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Topographical variation in lipid content and morphological structure of the blubber in the striped dolphin

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Summary: We investigate stratification patterns and topographical variations in the blubber of the striped dolphin (*Stenella coeruleoalba*) to gain insights into its regionally-specific functions. We collected blubber from 10 stranded striped dolphins (5 females and 5 males) from the eastern coast of Spain in 2007-2009, at 11 body positions. Histological measurements (adipocyte number and area) and blubber lipid content were analysed for each position. Histological measurements revealed stratification of blubber into outer, middle, and inner layers. Both the adipocyte number and area were largest in the middle layer. The adipocyte number was higher in the outer than the inner layer, whereas the adipocyte area was higher in the inner than the outer layer. The ventral anterior position did not follow this pattern, likely due to its proximity to the acoustic blubber, which is known to have a different biochemical composition. The stratification in morphological blubber characteristics most likely reflects functional differences. The outer layer may provide structural support and act as a mechanical barrier with a minor role in energy storage. The middle layer may be responsible for thermoregulation, and the inner layer could be responsible for energy mobilization, which is favoured by its proximity to the body core and a higher vascularization. In addition, an increasing gradient from dorsal to ventral positions was observed in the mean number of adipocytes and lipid content, with the exception of the caudal region. Although both ventral and dorsal blubber can have insulator and buoyancy functions, the ventral blubber may mainly serve as an energy reserve.

Keywords: *Stenella coeruleoalba*; cetacean; adipocytes; histology; stratification; lipid content.

Estructura morfológica y variación topográfica del contenido lipídico en la grasa del delfín listado

Resumen: En el presente estudio se investigaron los patrones de estratificación así como las variaciones topográficas en la grasa del delfín listado (*Stenella coeruleoalba*) con el fin de conocer mejor las posibles funciones regionales de este tejido. Se recolectaron muestras de grasa en 11 posiciones corporales diferentes de 10 delfines listados (5 hembras y 5 machos) varados en la costa este de España entre los años 2007 y 2009. Las medidas histológicas (número de adipocitos y área de los mismos) y el contenido lipídico se analizaron en cada una de las posiciones. Las medidas histológicas mostraron que la grasa se estratificaba en 3 capas: externa, media e interna. Tanto el número de adipocitos como su área eran mayores en la capa media. El número de adipocitos era superior en la capa externa que en la interna, mientras que el área de los adipocitos era mayor en la capa interna que en la externa. Sin embargo, la región ventral anterior no seguía este patrón, probablemente esto sea debido a que esta región se encuentra muy próxima a la grasa acústica, que es conocida por presentar una composición bioquímica diferente al resto de grasa corporal. La estratificación morfológica observada en la grasa probablemente esté reflejando una diferenciación funcional. La capa más externa podría dar soporte estructural y actuar como barrera mecánica, con un papel menos importante como almacén de reservas energéticas. La capa media podría ser responsable de la termorregulación, mientras que en la capa interna se produciría la movilización energética, lo cual se vería favorecido por su elevada vascularización y por estar en contacto con el interior del cuerpo. Además, se observó que tanto el número de adipocitos como el contenido lipídico seguían un gradiente creciente desde las posiciones dorsales a las ventrales, a excepción de la región caudal. Aunque tanto la grasa de la región dorsal como la de la ventral pueden presentar funciones de flotabilidad y aislamiento térmico, la grasa de la región ventral podría estar actuando principalmente como región de reserva energética.

Palabras clave: *Stenella coeruleoalba;* cetáceos; adipocitos; histología; estratificación; contenido lipídico.

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INTRODUCTION

Blubber is a characteristic and specialized tissue beneath the skin that is nearly continuous across the body of marine mammals. It is composed of numerous adipocytes, which play an important role in energy and glucose metabolism (Chen and Farese 2002), and it is surrounded by structural collagen and elastic fibres (Iverson 2009). This subcutaneous layer is recognized as the main tissue for energy storage, in the form of lipids, in this mammalian group (Aguilar and Borrell 1990, Koopman et al. 1996, 2002, Struntz et al. 2004, *inter alia*). In addition to serving as a depot of energy, it is also the primary thermal barrier in the aquatic environment (Worthy and Edwards 1990, Dunkin et al. 2005, Bagge et al. 2012), streamlines the body and facilitates hydrodynamic locomotion (Pabst 2000, Fish 2000, Hamilton et al. 2004), and contributes to water balance and provides buoyancy (Dearolf et al. 2000, Kipps et al. 2002, McLellan et al. 2002, Dunkin et al. 2010).

