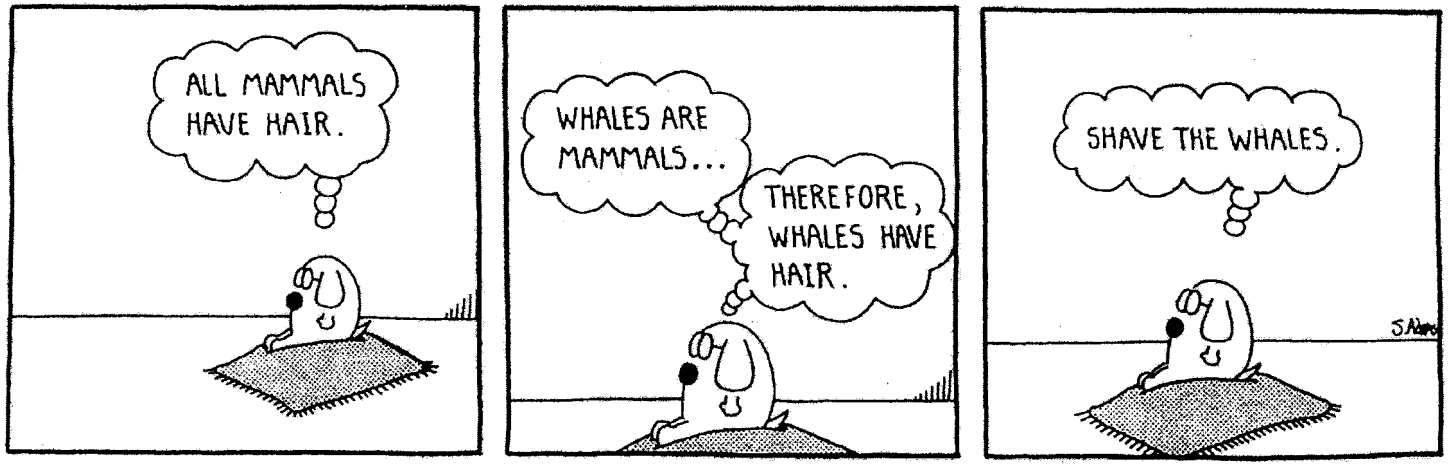
ALL MAMMALS HAVE HAIR.
WHALES ARE MAMMALS...
THEREFORE, WHALES HAVE HAIR.
SHAVE THE WHALES.

Structure, development and composition of the integument of the southern right whale, *Eubalaena australis*.

by

Desray Reeb

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Structure, development and composition of the integument of the southern right whale, *Eubalaena australis*.

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Abstract

The general architecture of the skin of the southern right whale, *Eubalaena australis*, is comparable to that described for other cetacean species. As found in bowhead whales, *Balaena mysticetus*, of the same family, southern right whales possess an acanthotic epidermis and a notably thick hypodermis. Epidermal rods and extensive papillomatosis support these unique characteristics. A "fat-free" reticular dermis makes the integument of southern right whales more like that of odontocetes than that described for balaenopterids. Skin samples taken in South African and Antarctic waters showed evidence of superficial moulting throughout the austral winter and in mid-summer. Unidentified "microflora" and fungal microbes were detected on the skin of whales sampled in both South African and Antarctic waters. The predominance of "microfloral"

aggregations on cows and calves in October/November suggests that these micro-organisms are acquired in coastal waters. A *Candida*-like invasive fungal infestation of the skin of a stranded neonate was recorded for the first time in this species and may be related to the demise of this animal. The film-forming diatom, *Bennettella* [*Cocconeis*] *ceticola*, was not detected on any skin samples. This may imply that southern right whales exhibit high cellular proliferation rates, which prevent diatomaceous films from forming. Neonatal southern right whales undergo a spectacular form of ecdysis approximately 6-7 days after birth. Histologically, "rough-skinned" neonates possess a distinct "fault line" above the distal tips of the dermal papillae, that becomes characterised by intercellular oedema, which causes all the cell layers above the plane to separate from those below it. The fatty acid composition of the dermal and hypodermal layers provides indications of prey species consumed as well as reflecting physiological processes within the digestive system of the southern right whale. Total lipid values in the blubber of late season cows and calves are reported for the first time. A new hand-held biopsy system for collecting deep-core integument samples from free-swimming balaenids is described. It is a practical and cheaper alternative to projectile systems and the head design allows for the collection of samples that can be used for multidisciplinary research on right whales (e.g. histology, toxicology and blubber composition studies).

Keywords: Southern right whale, integument, histology, ecdysis, microbial aggregations, *Candida* sp., blubber, fatty acid composition, total fatty acid, deep-core biopsy.

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CHAPTER 1

INTRODUCTION

The interest in describing anatomical structures and their consequent functions has encouraged the work of many investigators. Skin protects organisms from injury, maintains its homeostasis, adapts it to its environment, enhances or forms locomotory devices, secretes substances that attract or repel and fashions certain purely ornamental structures (Montagna, 1967).

The cetacean integument consists of three zones or layers namely, the epidermis, dermis and hypodermis (Parry, 1949; Yablokov, Bel'kovich & Borisov, 1974; Sokolov, 1982). Apart from the functions mentioned above, the epidermis provides a habitat for other marine organisms (Bennett, 1920; Hart, 1935; Hustedt, 1952; Nemoto, 1956; 1958; Nemoto, Brownell & Ishimaru, 1977; Haldiman, Abdelbaki, Al-Bagdadi, Duffield, Henk & Henry, 1981; Haldiman, Henk, Henry, Albert, Abdelbhaki & Duffield, 1985; Nagasawa, Holmes & Nemoto, 1990), the dermis provides structural support, while the hypodermis provides the animal with the ability to store tremendous amounts of energy in the form of lipid (thereby allowing it to sustain long periods of low food availability during migrations and lactation) (Ackman, Hingley, Eaton, Logan & Odense, 1975a; Ackman, Hingley, Eaton, Sipos & Mitchell, 1975b; Lockyer, McConnell & Waters, 1984; Aguilar & Borrell, 1990). The integument therefore potentially allows researchers to glean information covering many disciplines.

Southern right whales, *Eubalaena australis*, usually arrive in coastal waters off the southern Cape of South Africa (SA) during the months of May/June. Animals are present along the coast for approximately 6 months and the last animals usually depart for the summer feeding grounds in January. It is generally accepted that the animals mate and calve in these waters, with adult females in the year in which they calve, and the calves of the year, being the only fully represented components of the population. Calving occurs over a four-month period, peaking in August (Best, 1994). This species is generally slow-moving and is, on most occasions, tolerant of approaches by

boats. All these factors provide researchers with an opportunity to obtain developmental data from this section of the population.
This study aims to describe the structure, composition and development of the integument of southern right whales, *Eubalaena australis*, concentrating especially on seasonal and developmental changes in the superficial epidermis (exfoliation), and a qualitative comparison of the lipid composition of mothers and calves.

Historical catch data reveal that these whales tend to move south after leaving coastal waters, migrating to the region of the Subtropical Convergence in December and January, and later heading further south from February to April (Best, 1994). However, our present-day knowledge of where southern right whales go and what they do when they leave the Southern African coast is very limited, with only one confirmed match of an adult female seen both in the Antarctic and along our coast. Recent sightings of animals along the West Coast of Southern Africa (an area heavily impacted by historical and modern whaling operations) during summer months, as well as other "out of season" animals along the south coast, raise many questions about the present migratory behaviour of these animals. Satellite tracking devices are currently thought to be the only means by which some of these answers can be obtained. Designing a device for Balaenids (whose skin and blubber are thicker than any other cetaceans) that will successfully remain implanted in the animal will surely be assisted by accurate knowledge of the structure of the skin tissue of this species.

Considering the recent rapid changes in the environment, determining normal skin morphology is essential if an assessment of future damage due to, for example, biological (viral) or environmental contamination should ever be required. A comparison between skin samples taken in the southern summer and winter will add to the currently sparse knowledge surrounding possible seasonal moult in mysticetes. Tormosov, Mikhaliev, Best, Zemsky, Sekiguchi and Brownell (1998), using data provided by Soviet catches of right whales in the Antarctic, observed that (unlike most balaenopterids) southern right whales did not show seasonal variation in blubber thickness. This observation implies that this species does not show marked seasonality in feeding, although this interpretation is complicated by the fact that there

are structural differences between balaenid and balaenopterid blubber (Yablokov, Bel'kovich & Borisov, 1974).

The North Atlantic right whale, *Eubalaena glacialis*, is arguably the most threatened and rarest of the world's whales (IWC, 1998). Despite more than six decades of protection from whaling, the western North Atlantic right whale population is small (approximately 300 individuals), shows no clear signs of recovery (IWC, 1998) and may be declining (Caswell, Fujiwara & Brault, 1999), while the eastern North Atlantic population is so rare as to be only known to exist from a few recent sightings. Mortality caused by ship strikes and entanglements is known to be high (Kraus, 1990), and there is evidence that reproductive rates in the western North Atlantic are significantly lower than those observed in populations of the southern right whale (Best, Brandao & Butterworth, 2001). Adult females, which should be reproductively active, are not producing calves, and calf production is highly variable (31 recorded in 2001, 2 recorded in 2000). It has been noted that the North Atlantic animals are somewhat thinner in girth (*C. Miller, pers. comm.) and are in visibly worse general condition (scars, "bumps/growths" on the surface of the skin) when compared to the southern right whales off the southern African coastline. The current status of the North Atlantic right whale is a cause for great concern and as such the types of studies which can be done on this population are limited.

Evidence for the relationship between body-fat condition and reproductive performance in large whales has been provided by Lockyer (1986; 1987). According to the analyses done by Heyerdahl (1932, in Slijper, 1948) and Feltmann, Slijper & Vervoort (1948) in other mysticetes, it was concluded that the percentage of lipid was related to the blubber thickness, i.e. the thicker the blubber, the higher its fat content. It is an objective of this study to determine the lipid composition of southern right whale blubber, which has not been previously described. It is hoped this information will provide the basis for comparisons between this species and the North Atlantic right whale and possibly assist in ensuring the survival of the latter species.

Biomarkers are defined as chemical components of organisms, which can be analysed directly from the environment and ideally, can be interpreted both quantitatively and

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qualitatively in terms of *in situ* biomass (Sargent, Parkes, Mueller-Harvey & Henderson, 1987). Lipids are also particularly useful biomarkers since they are relatively easily extracted, identified and quantified as compared with other major biochemical constituents, such as protein and carbohydrate (Sargent *et al.*, 1987).

Earlier studies have drawn attention to the differences in fatty acid composition between northern and southern hemisphere whale oils (Lund, 1936; Lovern, 1942; Hilditch, 1956, Notevarp & Vonen, 1964 in Ackman, Epstein & Eaton, 1971; Notevarp & Fyrst, 1966 in Ackman *et al.* 1971), which have been considered to reflect differences in diet. In the Antarctic most baleen whales feed predominantly on euphausiids (almost exclusively *Euphausia superba*) as well as copepods and amphipods (Ackman & Eaton, 1966; Nemoto & Yoo, 1970; Hamner, Stone & Obst, 1988). A variety of organisms are usually included under the generic name "krill", but in the Southern Oceans the name *Euphausia superba* has been considered almost a synonym for krill (Bottino, 1974). However, the food of southern right whales (*E. australis*) found in South African coastal waters has, to date, not been isolated. Previous studies reported *E. superba* in the stomach of a right whale from South Georgia (Matthews, 1938) and other unspecified reports of "krill" have been made (Lönnberg, 1906); Tormosov *et al.*, 1998). Post-larva of lobster-krill (Matthews, 1932) and copepods (Payne, Brazier, Dorsey, Perkins, Rowntree & Titus, 1983; Tormosov *et al.*, 1998) are also known to be consumed by southern right whales. Determining the prey species of the various migratory groups of southern right whales has obvious implications for the management/conservation of this species.

Boat-based whale watching is practised in many places all over the world and although many studies have been conducted, there are no definite conclusions as to the long-term effects of such activity on the various whale species (IFAW, Tethys Research Institute and Europe Conservation, 1995). As a consequence, the newly founded, boat-based, whale-watching industry along the South African coastline is potentially a threat to the future of the southern right whale. As mentioned above, the animals that occur predominantly along the South African coastline are cows and their newborn calves. This time period is the critical part of the reproductive cycle, when stress should be kept to a minimum and suckling should not be interrupted (Bowen, Oftedal & Boness, 1992; Oftedal, Bowen & Boness, 1993). Although the current permit conditions prohibit permit-holders from

approaching cow/calf pairs, juveniles and adults are usually found further offshore and towards the middle and end of the whale season, cow-calf pairs are frequently all that is available in some of the bays. For this reason there is likely to be increasing pressure to relax this prohibition. Long-term monitoring of effects on a population (e.g. through aerial surveys) require many years of data collection to detect a significant change, by which time it may be too late. Identifying and monitoring the transfer of specific fatty acids from cows to their calves may be a method by which direct effects of boat-based whale-watching on these animals may be determined over the shorter term. Providing the baseline data for this method, is an objective of this study.

CHAPTER 2

STRUCTURE OF THE INTEGUMENT OF SOUTHERN RIGHT WHALES, *EUBALAENA AUSTRALIS*

2.1 Introduction

The physical features of a terrestrial and aquatic environment differ considerably in their relationship to a mammal's body surface. These pertain particularly to heat exchange and friction (as the agile aquatic behaviour of Cetacea demands a minimum of frictional resistance and a maximum of body streamlining); in addition, colouration and sensory perception may have differing adaptive significance in the two habitats. Integumentary structural features are therefore primarily related to meeting these environmental challenges (Simpson & Gardner, 1972; Ling, 1974).

A typical feature of the external body of cetaceans is the total absence of hair, though there are individual vibrissae on the heads of mysticetes. Most species of odontocetes have vibrissae on the head at various stages of embryonic development. The absence of sebaceous and sweat glands and the strong development of the epidermis and hypodermis are also characteristic of the skin of cetaceans (Parry, 1949; Yablokov, Bel'kovich & Borisov, 1974; Sokolov, 1982). Cetacean skin has traditionally been described as being "glabrous" or "smooth", although cutaneous ridges or furrows have been described on the surface of the skin of some species of cetaceans (Giacometti, 1967; Haun *et al.*, 1983 in Shoemaker & Ridgway, 1991; Geraci, St. Aubin & Hicks, 1986; Shoemaker & Ridgway, 1991). The function of these structures is unknown, though it has been suggested that they may play a role in tactile sensing or in the hydrodynamic characteristics of the animal or both (Shoemaker & Ridgway, 1991).

Histological study of the cetacean skin reveals fundamental changes in its structure, relative to other mammals, that are related to the adaptation to an aquatic mode of life (Sokolov & Kalashnikova, 1971). The reasons for this are referable to the specifics of the marine environment which is 800 times denser than air, where viscosity is approximately 40+ times greater compared to air, with 18-27 times greater heat capacity, and 1 atm increase in

pressure for every 10 meters of depth. It is expressly the density, heat capacity of the water environment and the pressure changes of dozens of atmospheres, when immersing, that determine the structure and function of the cetacean integument (Ling, 1974; Yablokov *et al.*, 1974).

The cetacean epidermis, unlike that of terrestrial mammals, consists of three layers with a stratum granulosum and stratum lucidum being absent. Histochemical analyses of the outermost layer have demonstrated the presence of keratin (Kleinenberg, Yablokov, Bel'kovich & Tarasevich, 1964; Palmer & Weddell, 1964; Sokolov & Kalashnikova, 1971; Spearman, 1972; Simpson & Gardner, 1972; Ling, 1974; Greenwood, Harrison & Whitting, 1974; Haldiman, Henk, Henry, Albert, Abdelbaki & Duffield, 1985; Haldiman & Tarpley, 1993) and as a consequence these authors have adopted the name stratum corneum. However, these analyses have also shown that the process of keratinisation is incomplete and hence Harrison & Thurley (1974), Albert, Migaki, Casey & Philo (1980), Migaki, (1981), Geraci *et al.* (1986) and St. Aubin, Smith & Geraci (1990) choose to refer to this layer as the stratum externum. The second layer, underlying the above-mentioned stratum, is generally referred to as the stratum spinosum (or prickle cell layer), although Harrison & Thurley (1974) subdivide the cells of this region into a lower stratum spinosum and an upper stratum intermedium. These two terms have also been used interchangeably by Geraci *et al.* (1986) to describe this layer. The innermost layer is the stratum basale or stratum germinativum (Parry, 1949; Sokolov & Kalashnikova, 1972; Harrison & Thurley, 1974; Ling, 1974; Hicks, St. Aubin, Geraci & Brown, 1985). Both names seem to be interchangeable and each is used in the literature. Ling (1974) also refers to this layer as the "Malphigian" layer.

In the literature, it is clear that "stratum corneum" is the more widely accepted term for the outermost layer, and although, as Haldiman *et al.* (1985) point out, "stratum externum" may be less confusing in terms of location within the epidermis, it ignores the occurrence of keratinisation. These authors add that the use of "stratum intermedium" in place of "stratum spinosum" is likewise topographically descriptive, but loses sight of the distinctive and numerous desmosomes that are present between the cells of this layer and, again, ignores the more conventional terminology in the *Nomina Histologica* (1980). For the purposes of this report, the epidermal layers will be referred to as (from outermost to

innermost) the strata corneum, spinosum and basale, in accordance with the *Nomina Histologica* (1980).

