obtained between blubber layers in our study may be due to the narrow range of environmental temperatures (19–26°C, a temperature difference of 7°C) on the KZN coast. For example, Montie et al. (2008) examined differences in blubber layers of subadults and adults from Charleston and the Indian River Lagoon and reported significant differences between the two geographic regions. The authors recorded consistently colder water temperatures at the station in Charleston Harbor compared to the station in the Indian River Lagoon (temperature differences of more than 10°C between the two regions), indicating that water temperature should influence blubber histomorphology. This was not the case in our study, leading us to conclude that warmer subtropical climates, such as those along the KZN coast, are less likely to affect blubber histomorphology when compared to more temperate climates, such as those in the studies conducted by Montie et al. (2008) and Ball et al. (2015).

In conclusion, our study showed that blubber stratification is not present in all cetaceans, and habitat (e.g., water temperature) and body size potentially influence blubber histomorphology. Other factors, such as sex and age class, were found to influence differences in the blubber morphology of *T. aduncus*. However, season played a less significant role in this study, which was attributed to the narrow temperature ranges between seasons in the subtropical habitat. This study represents the first investigation on blubber histomorphology in subtropical cetacean species and as such, additional information is required to better understand the structure, function, and physiology of blubber in the subtropics. One potential factor influencing blubber histomorphology not investigated in this study is blubber lipid content. Because lipid deposition and utilization are dynamic and are involved in the response to changing environmental and physiological conditions (Marón et al., 2021), it would influence the structure and function of adipocyte cells in the different blubber layers. Future studies should also take into consideration the reproductive state of the females and the temperature range of their study area, which could potentially affect blubber histomorphology.

Marine mammal body composition, defined as the "ratio of blubber mass to either total body mass or lean mass" (Adamczak et al., 2021), has recently been an important tool used as a proxy for the health and condition of individuals within a population (Adamczak et al., 2021; Castrillon et al., 2017; Castrillon & Nash, 2020; Derous et al., 2020; Marón et al., 2021). Recent global trends in climate change and overfishing have raised multiple issues concerning changes in the distribution and abundance of fish stocks (Barange et al., 2018; Castrillon & Nash, 2020). These changes can lead to lower prey availability for marine mammal populations, resulting in poorer health and condition (Simmonds & Isaac, 2007). Marine mammals are top predators of the marine food web and are increasingly being recognized as important indicators for ocean health (Fossi et al., 2020; Plön et al., 2021). Using body condition as a sentinel parameter to monitor ecosystem health, recent studies have used the adipocyte index (AI) obtained from biopsy samples of live humpback whales (*Megaptera novaeangliae*; Castrillon et al., 2017) as a rapid, nonlethal and low-cost method to monitor body condition. Thus, the AI could also serve as a proxy for body condition in dead odontocetes.

AUTHOR CONTRIBUTIONS

Natasha Roussouw: Data curation (supporting); formal analysis (lead); writing–original draft (lead); writing–review and editing (lead). **Tara van Vliet**: Data curation (lead); investigation (lead); methodology (supporting); visualization (supporting). **Kristina Naidoo**: Writing–review & editing (supporting). **Gideon Rossouw**: Supervision (supporting); visualization (supporting). **Stephanie Plön**: Conceptualization (lead); funding acquisition (lead); methodology (lead); project administration (lead); supervision (lead); visualization (lead); writing–original draft (supporting); writing–review & editing (supporting).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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