Some studies have shown that blubber in cetaceans is a histologically and biochemically structured tissue. Biochemically, the blubber structure is based on the lipid content, and the composition and distribution of fatty acids have been widely studied in cetacean species. Studies in balaenopterids and balaenids (Ackman et al. 1975, Aguilar and Borrell 1990, Moller et al. 2003, Olsen and Grahl-Nielsen 2003, Budget et al. 2008, *inter alia*) and to a lesser degree in odontocetes (Ackman 1971, Evans et al. 2003, Samuel and Worthy 2004, Smith and Worthy 2006, Koopman 2007, Koopman et al. 2002, Bagge et al. 2012 *inter alia*), have demonstrated that the lipid content and quantity and quality of fatty acids vary among blubber layers, indicating blubber stratification. Blubber was found to be histologically stratified in different layers in the harbour porpoise, *Phocoena phocoena*, (Koopman et al. 2002) and the bottlenose dolphin, *Tursiops truncatus* (Struntz et al. 2004, Montie et al. 2008), based on the size, shape, and metabolic characteristics of the adipocytes as well as on the abundance of collagen fibres.

The histological and biochemical structure of blubber can also vary greatly within the body of an individual in some cetacean species, and these differences are likely associated with different localized functions (Aguilar and Borrell 1990, 1994, Lockyer 1995, Olsen and Grahl-Nielsen 2003, Iverson 2009). In the harbour porpoise (*Phocoena phocoena*), the structure and composition of the thoracic-abdominal blubber area suggest an important role in insulation and energy storage, whereas in the area posterior to the dorsal fin or at the caudal peduncle, blubber may serve roles in maintaining hydrodynamic shape and other locomotory functions (Koopman 1998, Pabst et al. 1999). The study of how blubber reacts at different sites during fat mobilization periods may provide further insight into the adaptations of blubber structure as well as local functions.

In addition to general species characteristics, the structural and chemical composition of blubber is related to various physiological processes, including growth, age, nutritional condition, and reproduction (Koopman et al. 2002, Dunkin et al. 2005, Montie et al. 2008, Dunkin et al. 2010). The ultrastructure, thickness, and proximate composition of blubber can provide insight not only into the functional significance of the blubber itself, but also into aspects of individual foraging ecology, feeding habits and status, and environmental adaptations and species distribution (Herman et al. 2003, Smith and Worthy 2006, Bugde et al. 2008, Montie et al. 2008).

In order to understand the different blubber functions, this study investigates blubber morphology and topographical variations in the striped dolphin (*Stenella coeruleoalba*) by measuring histological characteristics and lipid content of blubber in different body positions. The results obtained will contribute with new data to increase the scarce blubber pattern information existing in small cetaceans and provide the first analysis in a pelagic and a relatively deep-diving delphinid.

MATERIALS AND METHODS

Sample collection

We collected blubber samples from 10 fresh carcasses of striped dolphins, 5 females and 5 males in different life history categories (Table 1), stranded along the eastern coast of Spain (western Mediterranean Sea) in 2007-2009. To minimize post-mortem degradation of blubber, only animals with a Smithsonian Institute code of 1 (live stranded and died naturally or by euthanasia) or 2 (fresh dead) (Geraci and Lounsbury 1993) were considered. Dolphins showed a normal to robust body condition, according to Cox et al. (1998) and McLellan et al. (2002) (Table 1). Blubber samples, including the entire thickness, were excised from a total of 11 body positions from each individual: four dorsal positions (1, 3, 6 and 9), three lateral positions (4, 7 and 10), and four ventral positions (2, 5, 8 and 11), as shown in Figure 1. These positions were chosen as representative of the entire body. Each sample was subdivided into two full-depth sub-samples immediately after collection; one sub-sample was fixed in 10% neutral buffered formalin (NBF) for histological analysis, whereas the other was preserved at –20°C to determine the tissue lipid content.