The stratum corneum varies in thickness between various odontocete (Bonin & Vladykov, 1940; Palmer & Weddell, 1964; Simpson & Gardner, 1972; Sokolov, 1982); Geraci *et al.*, 1986) and mysticete species. Giacometti (1967) stated that the stratum corneum of *B. physalus* was almost absent, except in the external genitalia and eyelids. Spearman (1972), however, found an extremely thick stratum corneum in a posterior dorsal sample of *B. physalus*, up to 200 μm in depth. These discrepancies are possibly due to the loss of superficial layers during collection and/or processing of samples. In bowhead whales (*Balaena mysticetus*) 12-60 layers are distinct, justifying the term "hyperkeratotic" for the bowhead's normal condition (Haldiman & Tarpley, 1993).

The stratum spinosum is indisputably the thickest region of the epidermis and the stratum basale consists of a single cell layer that maintains contact with the underlying basal lamina due to the presence of hemi-desmosomes. The stratum basale produces new cells that are pushed outward as additional cells are formed in order to maintain the normal epidermal thickness (Haldiman & Tarpley, 1993).

The epidermis is anchored to the underlying dermal connective tissue by uniformly long, moderately thick downward extensions, rete pegs or ridges, which are generally oriented parallel to the body axis. These ridges form slender flap-like projections between which dermal papillae are located (Giacometti, 1967; Simpson & Gardner, 1972; Ling, 1974; Geraci *et al.*, 1986) and are also a notable feature in the cetacean integument. The papillae in the dermal layer literally beset the epidermis over almost half its thickness and make it possible to increase the surface area of the germinal layer of the epidermis significantly, which is important for it to become so exceptionally thick (Yablokov *et al.*, 1974).

Some of the other most notable features of the marine mammal integument, reflecting varying degrees of contrast to that of terrestrial forms, lie in the dermis. The dermis is a thick bed of dense white fibrous connective tissue, blood vessels and adipose tissue (Haldiman & Tarpley, 1993). The dermis of cetaceans is also richly innervated (Parry,

1949; Palmer & Weddell, 1964; Giacometti, 1967; Simpson & Gardner, 1972; Haldiman *et al.*, 1985).

The papillary layer of the dermis sends long projections between corresponding rete pegs/ridges of the epidermis. The interdigitation between the epidermal ridges and dermal papillae is referred to as papillomatosis. By far the greatest constituent of cetacean dermis is collagen tissue, which is generally comprised of fairly stout bundles usually orientated parallel to the skin surface, but some are randomly arranged. At deeper levels the dermis becomes invaded by, and merges with, the fatty tissue of the hypodermis (Sokolov, 1962).

The nature of the hypodermis or blubber of marine mammals, with its often vast accumulation of fat cells, gives the integument, if not the entire anatomy, of these animals its most distinctive feature. Above, the hypodermis is continuous with the reticular layer of the dermis; below, it is separated from the panniculus carnosus by loose connective tissue (superficial fascia). The distinction between the end of the dermis and the beginning of the hypodermis is not always clear. Fat cells extend to some extent up to the epidermis and heavy collagen bundles ramify throughout the subcutaneous blubber (Parry, 1949; Simpson & Gardner, 1972; Ackman, Hingley, Eaton, Sipos & Mitchell, 1975b; Sokolov, 1982). Several authors have alluded to the structure of the hypodermis in cetaceans, the most extensive studies being of *D. leucas* (Bonin & Vladykov, 1940), *P. phocoena* and *B. physalus* (Parry, 1949) and *Eubalaena mysticetus* (Haldiman & Tarpley, 1993). These and other studies suggest that the hypodermis is architecturally simple and its basic structure similar in the different groups. It is usually represented by many fat cells, with thin membranes, between which there are sparse collagen fibres going in varied directions (Yablokov *et al.*, 1974). There is a paucity of collagen and elastic fibres in the hypodermis in odontocetes, in contrast to the mysticetes that possess a much denser connective tissue framework for supporting the subcutaneous fat layer (Sokolov, 1960). Parry (1949), however, found the structure of the blubber of *B. physalus* to be essentially similar to that of *P. phocoena* and did not remark on connective tissue differences.

In this thesis, the structure of the integument of the southern right whale is described and compared to previous descriptions of other cetacean species. Techniques include both light and electron microscopy, using material from neonates, calves, juveniles and adults, much

of which has been collected by biopsy from free-swimming animals. Possible seasonal changes in the shedding of superficial skin cells (i.e. exfoliation/desquamation) are examined using material from South Africa (July-November) and the Antarctic (January-February).

2.1.1 Definitions

There seems to be some uncertainty in the literature over what constitutes cetacean skin and/or integument and/or blubber. Generally speaking, integument and skin are used interchangeably to refer to the layered external covering of vertebrates. The epidermis is often erroneously referred to as skin, for in fact, this portion (pigmented or unpigmented) is only the outer epithelial part of the skin. Some authors (Parry, 1949; Bryden, 1964), probably following the terminology of whaling, include the entire integument of cetaceans under the term blubber, whereas most others (Sokolov, 1955; 1982; Durward & Rudall, 1958; Ling, 1974; Yablokov *et al.*, 1974; Ackman, Hingley, Eaton, Logan & Odense, 1975a; Berta & Sumich, 1999) refer only to hypodermal fat as blubber. Some of these authors also refer to blubber as "subcutaneous fat tissue".

Haldiman & Tarpley (1993) describe the integument or skin of bowhead whales as consisting of two layers: an outermost pigmented epidermis which covers a true dermis composed of connective tissue that includes a fatty blubber layer (the modified reticular layer of the dermis). These authors use dermis layer and blubber layer interchangably and the deeply located hypodermis of variable thickness, described above, is not considered part of the integument.

For the purposes of this study, "integument" refers to the epidermal, dermal and hypodermal layers. The pigmented uppermost layers of the integument are referred to as the epidermis. The generally accepted definition whereby the junction (although not always marked, as discussed above) between the dermis and hypodermis is defined by an increase in adipose tissue and a decrease in connective tissue, is followed in this text. "Blubber" refers to dermal and hypodermal layers. The term "skin" is generally used to describe the outermost covering of a whale's body, but, where contextually applicable, "skin" may also

refer to the superficial integumentary samples obtained using projectile-driven biopsy techniques.

The raised structures on the southern right whale head were noted by early observers (Ridewood, 1901). The term "callosity", from the root "callus" meaning "thickened", has been used to describe these patches of thickened skin on the heads of right whales, which are distinguishing features of the genus *Eubalaena* (Matthews, 1938; Esau, 1953; Omura, Ohsumi, Nemoto, Nasu & Kasuya, 1969; Payne *et al.*, 1983; Kraus, Crone & Knowlton, 1988).

In the text, the term "moult" is used to describe the periodic shedding of parts or all of a coat or an outer skin covering, which is then replaced by a new growth, as opposed to "exfoliation" and "desquamation" which refer to the regular removal of skin in flakes, scale or peel. "Sloughing" refers to the shedding or casting off of the outer epidermal layer, or parts thereof, and is regarded, in this text, as a synonym for moulting.

2.1.2 Taxonomy

Researchers and taxonomists have as yet not fully resolved the taxonomic status of right whales (Rice, 1998; IWC, 2000). The whales studied in this report are black right whales from the Southern Hemisphere and are referred to as southern right whales or *Eubalaena australis* following Rosenbaum, Brownell, Brown, Schaeff, Portway, White, Malik, Pastene, Patenaude, Baker, Goto, Best, Clapham, Hamilton, Moore, Payne, Rowntree, Tynan, Bannister & DeSalle (2000).

2.2 Materials and Method

2.2.1 Study area

Samples of integument (epidermis and blubber) from living southern right whales were collected during the August and October field seasons of 1998 and 1999, as well as during early November 2000. The study area included Walker Bay (Gansbaai), Struisbaai, De Hoop Marine Reserve and St. Sebastian Bay, all on the south coast of Southern Africa (Plate 1). Samples were taken from stranded animals in the above areas as well as in the Cape Peninsula, Dwarskersbos and Elands Bay, along the west coast of Southern Africa (Plate 1).

2.2.2 Sample collection

2.2.2.1 Biopsies

Integumentary samples from free-swimming southern right whales (35 cows and 63 calves) were collected along the South African coast, using a specially designed, hand-held deep-core biopsy system (Chapter 6).

Each animal was approached perpendicular to its long axis and sampled by inserting the biopsy head (on the end of a 9 m aluminium pole) into the dorso-lateral surface of the whale and immediately retracting it (Plate 2). Once a successful biopsy attempt was made, the sample was removed from the biopsy head, placed in foil and into a labelled plastic bag and then put into a cooler box with "blue ice". The biopsy heads were cleaned in 99% chloroform between samples, and the barbs reset or, if necessary, replaced. Back on land, the samples were measured, noting epidermal and blubber thicknesses. The pigmented epidermis was cut away from blubber samples (the cut was made on the blubber side of the intersection between the epidermis and dermis) using a sterile scalpel and the epidermis was immediately placed in a separate, labelled specimen bottle containing gluteraldehyde. The epidermal samples were left in the gluteraldehyde for a minimum of 3 days and a maximum

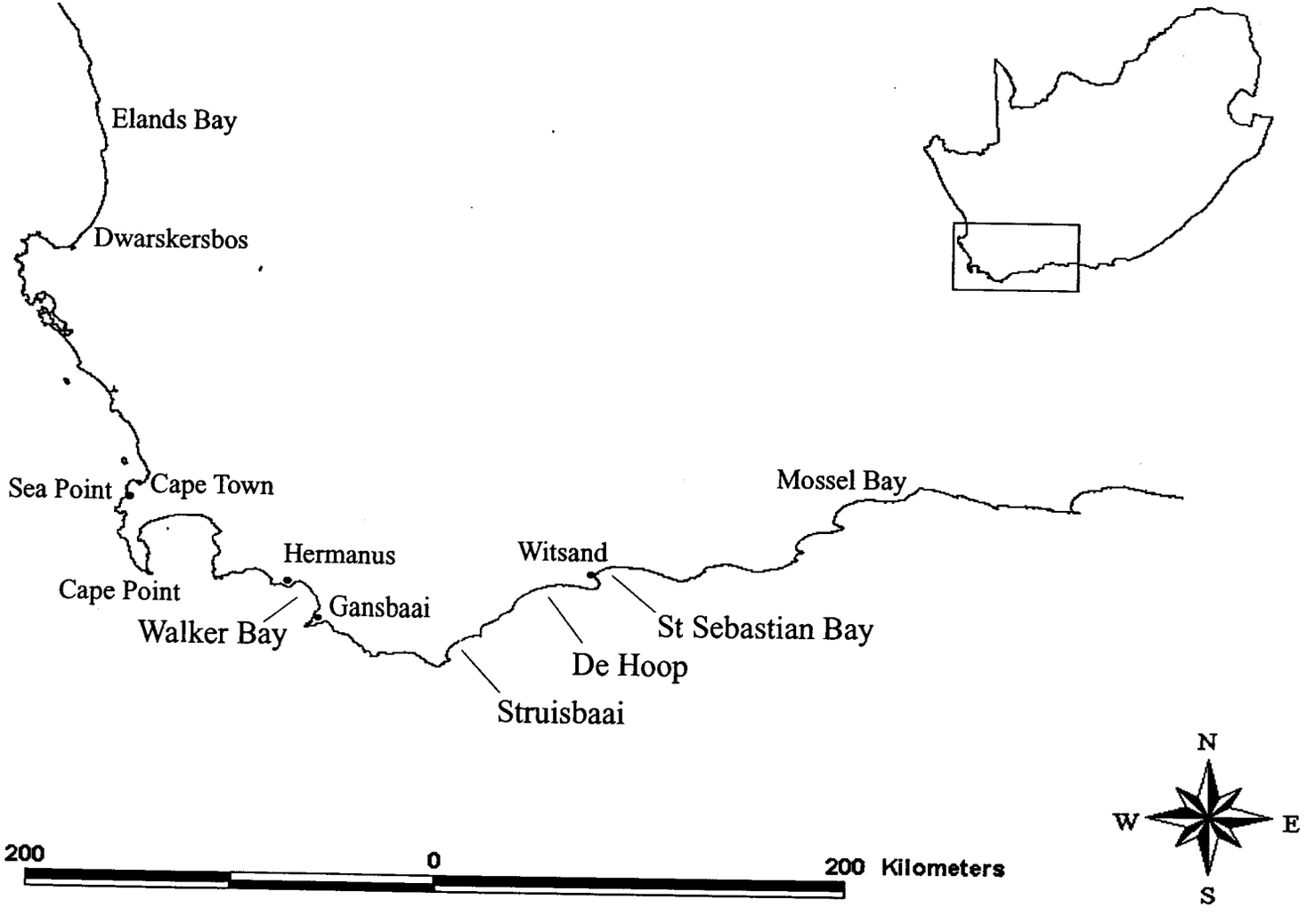

Plate 1: Map of sampling areas along the South African coastline.

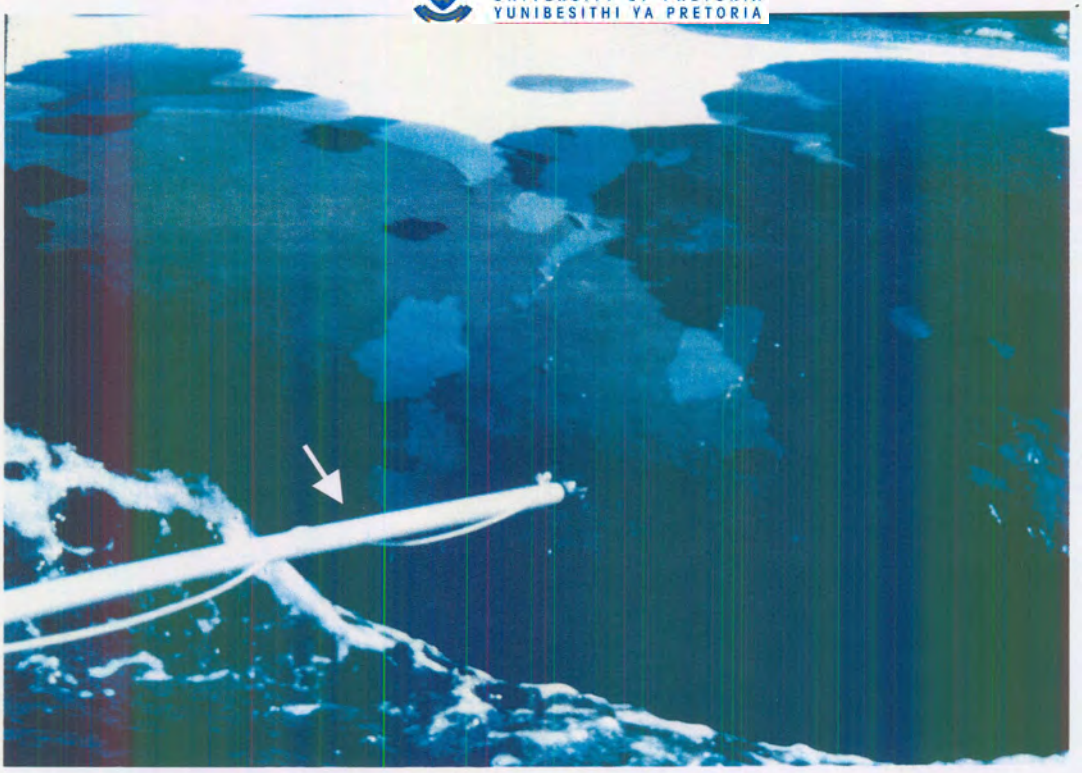

Plate 2: Biopsy pole (arrow) was inserted perpendicularly into the whale and immediately retracted. Note dorsally-located white and grey patches.

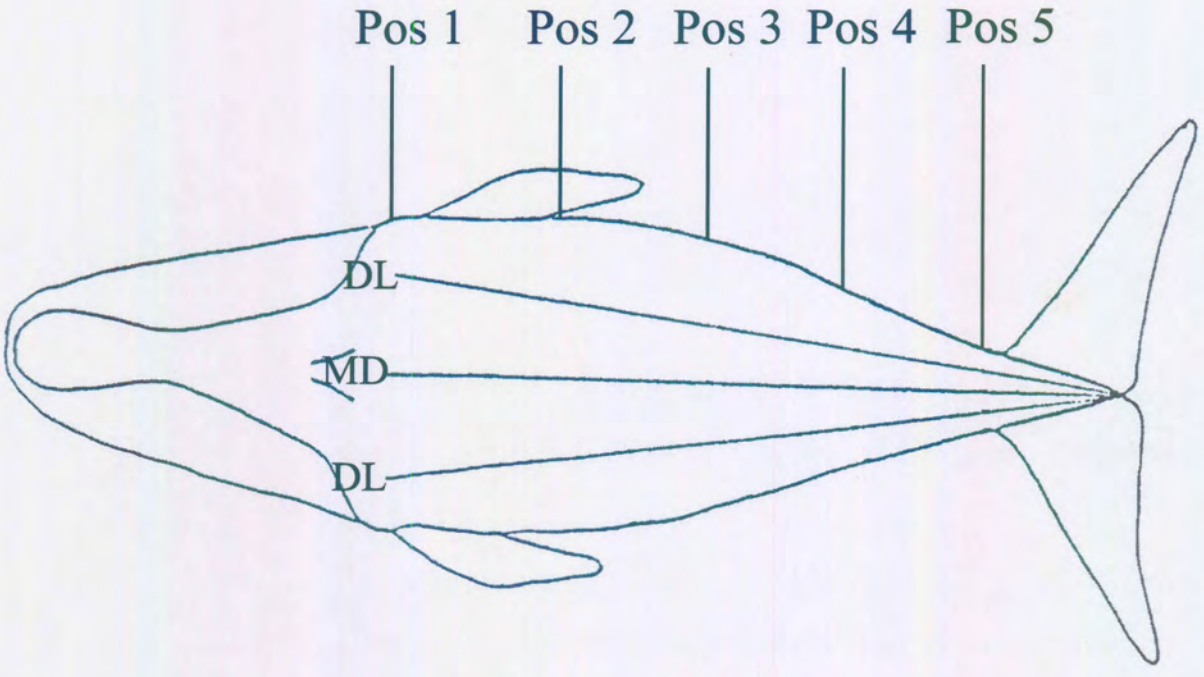

Plate 3: Sampling positions (Pos 1-5) along the bodies of stranded southern right whales. Dorso-lateral (DL) and mid-dorsal (MD) planes (lateral and mid-ventral planes are not indicated, but were sampled when possible).

orthophosphate + disodiumhydrogen orthophosphate anhydrous = water) until analysed.