Table 1. – Striped dolphin (*Stenella coeruleoalba*) specimens used in this study.

Dolphin ID code	Total body length (cm)	Sex	Life history category	Body condition
Scoe 2007-9	104	female	inactive	normal
Scoe 2008-10	185	female	resting	robust
Scoe 2008-6	189	female	resting	normal
Scoe 2008-8	190	female	lactating	normal
Scoe 2008-1	210	female	lactating	normal
Scoe 2008-7	185	male	immature	robust
Scoe 2008-2	194	male	mature	normal
Scoe 2008-11	204	male	mature	normal
Scoe 2008-9	205	male	mature	robust
Scoe 2009-1	224	male	mature	normal

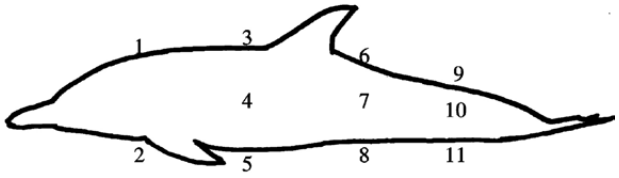

Fig. 1. – Blubber sampling locations in the striped dolphin (*S. coeruleoalba*).

Histological analysis

For morphological measurements, the tissue samples were fixed in 10% NBF, embedded in paraffin, cut into 10 µm sections, and stained with hematoxylin and eosin (H&E) following standard methods previously described (Chen and Farese 2002, Struntz et al. 2004, Montie et al. 2008). Slides were viewed with a BX-61 Olympus microscope and colour images were acquired with an Olympus DP-70 digital camera. Contiguous images were captured along the entire depth of the blubber, from the epidermis to the deep blubber layer that formed a transect every 2.25 mm at 40× magnification. The number of blubber depth intervals (represented each one by 2.15×1.60 mm image of blubber in histological measurements) varied among individuals and body positions and ranged from 4 (0.9 cm from the epidermis) to 10 (2.6 cm from the epidermis) (Figs 2A-2C). Naturally occurring landmarks in the blubber were used to avoid overlap of adjacent images.

Histological measurements

Images were analysed using Infinity Analyze 4.2 software (2007 Lumenera Corporation, Ottawa, Ontario, Canada). Within each 2.15×1.60 mm image of blubber, a 1×1 mm box was positioned approximately in the centre (Fig. 2D). The number of adipocytes was estimated by determining the number of cells that intersected each diagonal of the 1 mm^2 grid and then averaging the two cell counts, following the methods used by Montie et al. (2008). The size of the adipocytes (i.e. adipocyte areas) was measured using an area tool in the Infinity Analyze software and estimated in each

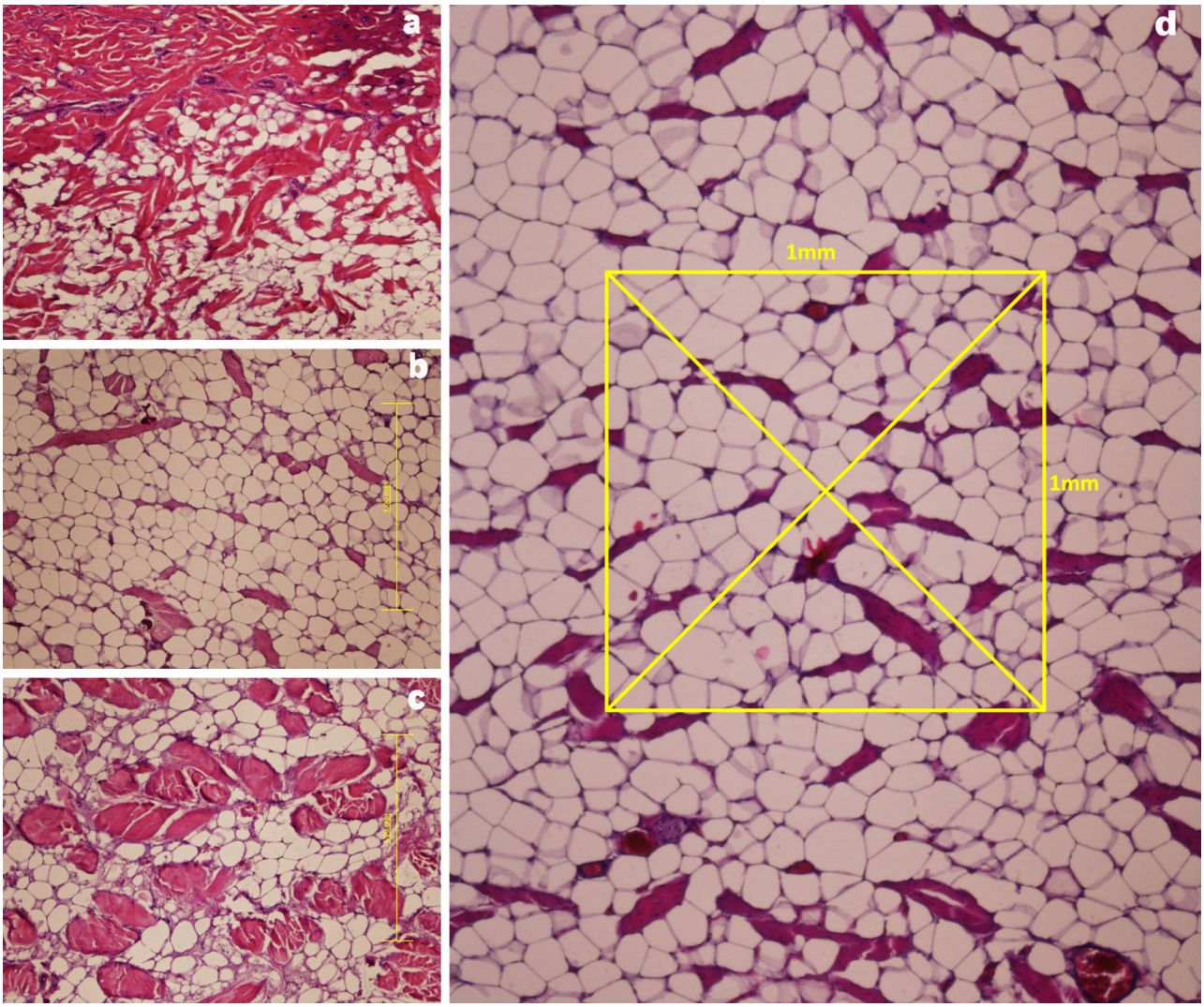

Fig. 2. – Representative hematoxylin and eosin slides of a blubber sample analysed showing the (A) outer, (B) middle, and (C) inner layers and (D) the 1×1 mm box containing diagonals used for quantitative histological analyses.

specific blubber image by averaging the areas of the cells that intersected the upper left diagonal. These cellular measurements (adipocyte number and size) were used to generate mean values for each blubber depth interval. For statistical analyses, the cellular measurements for each blubber depth interval were combined based on the categorization of each interval as part of the outer, middle, or inner layer and averaged for a layer-specific measurement.

The blubber layers limits were not discrete, but since the eosin stain intensity increases according to an increasing density of structural fibres, it allowed the layers to be differentiated, following previous studies in bottlenose dolphin (Struntz et al. 2004, Montie et al. 2008). Therefore, the outer layer extended from the dermal papillae to the boundary where the eosin stain decreased in intensity, the middle layer extended from the deep limit of the outer layer to the boundary where the eosin stain increased in intensity, and finally, the inner layer extended from the deep limit of the middle blubber layer to the subdermal sheath that separates the blubber from the underlying muscle (Montie et al. 2008).

Lipid content

The samples (1 g approx.) were ground with anhydrous sodium sulphate and extracted with n-hexane in a Soxhlet apparatus for 4 h. The solution obtained was concentrated to 40 ml and a portion of this extract (10 ml) was used to determine the quantity of extractable lipids by gravimetry (Aguilar and Borrell 1990). Lipid content was determined for the full-depth blubber sample and not for separated layers, because differences among them were only noticeable when the samples were stained. The results were expressed as a percentage of the tissue wet weight.