Skin samples (n = 14, but only 11 were suitable for histological analysis) were also obtained from living right whales in the Antarctic during the 1998/1999 IWC/SOWER Circumpolar Cruise between 25/01/1999-21/02/1999. Samples were collected using various techniques including crossbow, Paxarms biopsy gun and Japanese air gun. These samples were fixed as described for the above epidermal samples and exported from Japan under CITES permit number T-AG 99-100172(W).

2.2.2.2 Stranded animals

Total body length as well as blubber thickness measurements were taken from fresh/recently stranded animals (Table 1) and full core samples were placed in foil and frozen at -20 °C within a few hours of collection. Body girths, epidermal and blubber thickness measurements were taken from 5 positions along the mid-dorsal, lateral and mid-ventral surfaces from animals that stranded from 1998 onwards (Plate 3). Full core samples were taken from the same positions. Prior to 1998, mid-dorsal samples from stranded right whales were collected by members of the Whale Unit (Table 2).

Samples for histological analysis were fixed in 10 % buffered formalin and subsamples of the pigmented epidermis for EM analysis were fixed in gluteraldehyde (same procedure as for biopsy samples). In most instances, the positioning of the animal prohibited the collection of samples from both the mid-dorsal and mid-ventral surfaces and in other instances the location of the animal made it impossible to take measurements and collect samples from all positions along the various surfaces. On occasion, integumentary samples from other structures, e.g. callosities, flippers and flukes were opportunistically taken (Table 2).

Table 1: Total lengths, girths, epidermal and blubber thicknesses of southern right whales stranded along the Cape coast of South Africa 1998-2000.

Stranding #	98/09	00/10	00/12	99/05	00/09	00/11	00/14
Age	Neonate	Neonate	Neonate	Neonate	Neonate	Juvenile	Subadult
Sex	Female	Male	Male	Male	Male	Female	Male
Total length (m)	3.9	4.42	4.43	4.84	5.91	9.85	15.7
Girth – Position 1 (m)[1]	2.5	2.38	2.34	^	2.7	5.74	^
Girth – Position 2 (m)[1]	2.6	2.94	2.48	~2.3	2.88	7.12	~7.0
Girth – Position 3 (m)[1]	3.4	2.76	2.28	^	2.6	5.7	^
Girth – Position 4 (m)[1]	1.2	1.6	1.48	^	1.58	3.4	~4.88
Girth – Position 5 (m)[1]	0.8	0.93	0.78	^	0.99	1.5	~2.62
Epidermal thickness (cm)[1]							
Mid-Dorsal Position 1	1.2	0.9	1	0.9	^	^	^
Mid-Dorsal Position 2	1.3	0.8	1.3	0.6	^	^	^
Mid-Dorsal Position 3	1.3	0.9	1	1.3	^	^	^
Mid-Dorsal Position 4	1.4	0.9	1.5	1.5	^	^	^
Mid-Dorsal Position 5	1.3	1.1	0.8	0.5	^	^	^
Dorso-lateral Position 1	^	^	^	^	^	^	1.2
Dorso-lateral Position 2	^	^	^	^	^	^	1.3
Dorso-lateral Position 3	^	^	^	^	^	^	1.3
Dorso-lateral Position 4	^	^	^	^	^	^	1.1
Dorso-lateral Position 5	^	^	^	^	^	^	1.1
Lateral Position 1	^	1	1.4	^	1.1	1	^
Lateral Position 2	^	0.8	1.9	1.1	1.6	1.1	^
Lateral Position 3	^	1.3	2	1.3	1.5	1.2	^
Lateral Position 4	^	1.1	2.4	1.4	1.4	1.2	^
Lateral Position 5	^	1	1	0.9	0.9	0.8	^
Mid-ventral Position 1	^	1	^	2.2	1.3	^	^
Mid-ventral Position 2	^	1	^	1.4	1.7	^	^
Mid-ventral Position 3	^	*1.2	^	^	1.4*	^	^
Mid-ventral Position 4	1.4	1	^	1.2	1	^	^
Mid-ventral Position 5	^	1	^	0.6	1.2	^	^
Blubber thickness (cm)[1]							
Mid-Dorsal Position 1	1.7	4.6	2.5	3.7	^	^	^
Mid-Dorsal Position 2	1.5	3.2	3.1	1.5	^	^	^
Mid-Dorsal Position 3	2.8	5	3.5	3	^	^	^
Mid-Dorsal Position 4	4.5	8.7	4.3	5.5	^	^	^
Mid-Dorsal Position 5	7.3	10.3	5.6	7.3	^	^	^
Dorso-lateral Position 1	1.4	^	^	^	^	^	15.2
Dorso-lateral Position 2	1.9	^	^	^	^	^	16
Dorso-lateral Position 3	2.4	^	^	^	^	^	10.3
Dorso-lateral Position 4	3.8	^	^	^	^	^	13
Dorso-lateral Position 5	2	^	^	^	^	^	6.7
Lateral Position 1	3.1	6.7	1.1	^	4.7	18.1	^
Lateral Position 2	3.4	5.1	4.7	4.6	4.9	17.3	^

Table 1: continued

Stranding #	98/09	00/10	00/12	99/05	00/09	00/11	00/14
Lateral Position 3	3.3	5.4	3.2	3.8	6.3	14.6	^
Lateral Position 4	3.8	4.8	3.2	3	5.3	14.4	^
Lateral Position 5	2.8	4.5	1.8	9	3.4	5.6	^
Mid-ventral Position 1	^	7.7	^	5.6	5.3	^	^
Mid-ventral Position 2	4.9	5.7	^	3.7	4.3	^	^
Mid-ventral Position 3	5	*5.8	^	^	*6.4	^	^
Mid-ventral Position 4	6.7	8.7	^	6.7	8.5	^	^
Mid-ventral Position 5	7.3	6.2	^	4.8	8.8	^	^

[1] Positions as described in Plate 2.
* measurements taken just below genital aperture.
^ measurements unobtainable.

Table 2: Details of stranded southern right whales sampled for histological analysis

Sample #	Type	Date	Age	Location	Total length (m)	Gender
84/27	Mid-dorsal Pos 3 or 4	09/08/84	Juvenile	Gansbaai	9.25	?
86/32	Mid-dorsal Pos 3 or 4	09/02/86	Neonate	De Hoop	4.85	Male
89/30	Mid-dorsal Pos 3 or 4	12/06/89	Adult	Gansbaai	14.7	Male
90/29	Mid-dorsal Pos 3 or 4	16/08/90	Neonate	Hermanus	4.8	Female
91/18	Mid-dorsal Pos 3 or 4	13/09/91	Neonate	De Hoop	6.65	Male
94/12	Mid-dorsal Pos 3 or 4	22/09/94	Juvenile	Breede River	11.23	Female
98/09	Mid-dorsal Pos 1-5	20/08/98	Neonate	Witsand	3.9	Female
98/09	Mid-ventral Pos 4	20/08/98	Neonate	Witsand	3.9	Female
98/09	Callosity	20/08/98	Neonate	Witsand	3.9	Female
99/05	Mid-dorsal Pos 1-5	16/09/99	Neonate	Hermanus	4.84	Male
99/05	Right Lateral Pos 1-5	16/09/99	Neonate	Hermanus	4.84	Male
99/05	Mid-ventral Pos 1/2/4/5	16/09/99	Neonate	Hermanus	4.84	Male
99/05	Callosity	16/09/99	Neonate	Hermanus	4.84	Male
99/05	Fluke	16/09/99	Neonate	Hermanus	4.84	Male
00/09	Mid-dorsal Pos 3	24/07/00	Neonate	Witsand	5.91	Male
00/10	Mid lateral Pos 2	29/07/00	Neonate	Elands Bay	4.42	Male
00/11	Left lateral Pos 1-5	06/09/00	Juvenile	Sea Point	9.85	Female
00/11	Bonnet	06/09/00	Juvenile	Sea Point	9.85	Female
00/12	Mid-dorsal Pos 4	18/09/00	Neonate	Dwarskersbos	4.43	Male
00/12	Flipper, fluke, bonnet	18/09/00	Neonate	Dwarskersbos	4.43	Male
00/12	Lower lip	18/09/00	Neonate	Dwarskersbos	4.43	Male
00/14	Dorso-lateral Pos 1-5	13/10/00	Subadult	Cape Point	15.7	Male
00/14	Callosity	13/10/00	Subadult	Cape Point	15.7	Male

2.2.3 Histological preparations

Skin samples for light microscopy were prepared, embedded and stained according to standard histological procedures at the Department of Anatomical Pathology, Groote Schuur Hospital. A Leica "Jung Histokinette 2000" tissue processor was used, sections of 4-5 μ were cut on a microtome and adhered to APES coated slides. Mayer's Haematoxylin and Eosin were used to identify general histological structure. Weigert's Resourcin was used to stain for the presence of collagen and elastin fibres and Ayoub-Shklar to reveal keratin.

In order to investigate possible seasonal differences between cellular activity within the germinal layers, proliferating cell nuclear antigen (PCNA) staining was attempted on some Antarctic samples. The Avidin-Biotin Method (DAKO E 0354, DAKO P 0364) and Envision System (DAKO K 4001) were applied in the (Immunohistochemistry Laboratory) at Groote Schuur Hospital.

Samples for Transmission Electron Microscopy (TEM) were prepared at the Department of Anatomical Pathology, Groote Schuur Hospital and electron micrographs were taken using an Hitachi H600 Transmission Electron Microscope.

Samples for scanning electron microscopy (SEM) were removed from buffer and dehydrated through an ethanol (Merck AG EtOH) series (30%, 50%, 70%, 80%, 90%, 100%) for 2.5 hours in each solution. The samples were placed in two additional washes of absolute alcohol for 2.5 hours each. The samples were critical point dried from 100% EtOH in CO_2, mounted and coated with gold-palladium in a sputter coater, and viewed using a JEOL JSM-5200 Scanning Electron Microscope operating at 15kV.

2.2.4 Statistical analyses

The Student's t-test was used to compare normally distributed data and the Mann-Whitney Rank Sum Test was the nonparametric test used (SigmaStat for Windows, Jandel Scientific Software) to statistically describe any variation in epidermal and blubber thicknesses between different age groups. An alpha value of 0.05 was used.

2.3 Results

2.3.1 General characteristics of southern right whale skin

Although many samples were obtained from live animals, stranded animals provided the opportunity for close study of the gross appearance of southern right whales. Raised areas of skin ("bumps") occur on the rostrum and caudal to the blowholes of both live (Plate 4) and stranded southern right whales. These structures were also present in a regular row along both sides of the mandible, each side of the chin and immediately above each eye. A stiff, single hair usually emerges from each of the smaller "bumps" and, in the case of the bonnet, several individual hairs arose from several coalesced bumps. Associated with these structures, in older calves, juveniles and adults, were barnacles (*Tubicinella* sp.) (Plate 5a) and small amphipod crustaceans (*Cyamus* sp.) (Plate 5b), which gave these structures varieties of white, yellow and orange colouration. The resulting structures (bumps) are referred to as "callosities" (Matthews, 1938). Projections of pigmented skin seemed to form "epidermal stalks" between callosities. These stalks varied in length, but were generally only long enough to project above the surrounding barnacles. According to Payne *et al.* (1983), these stalks were probably formed when callosity projections extended into water layers that were moving too fast to be suitable habitat for cyamids (Page 43). The chin possessed a number of similar hairs that are not associated with callosities.

The gross appearance of the general epidermal surface of non-calves was usually smooth. Fresh epidermis had a smooth, rubbery consistency, reminiscent of neoprene material. The head and body were generally black in colour, with variably sized white areas on the belly and sometimes on the back (Plate 4). Variations in epidermal skin colour do, however, occur with some adults being predominantly grey in colour and others having dorsally-located grey and/or white blazes (Plates 2 and 4). Some calves are born partially

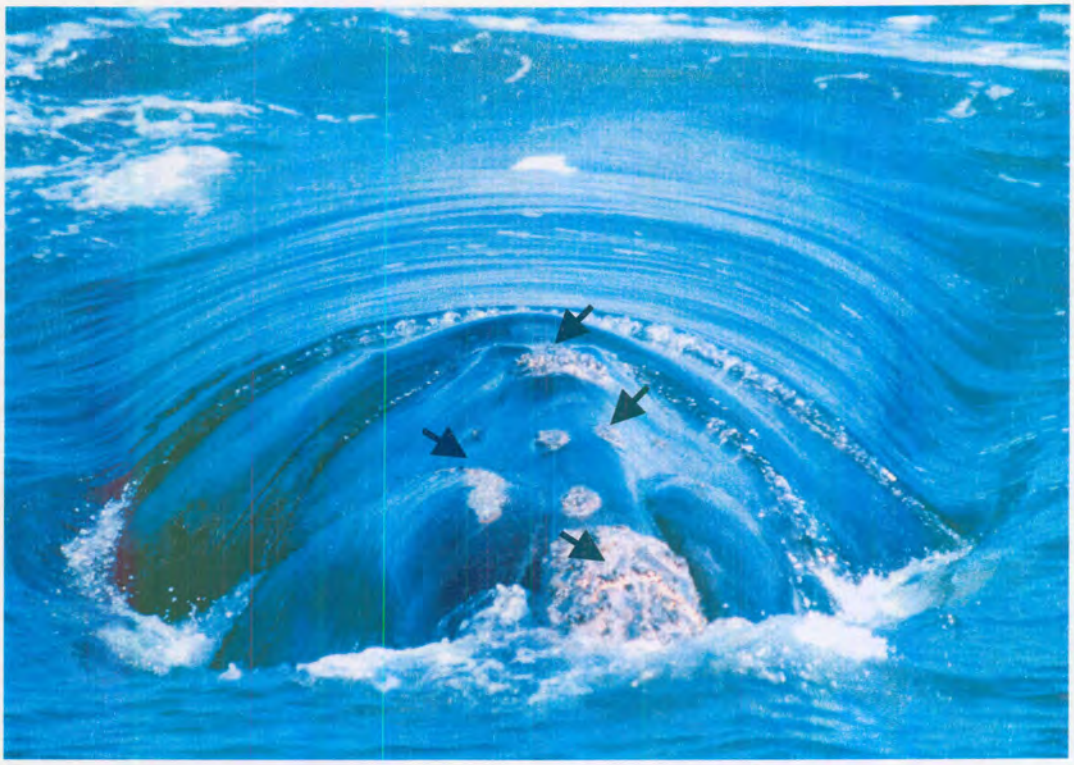

Plate 4: Raised areas of skin (arrows) on the rostrum and mandible of an adult southern right whale. Note white patch on the mid-dorsal surface used for individual identification.

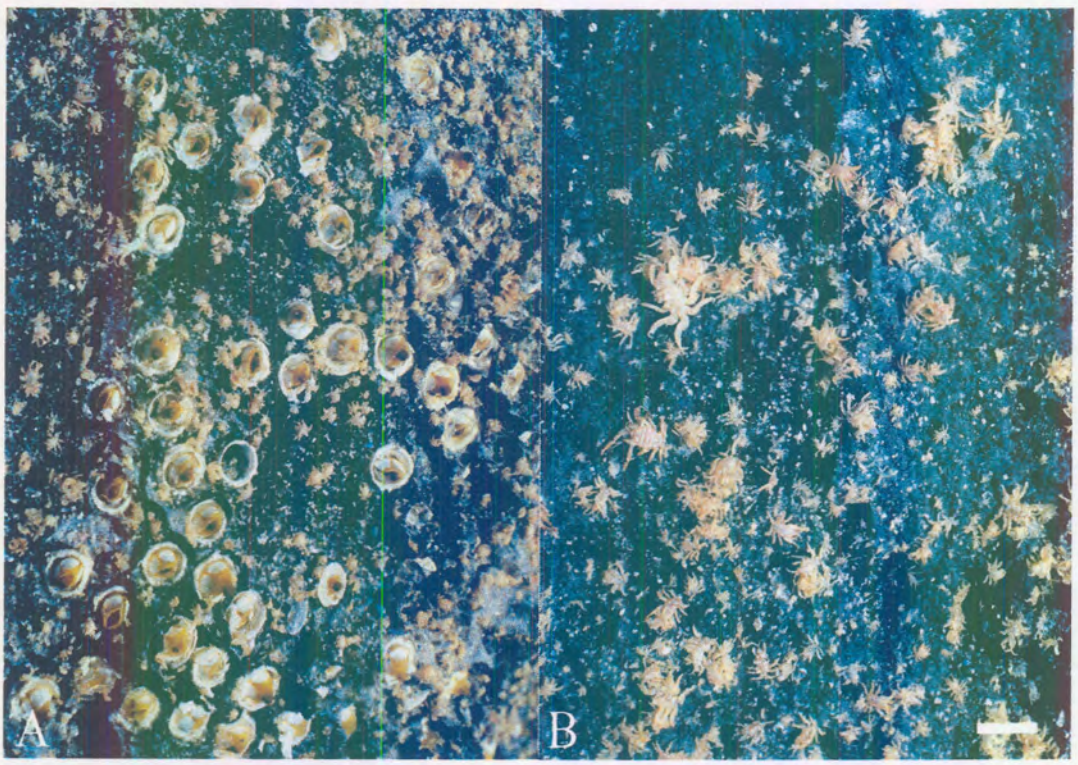

Plate 5: Barnacles (a) and cyamids (b) on the bonnet callosity of a southern right whale. Scale bar = 1.5 cm.

albinistic but as the animal ages, the white pigmentation tends to become dark grey (Plate 6).

Discolouration of the skin surface, due to diatom films, was not seen on any animals. The outermost layer of the epidermis is, however, a macroscopic, thin superficial layer of cells that separates easily from the rest of the epidermis (the stratum corneum) and is continually being sloughed. Sloughed areas, where the stratum corneum has been recently shed, give the skin a grey-patchy look, as frequently seen in adult right whales off South Africa (Plate 7).

The epidermis is usually heavily pigmented, and is noticeably thick (Plate 8). It varies slightly in thickness around the body, as seen from stranded animals (Table 1) and between calves and adults. The average epidermal thickness for stranded neonates, along the mid-dorsal, lateral and mid-ventral planes was 1.13 $\pm$ 0.2 cm (n =4), 1.52 $\pm$ 0.3 cm (n=4), and 1.14 $\pm$ 0.2 cm (n =4), respectively. In comparison, one juvenile female measured had an average epidermal thickness of 0.86 cm along the lateral plane and one subadult male had an average epidermal thickness of 1.2 cm along the dorso-lateral plane. Epidermal thicknesses at different positions along the mid-dorsal and lateral planes of stranded neonates were not significantly different ($p > 0.05$). The mean ($\pm$ S.E.) epidermal thickness of calves biopsied in August/September [1.57 $\pm$ 0.13 cm (n = 20)] did not differ from those biopsied in October/November [1.39 $\pm$ 0.07 cm (n = 19)] ($p = 0.261$). The corresponding adult measurements were 1.42 $\pm$ 0.09 cm (n = 13) and 1.43 $\pm$ 0.08 cm (n = 9, $p = 0.934$), respectively. The differences between early season calves and early season adults ($p = 0.552$), early season calves and late season adults ($p = 0.587$), late season calves and early season adults ($p = 0.851$) and late season calves and late season adults ($p = 0.725$) were not significant.

The dorsal epidermis of the fluke of a neonate (1 cm) was slightly thicker than the ventral epidermis (0.5 cm) (Plate 9). The core of the flipper and fluke (Plate 9) possessed more abundant coarse, white connective tissue strands than any other body region studied.

Plate 6: A partially albinistic southern right whale calf that has become grey with age.

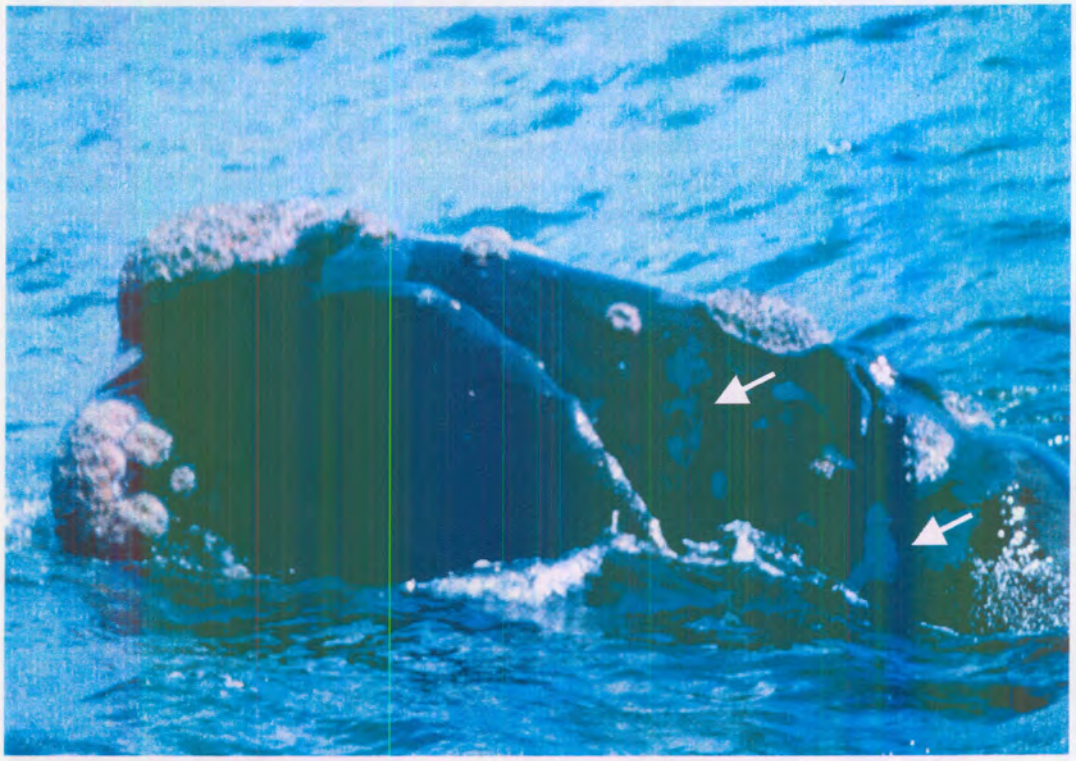

Plate 7: Grey patches (arrows) caused by sloughing of the superficial stratum corneum on the head of an adult southern right whale.

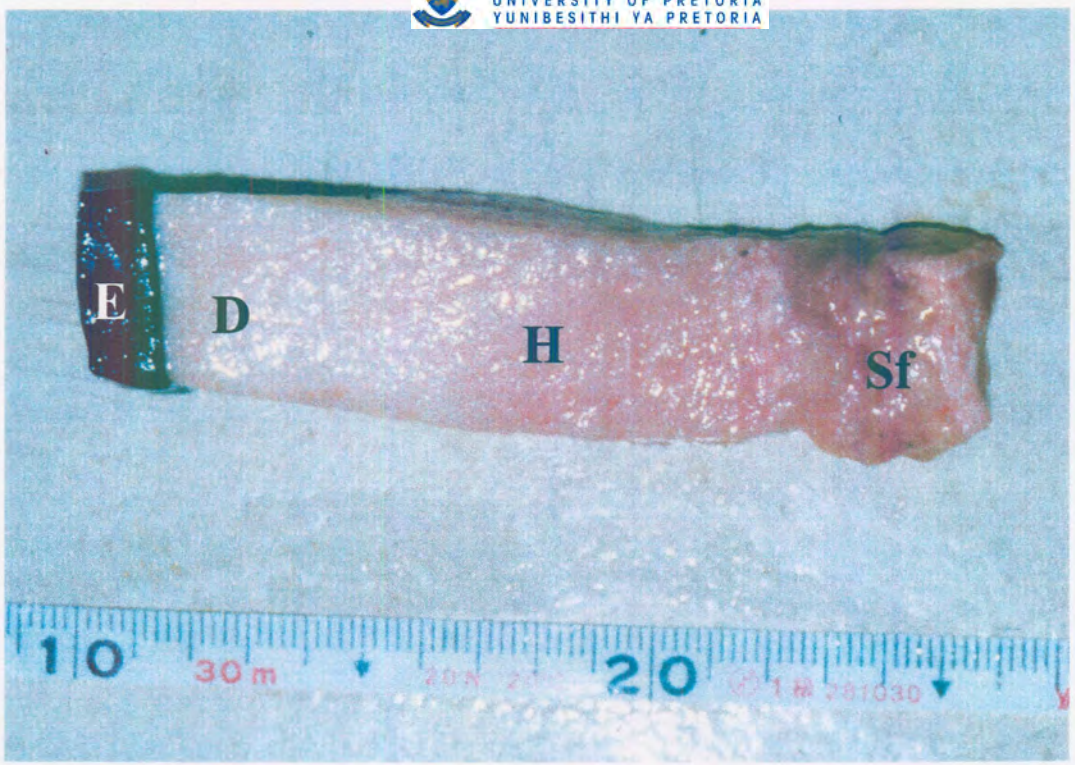

Plate 8: A core sample taken through the integument of a neonatal southern right whale. The epidermal layers (E) are heavily pigmented and noticeably thick. Dermal layer (D), hypodermal layer (H), superficial fascia (Sf) are shown.

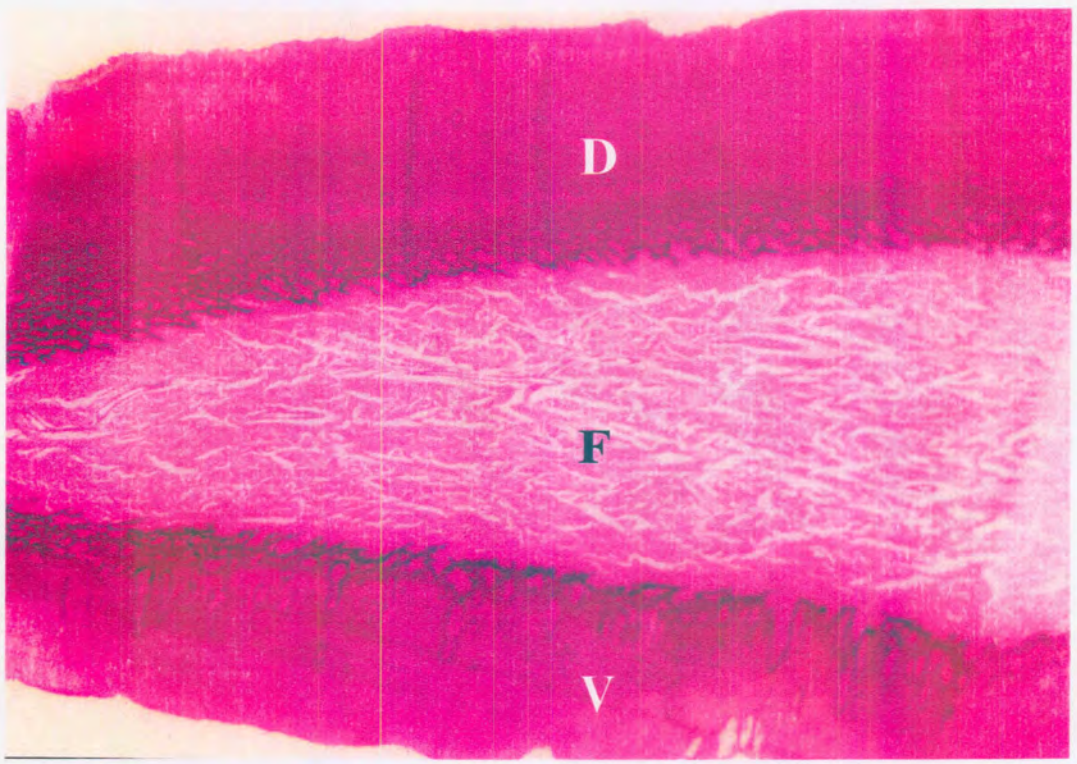

Plate 9: Longitudinal section through the fluke tip of a southern right whale calf. Note the thicker dorsal epidermis (D) compared to the ventral epidermis (V) and extensive collagen fibres (F), coloured light pink, ramifying through core of fluke. (H/E, Mag 8X).

The skin of one freshly stranded neonate (99/05) had an unusual appearance (Plate 10). On close inspection of the skin, it seemed as if the dermal papillae were exposed and the entire epidermis was missing, lost or not yet properly formed in some regions. Histological preparations of 15 skin samples from all over the body of this neonate confirmed the presence of stratum spinosal cells, but the absence of the entire stratum corneum in all but one sample (position 3 on the right lateral plane) (Plate 11), which is a pathological condition (*M. Duffield, pers. comm.).

2.3.2 Microscopic characteristics of southern right whale skin

2.3.2.1 Superficial epidermal features

Cutaneous ridges or furrows were absent on the epidermal surfaces of all samples and upon gross examination, the skin of adults and darkly-coloured calves appeared smooth and uniform in colour. However, scanning electron microscopy clearly showed flaking of the surface squamous keratinocytes (Plate 12) in both early and late season adults and calves, as well as in animals sampled in the Antarctic (Plate 13). The surface cells may desquamate individually or in sheets, with the cells showing close apposition to each other. Distinct pentagonally-shaped, cell junctions and deep surface ridges form a honeycomb-like pattern that resembles those of terrestrial mammals (Plate 14). The texture of the skin in areas where epidermal sloughing has occurred looks uniformly pitted, exposing the disconnected and freed intercellular boundaries (Plate 15). Sloughing occurred in multiple layers (Plate 16).

Differences in the appearance of the skin of calves were noted and they were grouped accordingly. Calves with dark, smooth-looking skin were termed "smooth-skinned" (Plate 17) and calves with light grey and seemingly "broken" skin (Plate 18) were termed "rough-skinned (Chapter 2). The skin of early season, smooth-skinned calves possessed patches of smooth skin with no honeycomb patterns visible on the surface; these patterns gave way to the exposed, pitted surface of recently sloughed areas (Plate 19) as seen in adults (Plate 15). The presence of randomly dispersed white/grey dots on the superficial epidermis (Plate 20a) was also noted on all smooth-skinned animals,

*Department of Anatomical Pathology, University of Cape Town, Groote Schuur Hospital, Cape Town, South Africa.

Plate 10: Longitudinal section through the fluke of a southern right whale calf (99/05). Note the unusual, corrugated appearance of the dorsal (d) and ventral (v) epidermis (arrows).

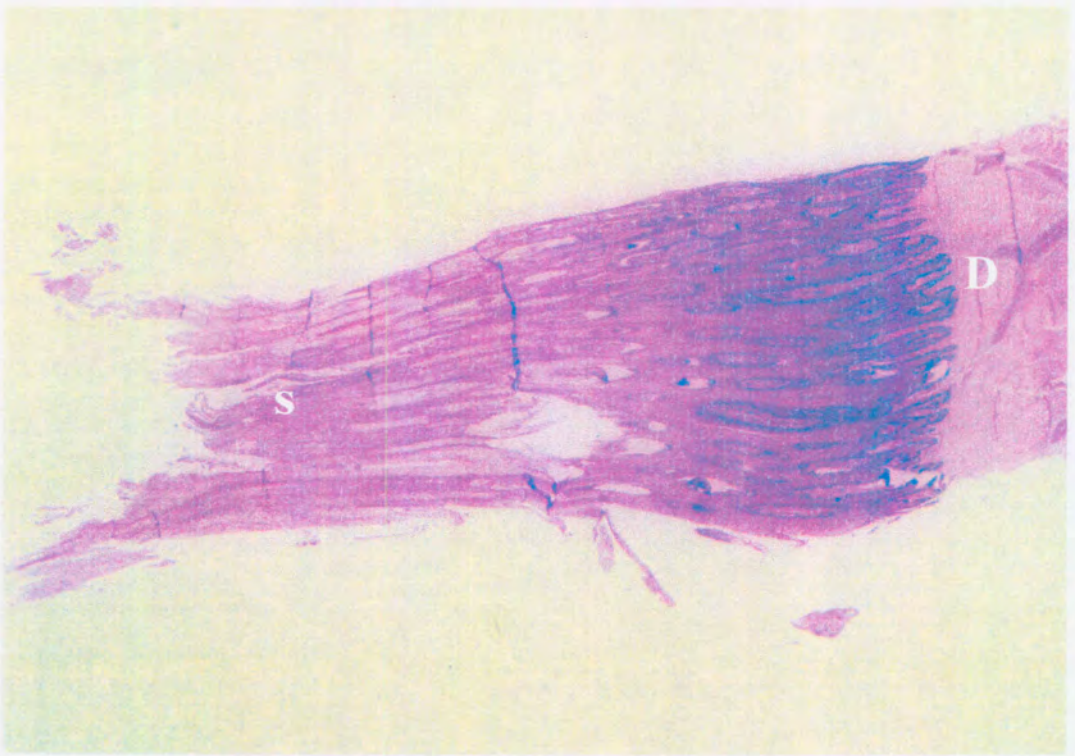

Plate 11: Longitudinal section through the epidermis of a southern right whale calf (99/05) indicates the presence of flattened stratum spinosum cells (s) and the absence of the stratum corneum. Dermal layer (D). (H/E, Mag 10X).