Statistical analyses

Before analysing the data, normality was tested with the Kolmogorov-Smirnoff test and the homogeneity of variances was tested with Levene's test. We first investigated variations in mean adipocyte number and area among blubber layers and body position with mixed-effects ANOVA, considering individual as a random variable and blubber layer and body position as fixed variables. Blubber lipid content variation among body positions was investigated through a one-way ANOVA. When differences were detected in ANOVA analyses, they were explored through a post-hoc Tukey test. Statistical significance was considered at a p value <0.05 for all analyses. Statistical analyses of the data were performed using the SPSS 15.0 statistical package (IBM, USA).

The mean adipocyte number and blubber lipid content were distributed normally and displayed homogenous variance ($p>0.202$ for both), whereas the mean adipocyte areas were log-transformed to fulfil the assumptions of normality.

Differences across sex and life history status of the individuals could not be detected, probably due to the limited group sizes across these categories.

RESULTS

Histological analysis showed that the blubber of striped dolphins was morphologically stratified into "outer", "middle", and "inner" layers (see Fig. 2).

Blubber stratification

Mean adipocyte number varied significantly among blubber layers ($p<0.001$) and body positions ($p=0.002$) (Fig. 3), and the interaction between the blubber layer and body position was significant ($p=0.008$). However, graphical analyses of the data showed that blubber in position 2 (Fig. 3B) differed in the adipocyte number pattern from the rest of the body positions (Fig. 3A). By excluding body position 2 from the ANOVA analysis, we found that the interaction between the body position and blubber layer was not significant ($p=0.213$), which indicated that the blubber stratification pattern for adipocyte number was the same in all body positions considered, with the exception of position 2. According to that irregular behaviour, statistical analyses for adipocyte number were carried out by excluding position 2 and considering it as a separate position. The number of adipocytes (mean ± sd) was highest in the middle layer (29.36±5.82 cells per field), intermediate in the outer layer (25.49±7.54 cells per field), and lowest in the inner layer (22.49±8.36 cells per field) ($p<0.008$; Fig. 3A). In position 2, the number of adipocytes was highest in the outer layer (34.85±6.15 cells per field), intermediate in the middle (27.13±5.97 cells per field), and lowest in the inner layer (20.57±6.26 cells per field) ($p<0.001$; Fig. 3B).

Mean adipocyte areas varied significantly among blubber layers ($p<0.001$) and body positions ($p=0.001$), and the interaction between them was not significant ($p=0.051$). The middle layer showed the largest mean adipocyte area (2957.80±1142.47 μm^2), the inner layer showed an intermediate mean area (2051.46±1009.55 μm^2), and the outer layer showed the smallest adipocyte area (1234.55±524.41 μm^2) (Fig. 3C).

Differences across body positions

Although an increasing gradient was observed in the mean number of adipocytes from dorsal to ventral positions, with the exception of the caudal region, significant differences were only detected between dorsal position 6 and ventral positions 5 and 8 ($p<0.009$ for both). Ventral position 5 also showed a higher mean adipocyte number than dorsal position 9 ($p=0.040$). A comparison of the mean adipocyte area among body positions indicated that only position 2 showed significantly larger adipocytes than positions 5, 6, 8, 9 and 11 ($p<0.011$).

Blubber lipid content increased from dorsal to ventral positions, with the exception of the caudal region, following the same pattern as the mean adipocyte number. In the ventral region, a decreasing anterior-posterior gradient was observed. Interestingly, higher lipid concentrations were present in the zone composed by positions 2, 3, 4, 5 and 8, indicating that position 2