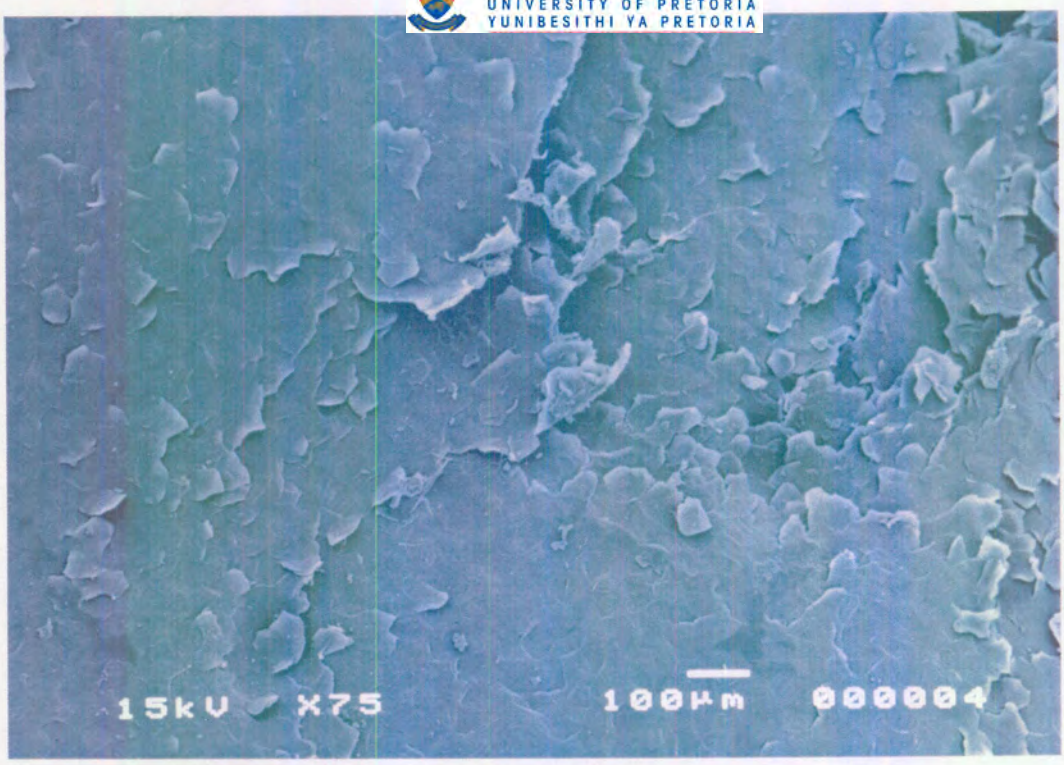

Plate 12: SEM showing the flaking of superficial squamosal keratinocytes of a late season southern right whale calf. (Mag 75X).

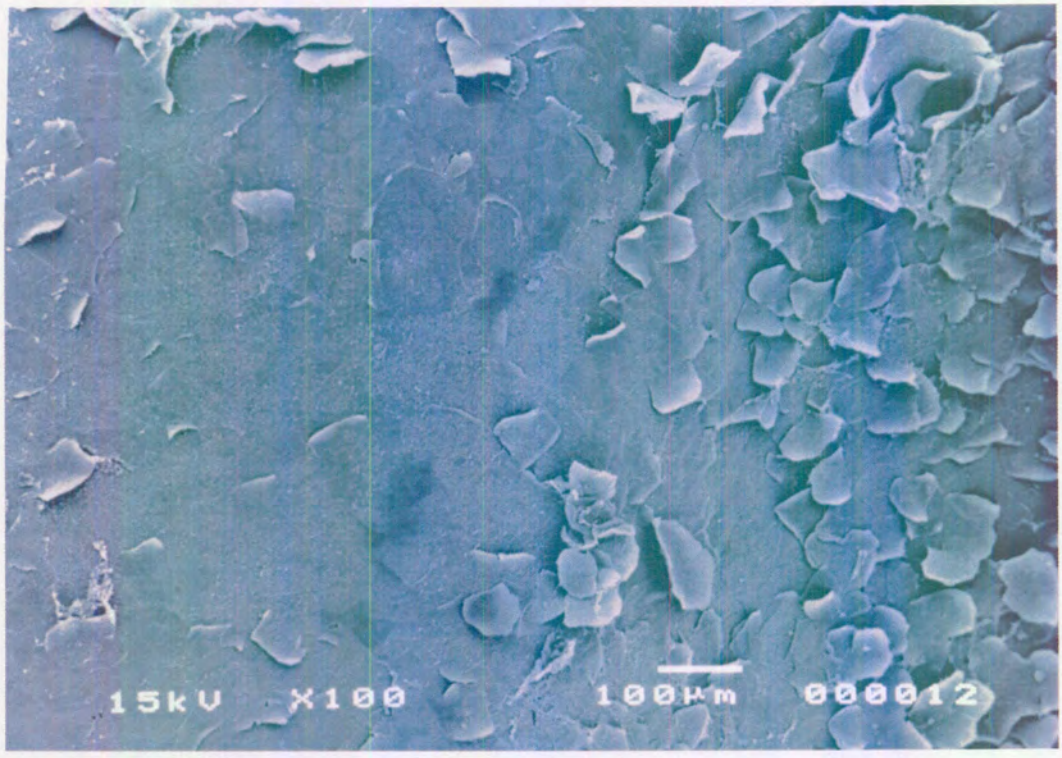

Plate 13: SEM showing the flaking of superficial squamosal keratinocytes of an adult southern right whale sampled in Antarctic waters. (Mag 100X).

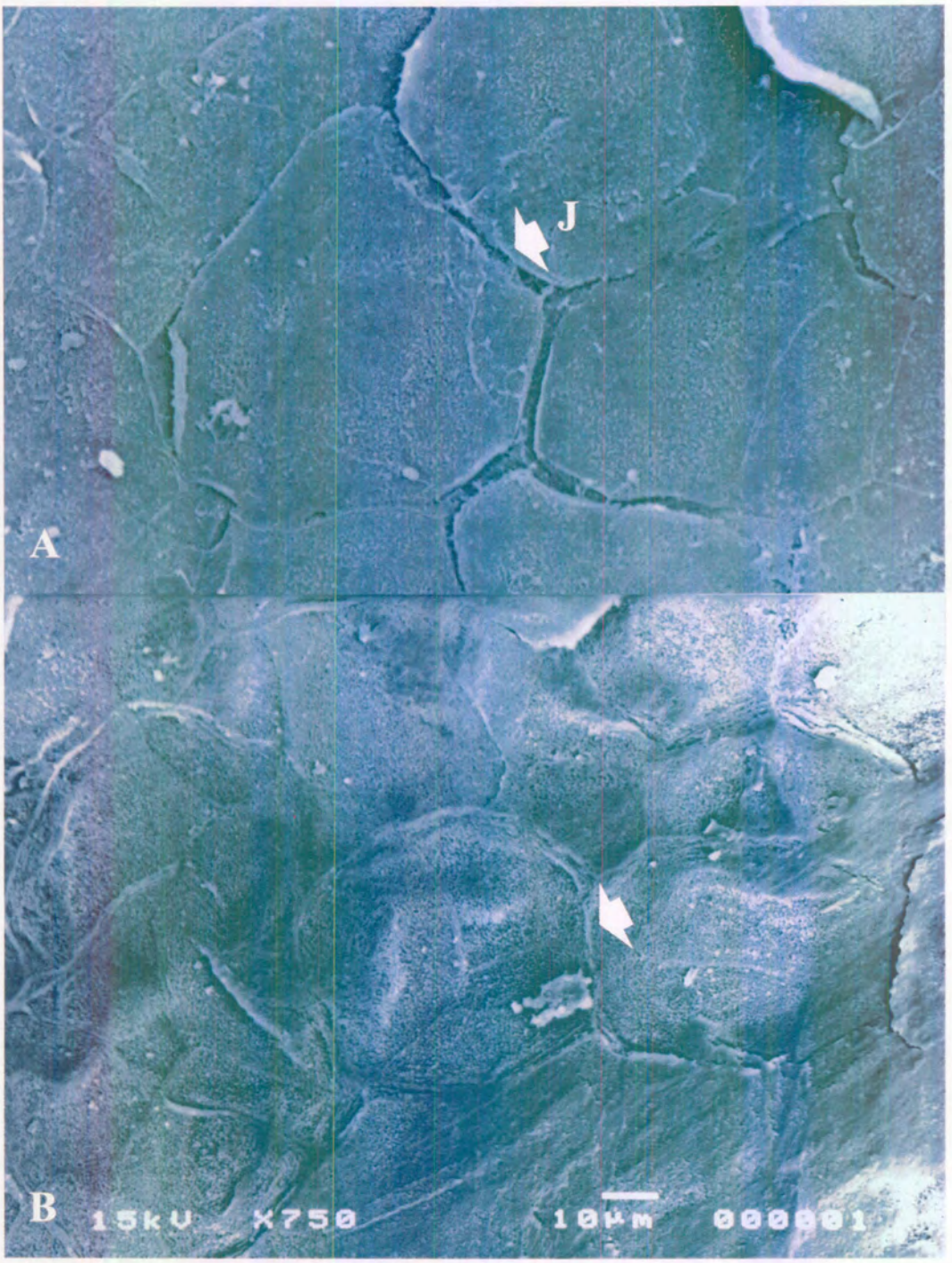

Plate 14: SEM of superficial keratinocytes with distinct pentagonally-shaped cell junctions (j) forming deep surface ridges in a honeycomb-like pattern (arrows). A, individual epidermal cells in the process of sloughing; B, cell boundaries formed by epidermal cells that have already sloughed. (Mag 750X).

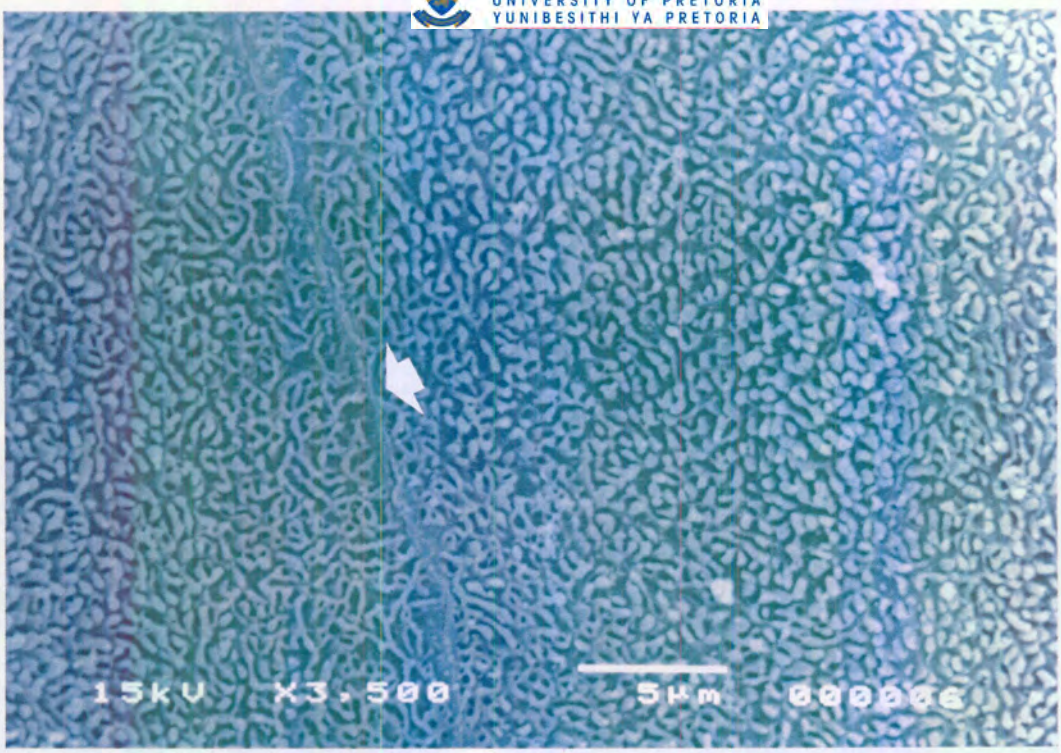

Plate 15: SEM showing the uniformly pitted appearance of the superficial epidermis exposing the disconnected and freed intercellular boundaries (arrow), after sloughing has occurred. (Mag 3 500X).

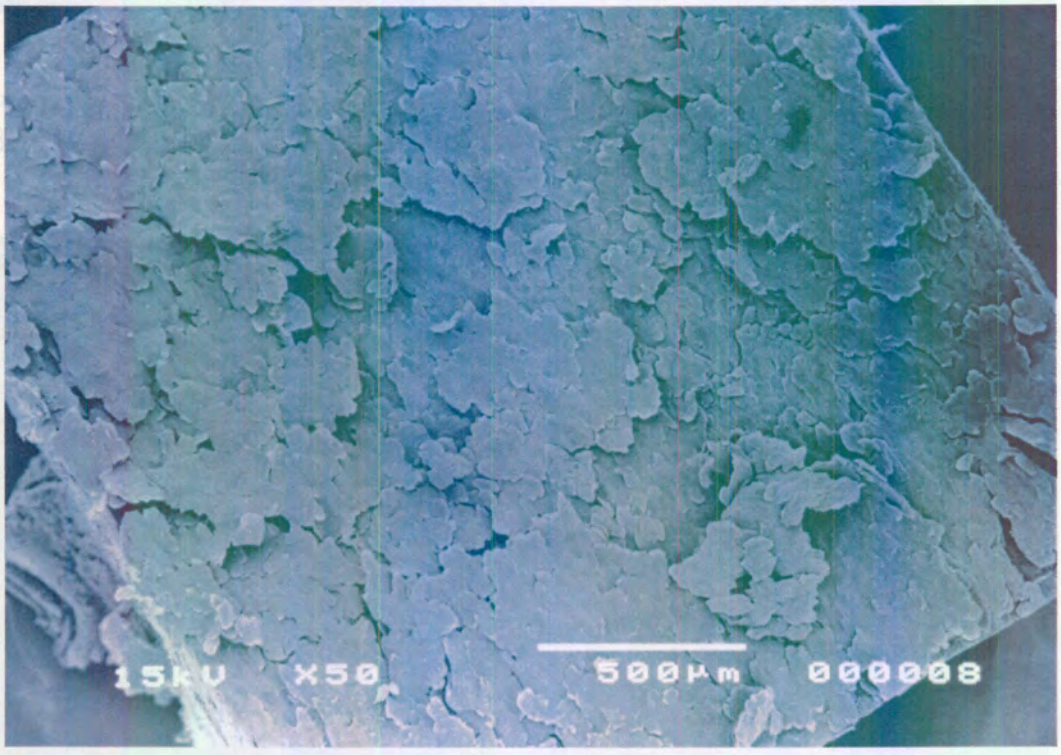

Plate 16: SEM exposing the multi-layered superficial epidermal moult of a subadult southern right whale. (Mag 50X).

Plate 17: Southern right whale cow and "smooth-skinned" calf. Note the smooth dark skin of the calf.

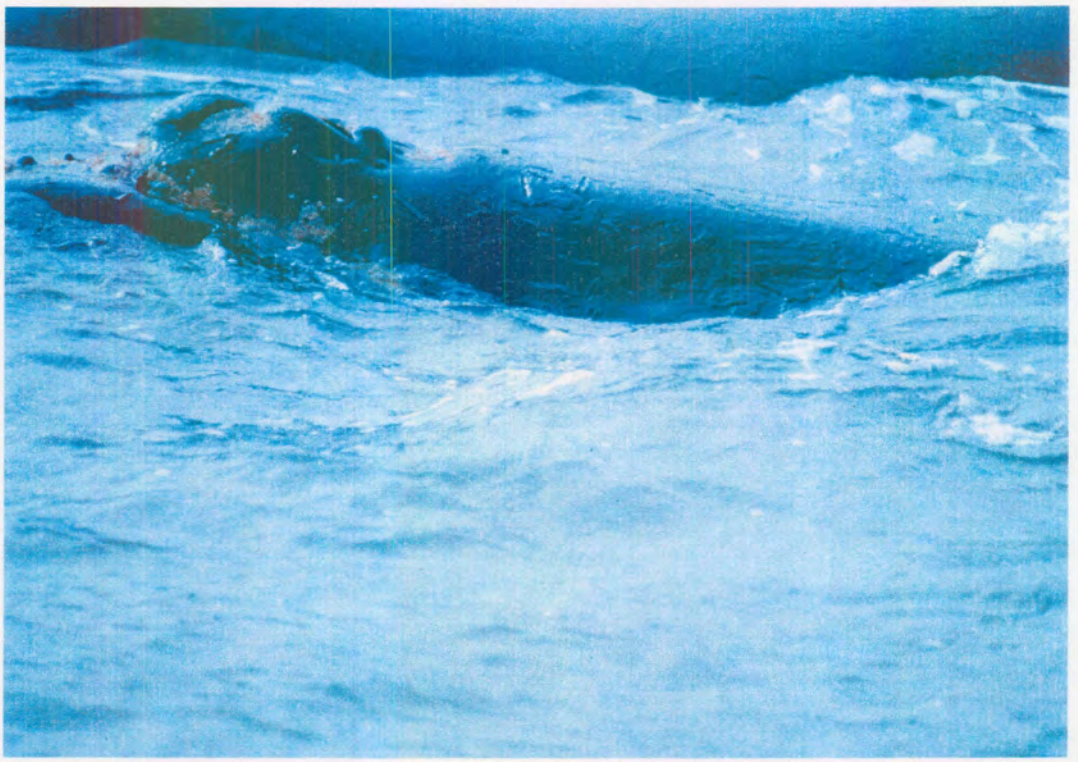

Plate 18: Southern right whale cow and "rough-skinned" calf. Note the light grey colour and rough, broken appearance of the calf's skin.