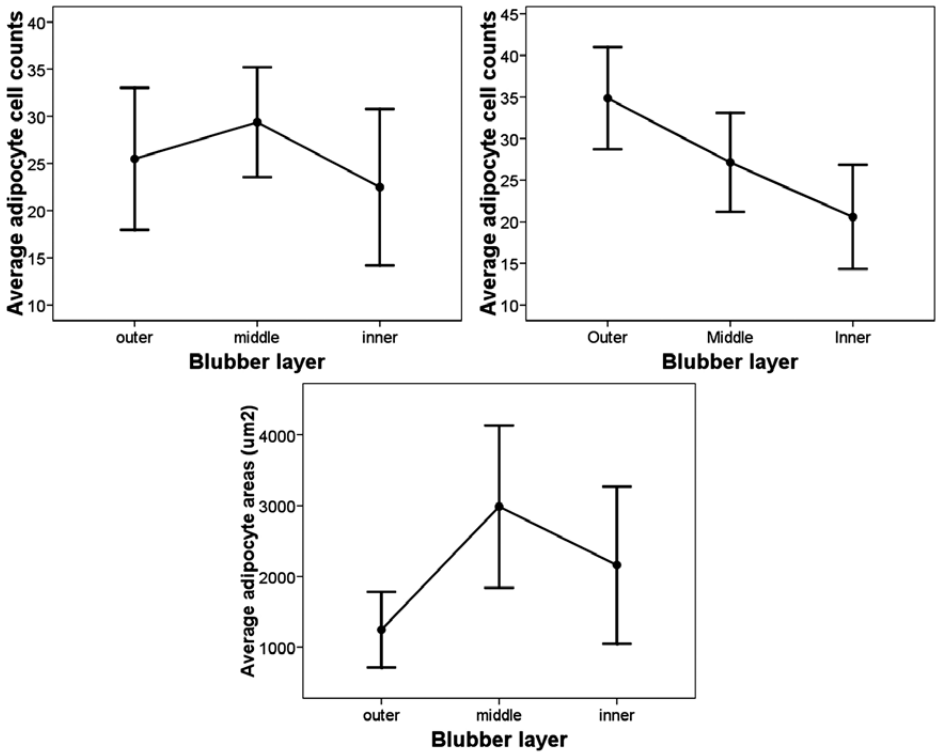

Fig. 3. – The pattern of mean adipocyte number in blubber layers for all positions except 2 (A), position 2 (B), and average adipocyte areas in each layer for all positions (C).

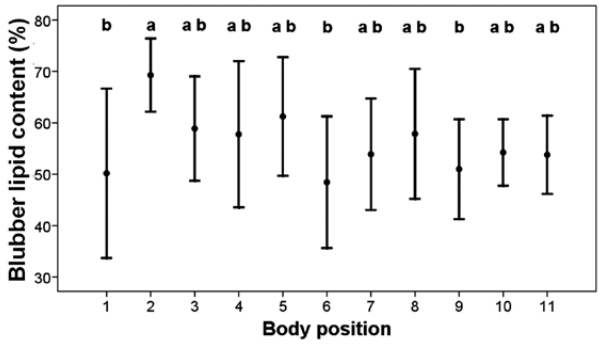

Fig. 4. – Mean and standard deviation of blubber lipid content (%) in the 11 body positions. The letters on top depict homogeneous statistical sub-grouping by Tukey test.

had the highest lipid concentration among all positions. However, significant differences were only detected between ventral position 2 and dorsal positions 1, 6 and 9 (Fig. 4). If position 2 was omitted from the analysis, significant differences among dorsal positions were not detected.

DISCUSSION

Blubber stratification

Histological analyses performed in the present study indicate that blubber of the striped dolphin can be described as being morphologically stratified (Figs 2 and 3). The three layers (outer, middle and inner) were characterized by differences in both the number and size of the adipocytes. The outer blubber layer contained the smallest adipocytes and had an intermediate number of cells. The middle layer contained the largest and most abundant adipocytes, and the inner layer contained adipocytes of intermediate size and had the lowest cell number. This pattern was consistent for all 11 body positions examined (Figs 3A, C), with the exception of position 2, in which the number of adipocytes decreased progressively from the outer to the inner layer (Fig. 3B). Although structural fibre areas were not analysed in our study, the gross examination of histological images confirmed that the structural fibres were highest in the outer layer, lowest in the middle, and intermediate in the inner layer. Adipocyte numbers seem to be inversely related to the presence of structural fibres in each blubber layer, according to Montie et al. (2008).

Stratification results obtained in the present study could be related to heterogeneous functions of the different layers. The lower number of adipocytes and intermediate cell sizes in the outer layer, as well as the higher presence of structural fibres, seems to indicate that this layer plays an important role in structure and a minor role in energy storage. This layer could provide important structural support to epidermis and dermis and acts as a mechanical barrier. In fin whales, the outer layer was found to be a stable layer and not influenced by age, sex or reproductive state (Aguilar and Borrell 1990). The large and numerous adipocytes in the middle layer and the lower presence of structural fibres suggest that it acts as a reservoir of lipids. This higher lipid content could be related to thermoregulation or buoyancy functions. The middle situation of this lipid reservoir in blubber could be related to its