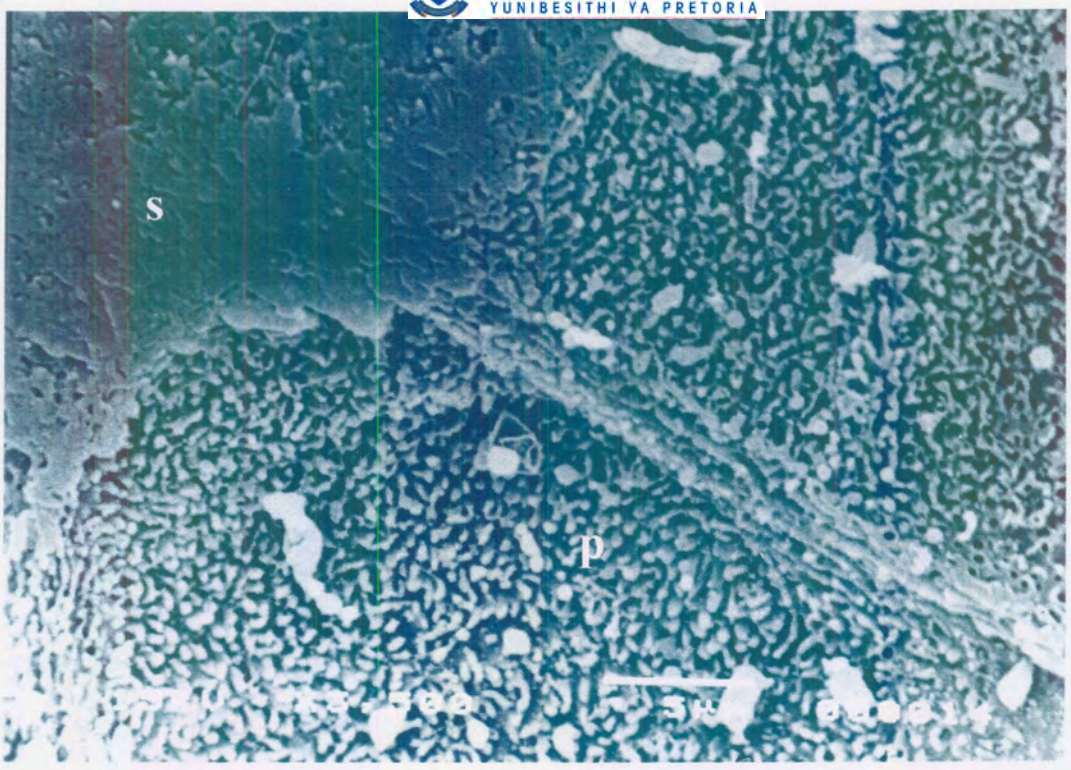

Plate 19: SEM of the skin of an early season, smooth-skinned calf. Note the patches of smooth skin (s) with no honeycomb patterns visible which give way to the exposed, pitted surface of recently sloughed areas (p). (Mag 3 500X).

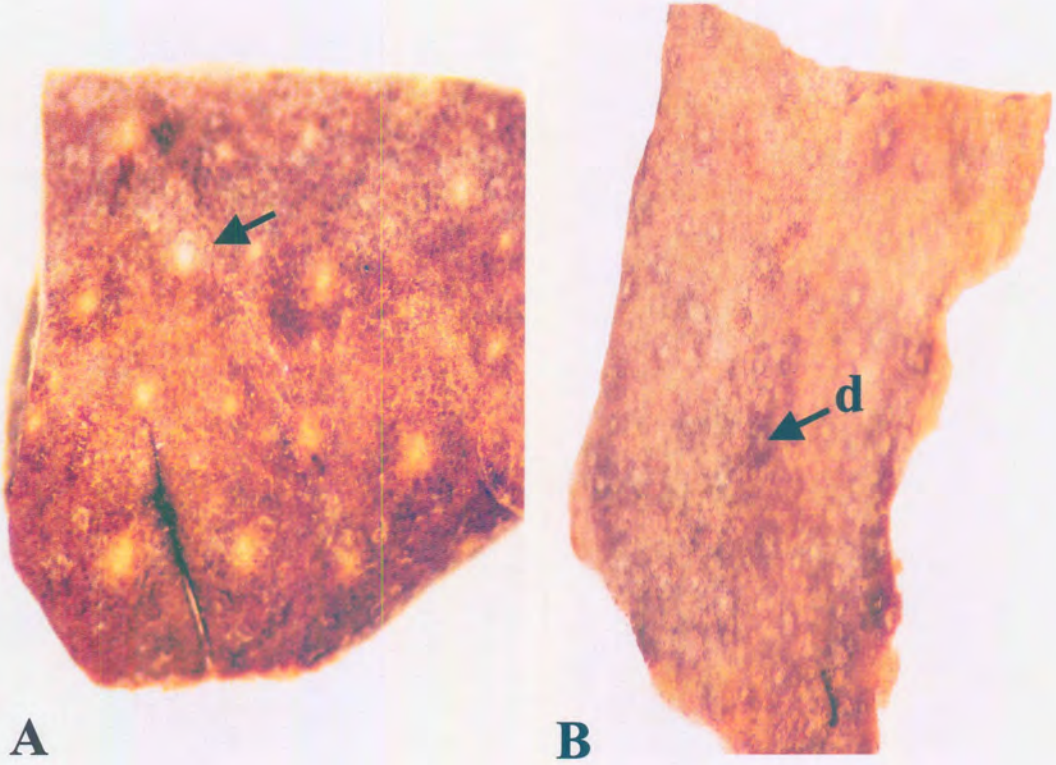

Plate 20: "Dots" on the surface of the skin of a southern right whale sampled in the Antarctic (a) and a brindle-coloured southern right whale calf (b) showing the distinct dark ring (d) around the"dots".

although brindle/grey-coloured animals (Schaeff *et al.*, 2000) seem to have a distinct dark ring around each dot (Plate 20b).

Rough-skinned calves did not show the typical sloughing features as described above, instead, the surface skin of these animals was very uneven (Plate 21), with no visible dots. However, scanning electron microscopy revealed keratinocytes forming rosettes around, and superficial to, dermal papillae. The dermal papillae were located in the centre of the rosettes on the skin of rough-skinned calves (Plate 21) which, together, presumably form the superficial dots seen on smooth-skinned animals. Rosettes were also exposed in samples from a stranded adult (89/30) that possessed no superficial epidermal layers (Plate 22), probably due to autolysis/decomposition.

2.3.2.2 Histological and ultrastructural epidermal features

Since the ultrastructural features of the southern right whale calf epidermis have been reviewed in detail by Pfeiffer & Rowntree (1996), this description provides only a limited review as well as adding information on animals from other age groups.

When analysed histologically, many of the biopsied and stranded samples seemed to have lost the outermost epidermal layers (stratum corneum) or only small portions of this layer were present, which indicates the friable nature of this layer. However, all sections that possessed stratum corneum cells (from both rough and smooth-skinned calves and juvenile and subadult samples) revealed the presence of keratin in these superficial cells. The epidermis in both adults and calves consisted entirely of stratified squamous epithelium making up three distinct layers, namely (from outermost to innermost), stratum corneum, stratum spinosum and stratum basale (Plates 23a and 23b). Neither a stratum granulosum nor a stratum lucidum was observed, the absence of which has been described for other cetaceans (Plate 24) (Page 7).

The stratum corneum consisted of multiple layers of stratified, squamous keratinocytes, with their long axes parallel to the skin surface. Most cells possessed flattened, moribund nuclei, due to the keratin in their cytoplasm. The presence of

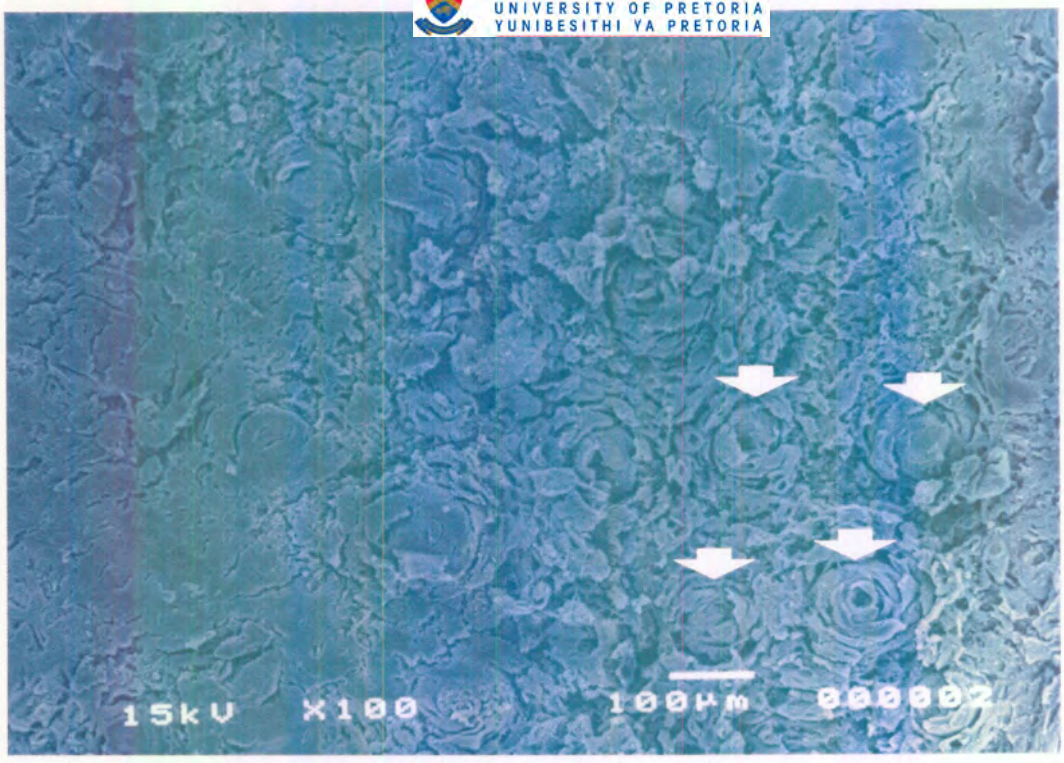

Plate 21: SEM showing the irregular nature of the superficial epidermis of a "rough-skinned" southern right whale calf. Note the exposed keratinocyte rosettes around and superficial to the dermal papillae (arrows). (Mag 100x).

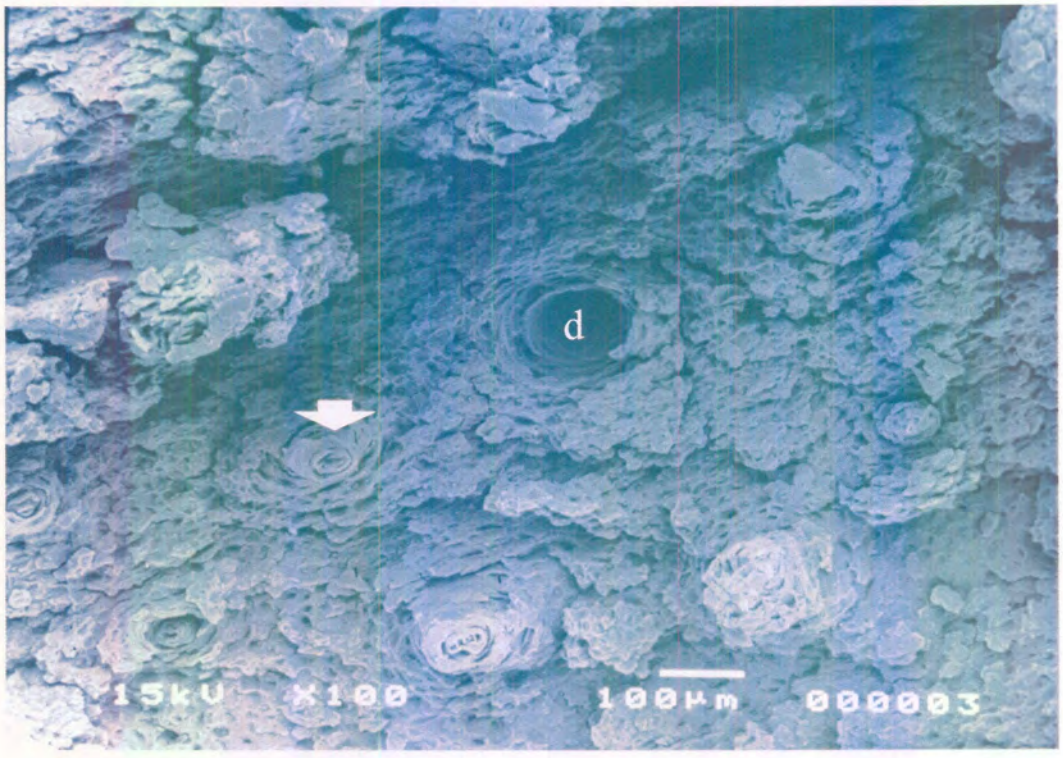

Plate 22: SEM of exposed keratinocyte rosettes around and superficial to the dermal papillae (arrow) of the skin of a stranded adult southern right whale (89/30). Decomposed dermal papilla (d). Absence of stratum corneum, probably due to decomposition. (Mag 100x).

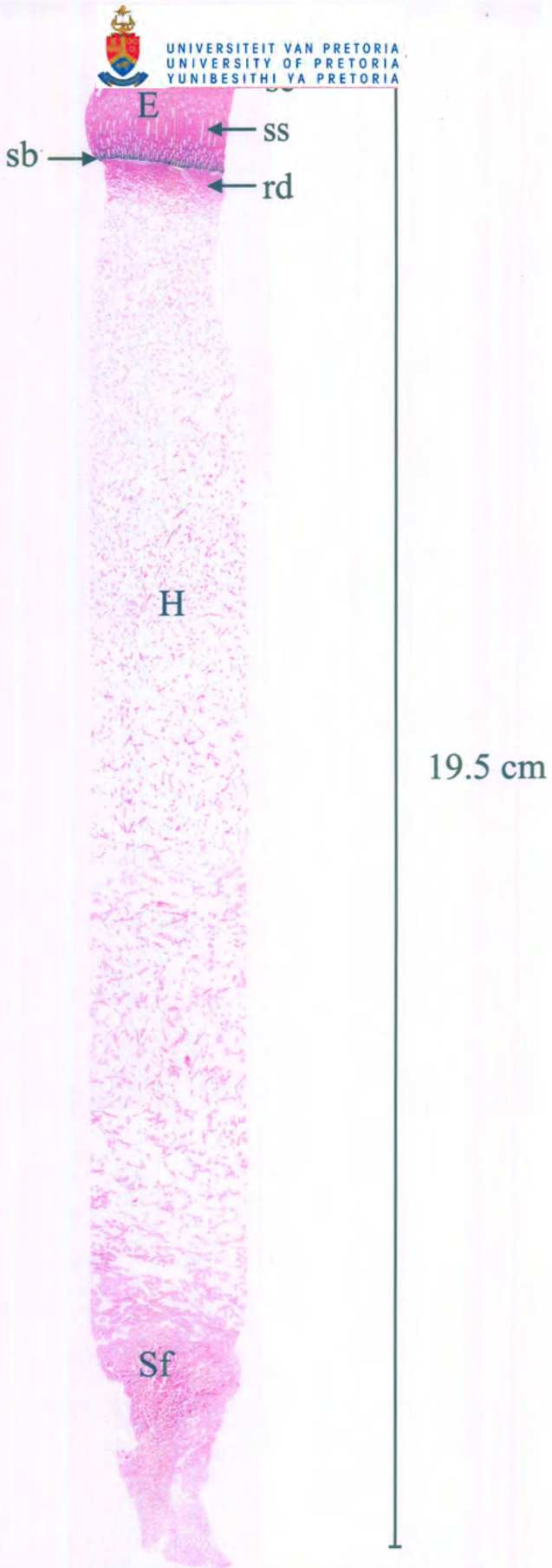

Plate 23a: Longitudinal section through the integument of a juvenile southern right whale (00/11), killed by a boat collision. Epidermis (E), stratum corneum (sc), stratum spinosum (ss), stratum basale (sb), reticular dermis (rd), hypodermis (H) infiltrated with collagen fibres (pink lines) and adipocytes (white spaces), superficial fascia (Sf). Note increase in the concentration of adipocytes in a proximal direction. (H/E, whole mount).