preservation, in order to ensure these vital functions for the survival of these animals. These findings are in agreement with those of Montie et al. (2008), who found that changes in blubber, which are related to thermoregulation, affect the adipocytes of the middle layer in bottlenose dolphins and therefore suggest that this blubber layer provides greater thermoregulation than the other layers. Bagge et al. (2012) proposed that blubber lipid content may act as a dynamic thermal buffer, with the capacity to store and release heat, although this statement does not strictly refer to any particular blubber layer. In the current study, the inner layer showed smaller adipocytes than the middle layer but larger ones than the outer layer. The lower number of adypocites in the inner layer could be related to the higher degree of structural fibres present in this layer, compared with the middle layer. It has been proposed that the inner layer is a metabolically active layer that is responsible for energy mobilization in high energy-demanding processes in some cetaceans, such as lactation or starvation (Aguilar and Borrell 1990, Koopman et al. 2002, 2003, Struntz et al. 2004, Montie et al. 2008, *inter alia*). This is in accordance with our observations, if the smaller adipocytes in the inner layer are considered to be a representation of lipid loss compared with the larger adipocytes in the middle layer. The proximity of the inner layer to the body core, the assumed higher vascularization in this layer, and the temperature gradient through the depth of the blubber (Castellini 2009, Bagge et al. 2012) would facilitate lipid mobilization from this layer into the circulation. Furthermore, Struntz et al. (2004) found that in bottlenose dolphins significantly greater levels of long-chain fatty acids are present in the inner layer, providing more energy than the short-chain fatty acids that are more abundant in the middle and outer layers. Lipid composition stratification provides strong support for functional differences across layers.

Morphological stratification has previously been addressed in a few cetacean species, such as harbour porpoises (*Phocoena phocoena*) (Koopman et al. 2002) and the bottlenose dolphins (*Tursiops truncatus*) (Struntz et al. 2004, Montie et al. 2008). The results obtained in the present study are consistent with those obtained for the bottlenose dolphins (Struntz et al. 2004, Montie et al. 2008) and similar to those from harbour porpoises (Koopman et al. 2002), although in the latter study only the outer and inner layers were described. Heterogeneous functions of the different blubber layers based on its stratification have been documented in different cetacean species (Aguilar and Borrell 1990, Koopman et al. 2002, Koopman 2007, Samuel and Worthy 2004).

Although the adipocyte area pattern of ventral position 2 was similar to that of other positions, we observed that this position differed from the rest in its stratification pattern, with a decreasing number of adipocytes from the outer to inner layer. This ventral position is located at the posterior end of the lower jaw and in front of and between the anterior insertions of flippers (Fig. 1). In odontocetes, the melon and the large fat bodies found in and around the mandibular region serve as part of the acoustic pathway and are known as the acoustical window for sound transmission (Norris et al. 1961, Norris 1968, Norris and Harvey 1974). The biochemical composition of these "acoustic fats bodies" is different to that of body blubber (Varanasi and Malins 1970, Ackman et al. 1971, Litchfield et al. 1975, Koopman et al. 2006). Acoustic fat bodies contain a high quantity of lipids with high concentrations of unusual endogenous lipids, and their biochemical composition does not seem to be influenced by diet. These fat bodies do not change in lipid content or composition during fasting and starvation, are quite metabolically stable (Pond 1998, Cranford et al. 1996, Koopman et al. 2002, 2003), and do not exhibit biochemical stratification (Zahorodny Duggan et al. 2009), although acoustic fat depots showed a biochemical composition gradient to channel sound toward ears (Koopman et al. 2006). Some studies have addressed the morphology of melon and acoustic fat depots in the striped dolphin (Scano et al. 2005, Maxia et al. 2007), but performed no comparison with morphological structure from body blubber. Although blubber from position 2 does not properly correspond to the acoustic fat region, it is clearly adjacent to the jaw. The fact that position 2 is located in a transition area between two blubber zones with different biochemical compositions, such as acoustic fat and body blubber, would explain the observed differences in the stratification pattern compared with other body positions.