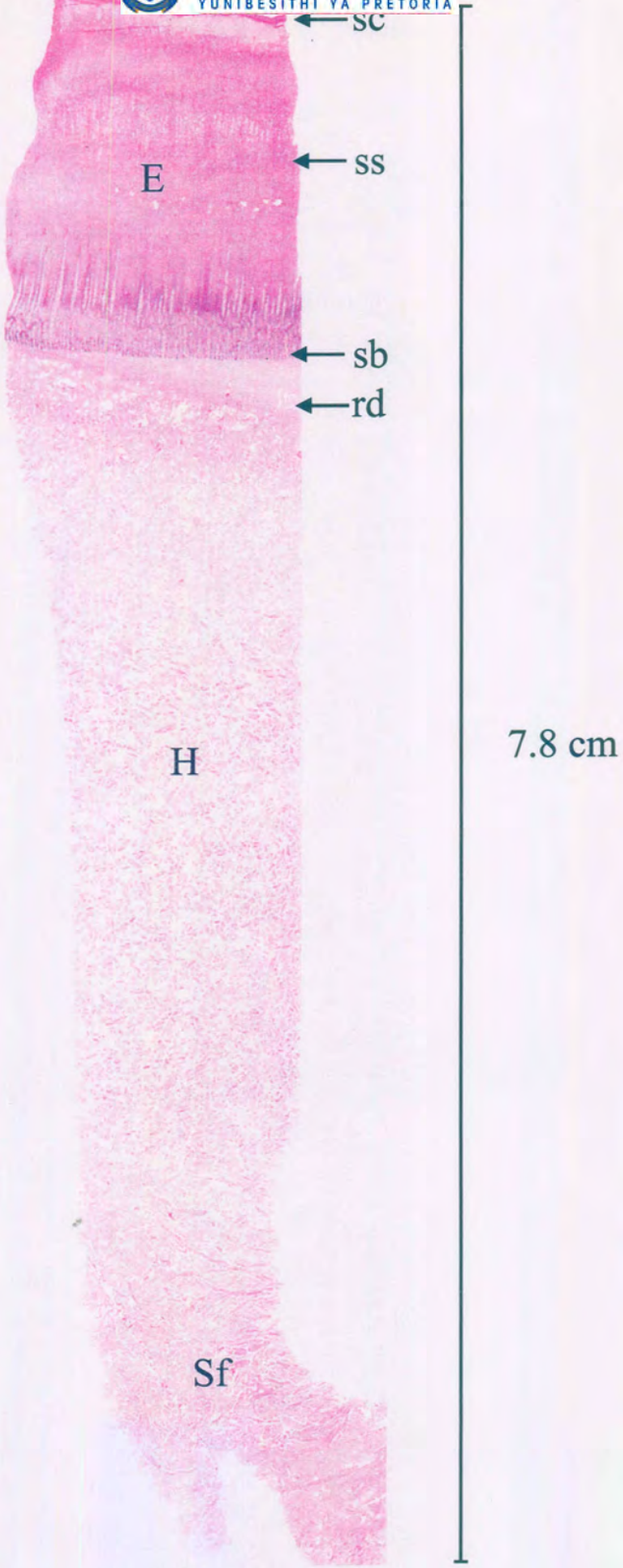

Plate 23b: Longitudinal section through the integument of a neonatal southern right whale (00/09). Epidermis (E), stratum corneum (sc), stratum spinosum (ss), stratum basale (sb), reticular dermis (rd), hypodermis (H) infiltrated with collagen fibres (pink lines) and adipocytes (white spaces), superficial fascia (Sf). Note the lower concentration of adipocytes compared to Plate 23a. (H/E, whole mount).

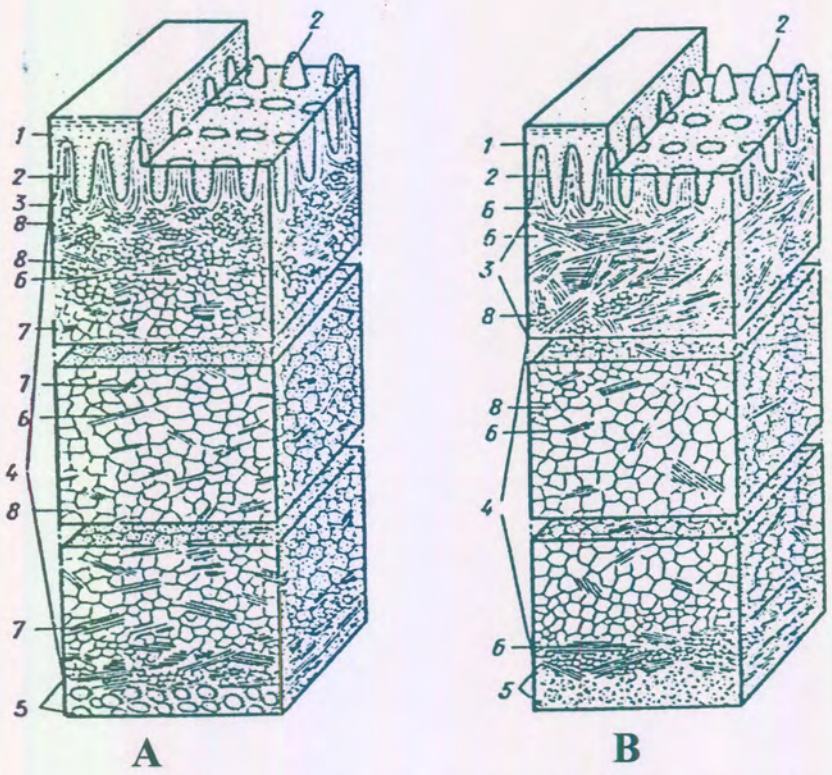

Plate 24: Structure of the integument of rorquals (A) and toothed cetacea (B). Legend: 1 = epidermis, 2 = dermal papillae, 3 = dermis, 4 = hypodermis, 5 = subcutaneous musculature, 6 = bundles of collagen fibres, 7 = bundles of elastin fibres, 8 = adipocytes (from V. Sokolov, 1955).

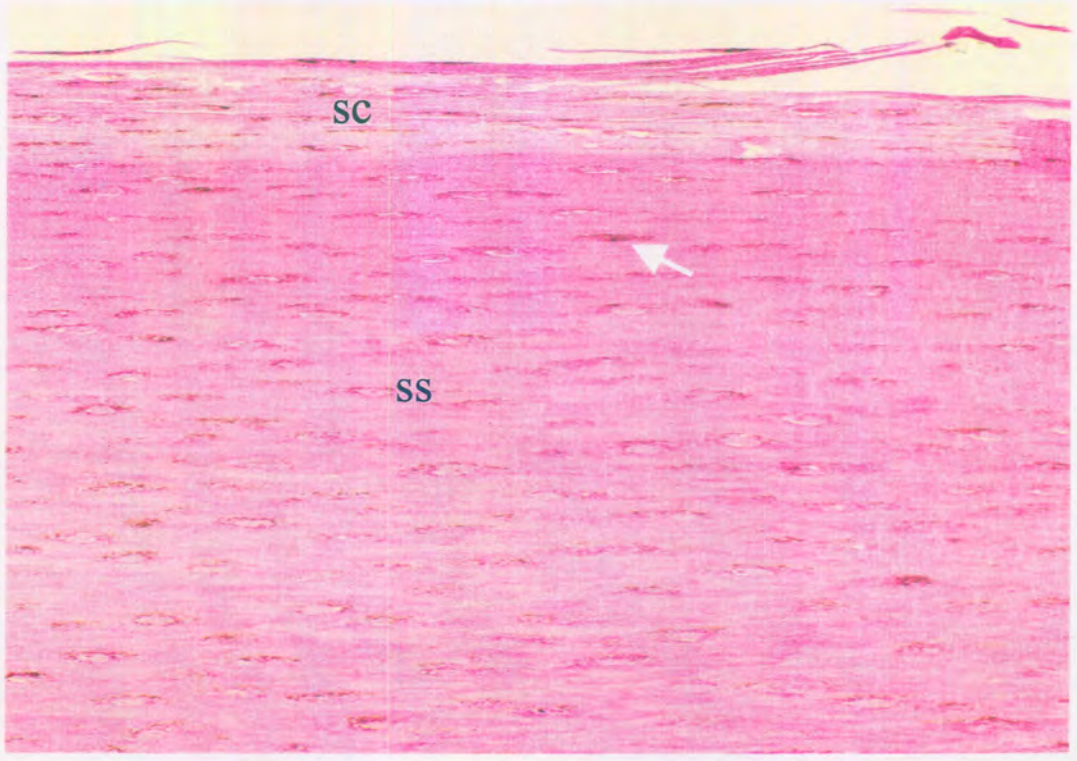

Plate 25: Stratum corneum (sc) and stratum spinosum (ss) layers of the epidermis of an adult southern right whale. Note melanin granules stained black (arrow). (H/E, Mag 200X).

nuclei indicated that the process of keratinisation in these cells was not complete and the nature of this layer could therefore be described as parakeratotic. Within pigmented epidermis, most of the stratum corneum cells possessed melanin granules (Plate 25), which were usually located at the base of the cells, usually surrounding the nuclei.

The epidermal cells deep to the parakeratotic stratum corneum comprised a typically mammalian stratum spinosum. The stratum spinosum was the most extensive of all the epidermal layers. The spinosal cells were rounded, oval or polyhedral in shape, becoming increasingly flattened near the stratum corneum (Plate 25). At all the body positions studied, tightly packed spinosal cells occurred along the sides and tips of the dermal papillae (Plate 26), possibly forming the rosettes mentioned above.

Melanin granules were present in the cytoplasm of most cells, which occurred near the nucleus (Plate 27). The nuclei in this layer were more complete in presentation and occurred in a larger number of cells when compared to the nuclei of the stratum corneum cells. When viewed using light microscopy, the cell boundaries in this region appeared very thick. Transmission electron microscopy revealed that these thick boundaries were formed by highly folded cell membranes and desmosomes (Plates 27 and 28). Tonofilaments, arranged in parallel bundles, were present in all cells in the stratum spinosum (Plate 28). Ultrastructurally the large spinosal cell nuclei were irregular in shape with generally centrally located nucleoli (Plate 27). Lipid droplets and large groups of glycogen granules were present in the cytoplasm of spinosal cells (Plate 29). Collagen and elastin fibres were more abundant in the cells of the stratum spinosum of flukes and flippers than in any other location.

The stratum basale of the epidermis consisted of a layer of variably shaped keratinocytes which interdigitate with the basal lamina separating the dermis and epidermis (Plate 30). The basal cells had basally located, oval nuclei and contained numerous mitochondria, tonofilaments associated with desmosomes, and lipid droplets. The surfaces of adjacent cells interdigitated extensively, with numerous desmosomes present. These interdigitations produced wide areas of apparent intercellular space, that were caused by the interfolding of the undulating adjacent

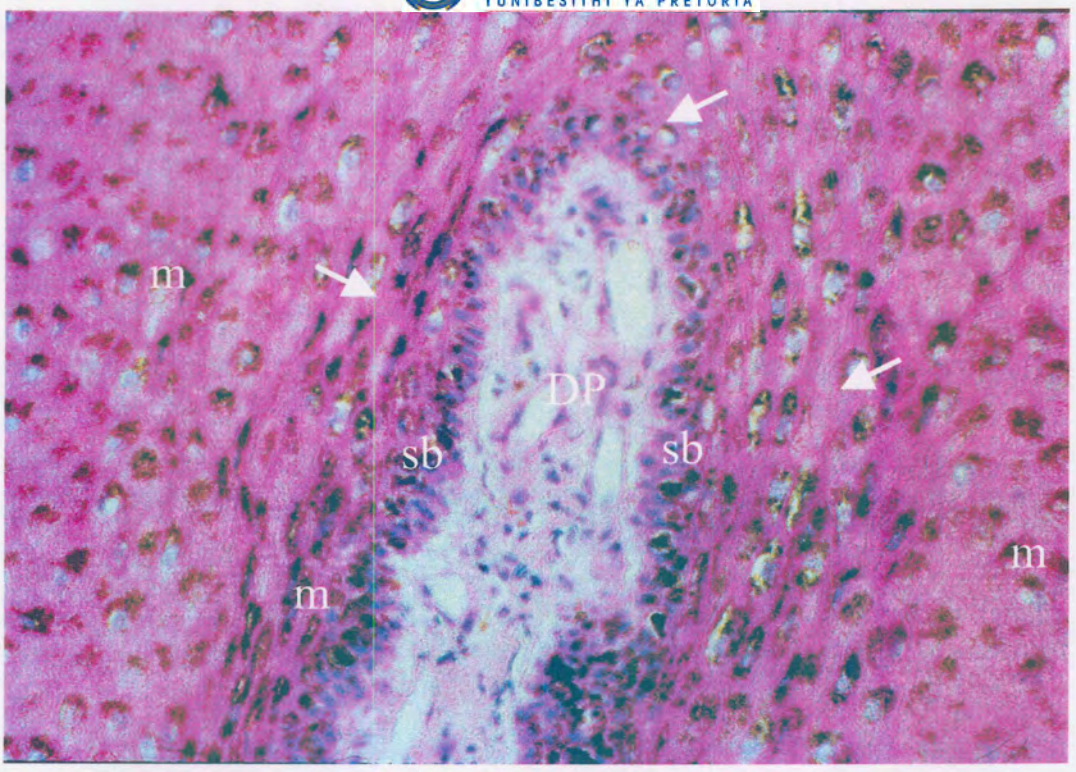

Plate 26: A dermal papilla (DP) protruding into the stratum spinosum of a non-calf southern right whale. Note flattened stratum spinosum cells along the sides and tip of the papilla (arrows), melanin granules (m) stained black and stratum basale. (H/E, Mag 200x).

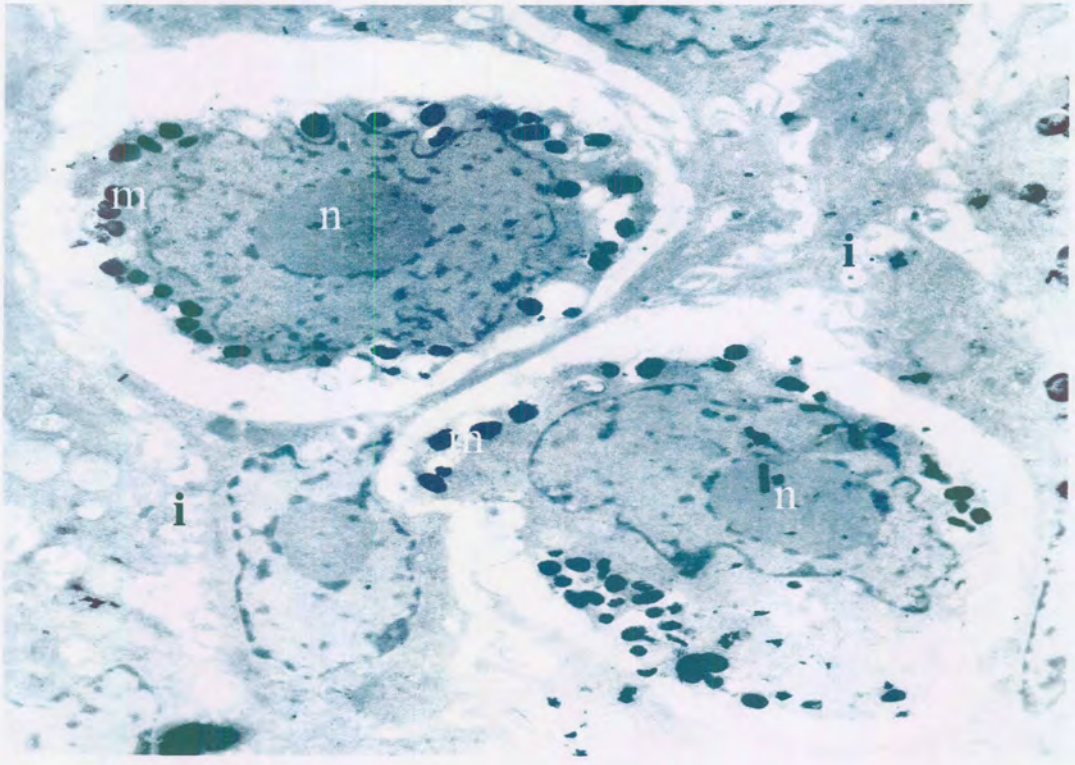

Plate 27: TEM showing the presence of melanin granules (m - black dots) around the nuclei of stratum spinosal cells. Note the thick cell boundaries formed by inter-folding cell membranes (i), nucleolus (n). (Mag 12 000X).

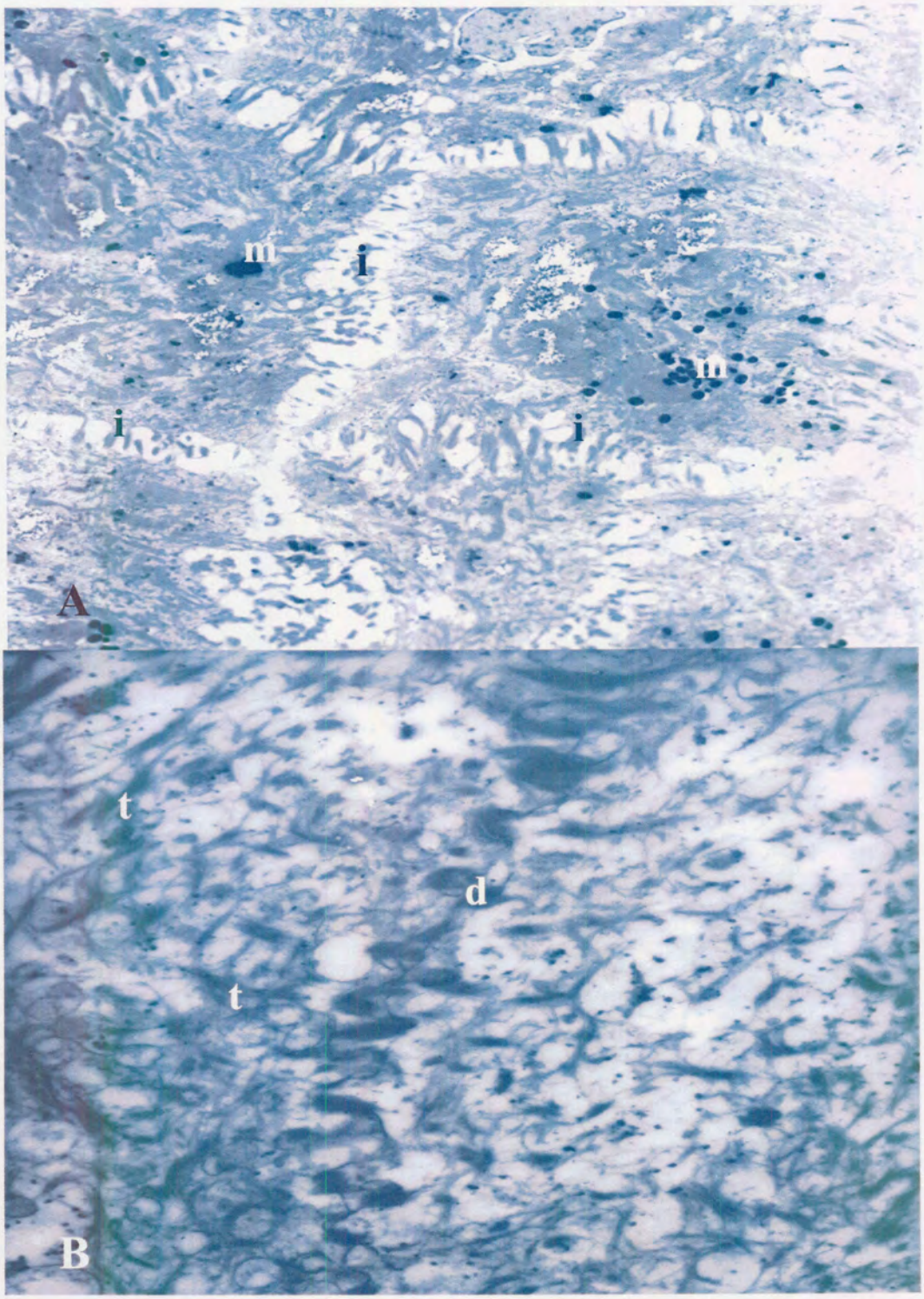

Plate 28: TEM showing the extensive interfolding of stratum spinosum cell membranes (i) connected by desmosomes (d). Tonofilaments (t) are present in parallel bundles within these cells, melanin granules (m) are also present. (A = Mag 4 500X, B = Mag 5 000X).