Differences across body positions

Topographical differences were detected among the body positions considered in the striped dolphin blubber. An increasing gradient from dorsal to ventral positions was observed in the number of adipocytes and blubber lipid content, although the differences were only significant between specific positions. Dorsal positions 6 and 9 showed a lower adipocyte number and lipid content than ventral positions 5 and 8. These results indicate that blubber from the ventral region has a high number of adipocytes and lipid concentration and most likely responds to the function of energy storage in the form of lipids. The information available on topographical variation in histological structure, composition and physicochemical properties of blubber indicates that it has different functions depending on the body location. In balaenopterids, the anterior ventral blubber forms the semi-elastic feeding grooves, which permit the distension of the mouth and throat while feeding. This implies that blubber from this region is composed of abundant structural collagen and a lower lipid content than the dorsal posterior region in fin (*Balaenoptera physalus*), sei (*B. borealis*), and minke whales (*B. acutorostrata*) (Watanabe and Suzuki 1950a, Lockyer et al. 1984, 1985, Kvadsheim et al. 1996). These findings also indicate that the dorsal posterior region is the main body location for energy storage in balaneopterids (Lockyer et al. 1985). Although the anterior ventral region has no specialized feeding functions in sperm whales (*Physeter macrocephalus*), the dorsal posterior and lateral regions of

the body also display the highest lipid content (Watanabe and Suzuki 1950b, Lockyer 1991). It has been suggested that since the blubber layer is also thicker in the dorsal area in balaenopterids and sperm whales, lipids may accumulate in this region to shape the body and possibly regulate buoyancy (Lockyer et al. 1984, Iverson 2009). Blubber is less dense than water, aiding the body by decreasing the overall density of the body and therefore increasing its buoyancy (Taylor 1994, Kipps et al. 2002). The information currently available on small cetaceans is comparatively limited. In pilot whales (*Globicephala melas*), topographical variation in lipid content is nonexistent or at least masked by other variables, such as seasonal variation (Lockyer 1993). In harbour porpoises, the variation pattern of lipid content has been found to be either nonexistent (Tilbury et al. 1997) or to be opposite to that found in large cetaceans, with the lipid content in the harbour porpoise being lowest in the blubber of the dorsal posterior region and highest in the anterior ventral region (Calambokidis 1986, Ishaq et al. 2000).

In harbour porpoises, blubber has been divided into two functional components based on significant differences in blubber characteristics between the thorax and abdomen, which were found when individuals in different reproductive classes as well as healthy vs. starved porpoises were compared (Koopman 1998, Koopman et al. 2002). A line of division occurring at the level of the anus can separate the blubber into two functional components, with one anterior and one posterior. Both parts have insulator and buoyancy functions, but the posterior section mainly serves as a structural element to streamline the body (Hamilton et al. 2004). The blubber from the anterior section functions primarily as an insulator, because the main muscles and organs are concentrated at this site, and secondly as a limited short-term energy reserve (Koopman 1998). In the common dolphin (*Delphinus* sp.), the lipid content progressively decreases from head to tail and from belly to back (Tornero et al. 2006), matching the pattern observed in harbour porpoises. These findings suggest that in common dolphins the anterior ventral region is also more important for insulation and lipid storage than the dorsal posterior region, perhaps to provide thermal protection to the viscera (Tornero et al. 2006). According to our study and the results obtained for harbour porpoises and common dolphins, both the number of adipocytes and the lipid content in the blubber of striped dolphins increased from the dorsal to the medio-ventral region, indicating that this zone has a more important role in lipid storage and insulation.

As mentioned in the Material and Methods section, differences could not be detected between different life history groups due to the limited group sizes, although some studies have found differences in blubber characteristics between reproductive states (Samuel and Worthy 2004, Dunkin et al. 2005, Montie et al. 2008, Noren and Wells 2009). It is known that pregnancy and lactation are conditions that may affect the dynamics of blubber lipids in cetaceans: higher lipid content and swelling of adipocytes are associated with pregnancy, whereas lower lipid content and shrinkage of adipocytes are associated with lactation (Aguilar and Borrell 1990, Struntz et al. 2004, Dunkin et al. 2005, Montie et al. 2008).

Further investigations are needed to confirm whether these types of variations may also be present in the striped dolphin and to address biochemical stratification in blubber to support some of the functions proposed in the present study for blubber layers and topographical regions in the striped dolphin.

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