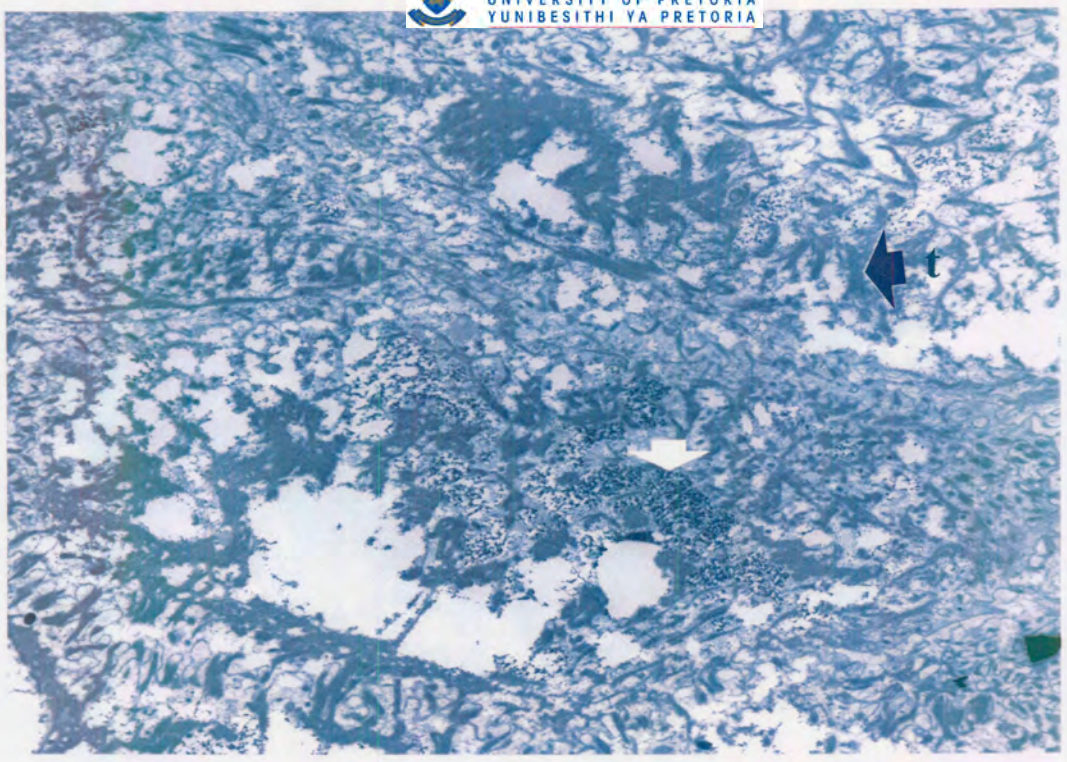

Plate 29: TEM showing large groups of glycogen granules (arrow) in the cytoplasm of spinosal cells. Tonofilaments (t with arrow). (Mag 6 000X).

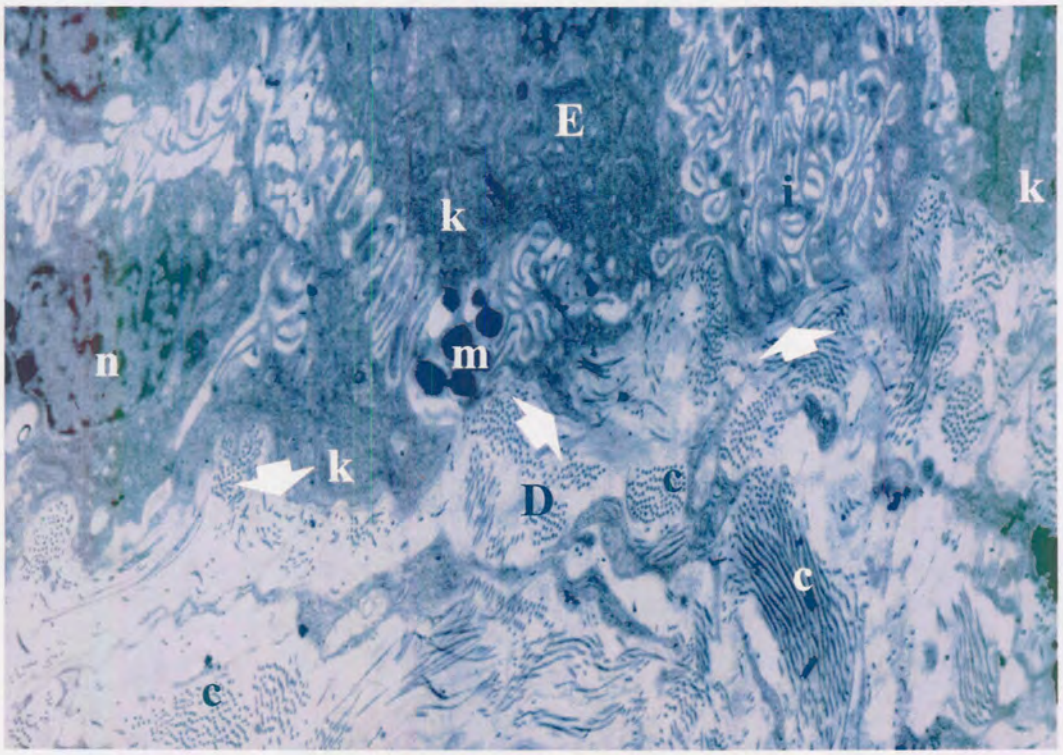

Plate 30: The stratum basale of the epidermis consisted of a layer of variably shaped keratinocytes (k) which interdigitate with the basal lamina (arrows) separating the epidermis (E) and the dermis (D). Melanin granules (m), nucleolus (n), interconnecting cell membranes (i), collagen fibres (c). (Mag 7 500X).

cell membranes, with greater numbers of desmosomes than found in either of the other strata (Plate 30). In pigmented areas, melanocytes were present among the basal cells (Plate 30) and occasionally in the first few layers of the stratum spinosum. These specialised cells were large and well developed with typical dendritic processes and melanosomes.

Melanosomes, and consequently melanin granules, were most abundant in the stratum basale. A basement membrane separated the dermis and the epidermis but numerous membranous undulations of the basal cells maintained contact with the basal lamina and these layers (Plate 30).

Histologically, the only detectable difference between the epidermal strata of grey adults, partially albinistic calves and dark-skinned animals was that the concentration of melanin granules was visibly reduced in the two former colour forms. Smooth-skinned calves and adults (Plates 25) appeared to possess more melanin granules than rough-skinned calves (Plate 31).

2.3.2.3 Histological and ultrastructural dermal and hypodermal features

The dermis was divided into a papillary and reticular layer (Plate 32). Highly elongated macroscopic dermal papillae interdigitated extensively and distinctly with epidermal rete and were abundant throughout the integument along the body. The basal margin of the papillary dermis was composed of scattered adipocytes that infiltrated irregular, white fibrous connective tissue strands. Extensive vascularisation and innervation were evident, with nerves extending from the hypodermis to the base of the dermal papillae and some blood vessels and nerve fibres extending along the dermal papillae (Plates 33 and 34). The reticular dermis consisted of tightly packed collagen fibres lying parallel to the long axis of the whale's body (Plates 23 and 34). These fibres formed a thick network with essentially no adipocytes present, effectively creating a narrow "fat-free" band/zone (Plates 23 and 34). Collagen fibres from this zone extended into the dermal papillae. Few elastin fibres extended through this layer. Deep to this layer, the hypodermis was defined by the increased presence of adipocytes. Adipocyte cell size was not measured, but together with the numbers of

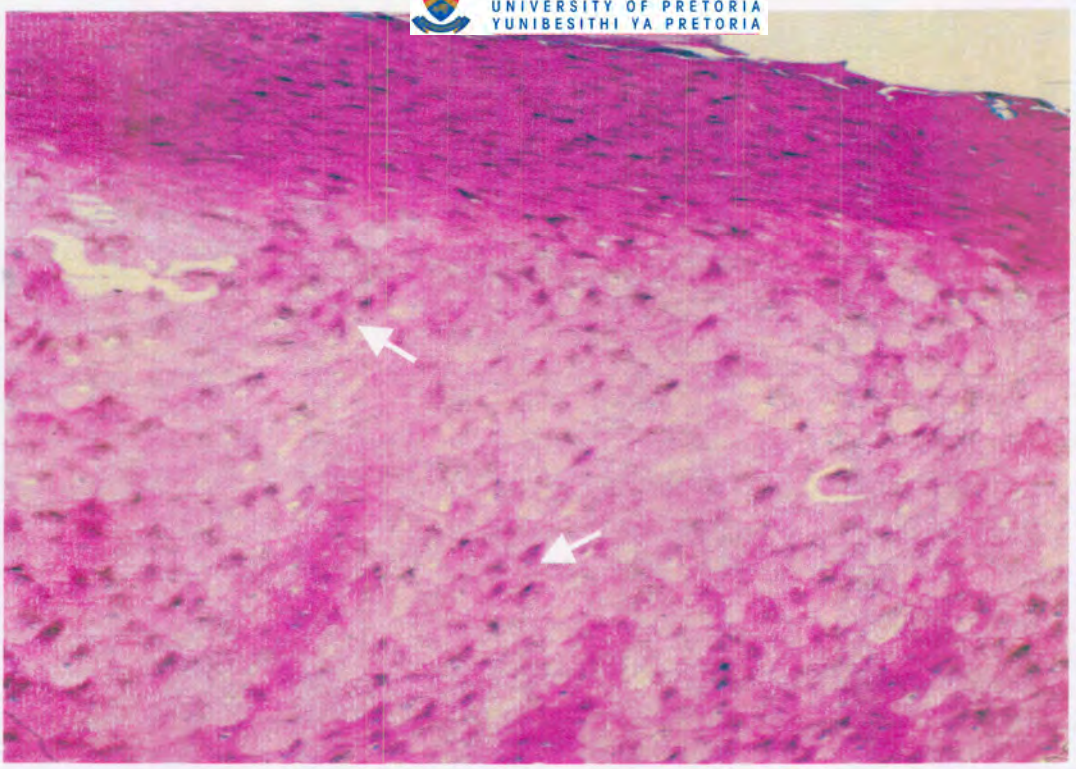

Plate 31: Stratum spinosum and stratum corneum cells of the epidermis of a "rough-skinned" southern right whale calf (00/09). Note reduced concentrations of melanin granules (arrows) compared to Figure 25. (H/E, Mag 100X).

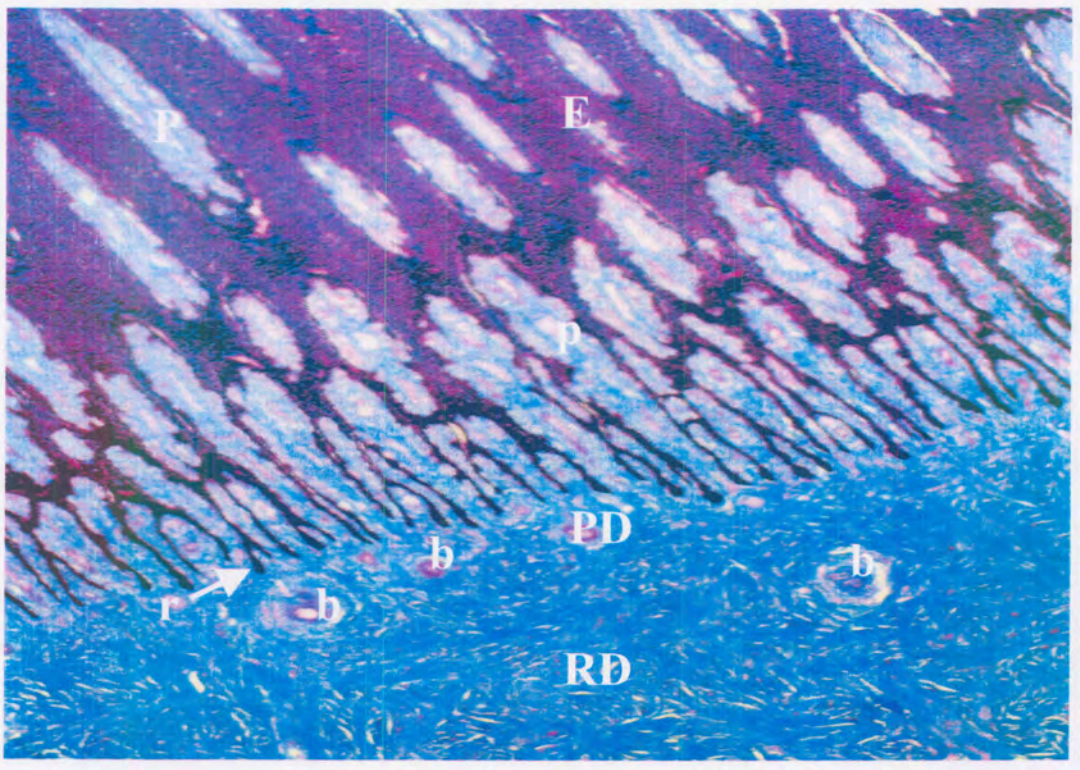

Plate 32: The skin of a neonatal southern right whale calf (98/09). Dermal papillae (p) reach from the base of the papillary dermis (PD) into the epidermis (E) and epidermal rete (r) interdigitate with the dermal papillae. The reticular dermis (RD) consists of dense collagen fibres (dark blue) with blood vessels (b) coursing through both layers. (Ayoub-Shklar, Mag 25X).

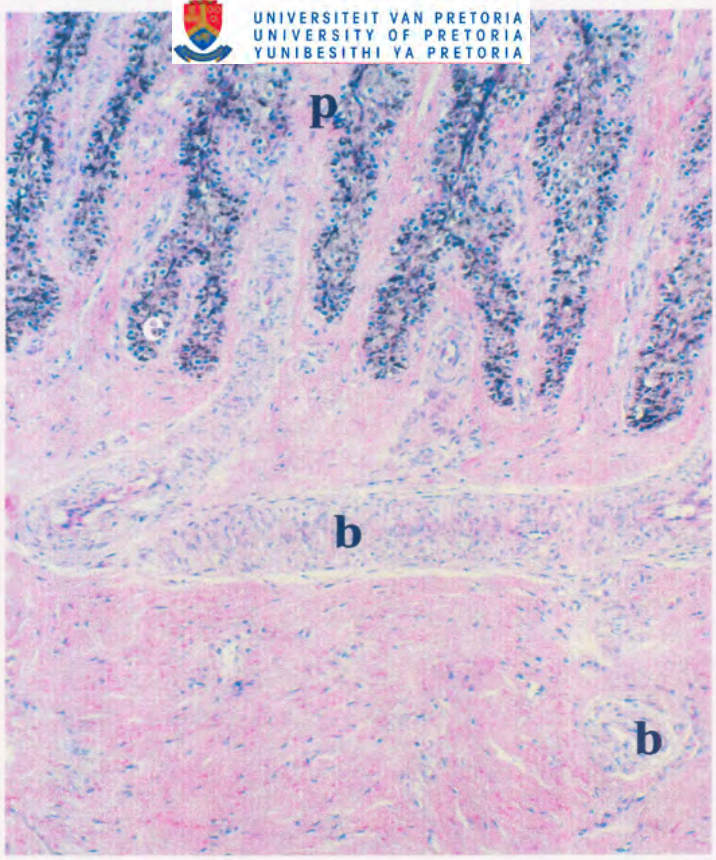

Plate 33: Blood vessels (b) extending from the papillary dermis into dermal papillae (p) and between epidermal rete (e) of the skin of a neonatal southern right whale (00/09). (H/E, Mag 100X).

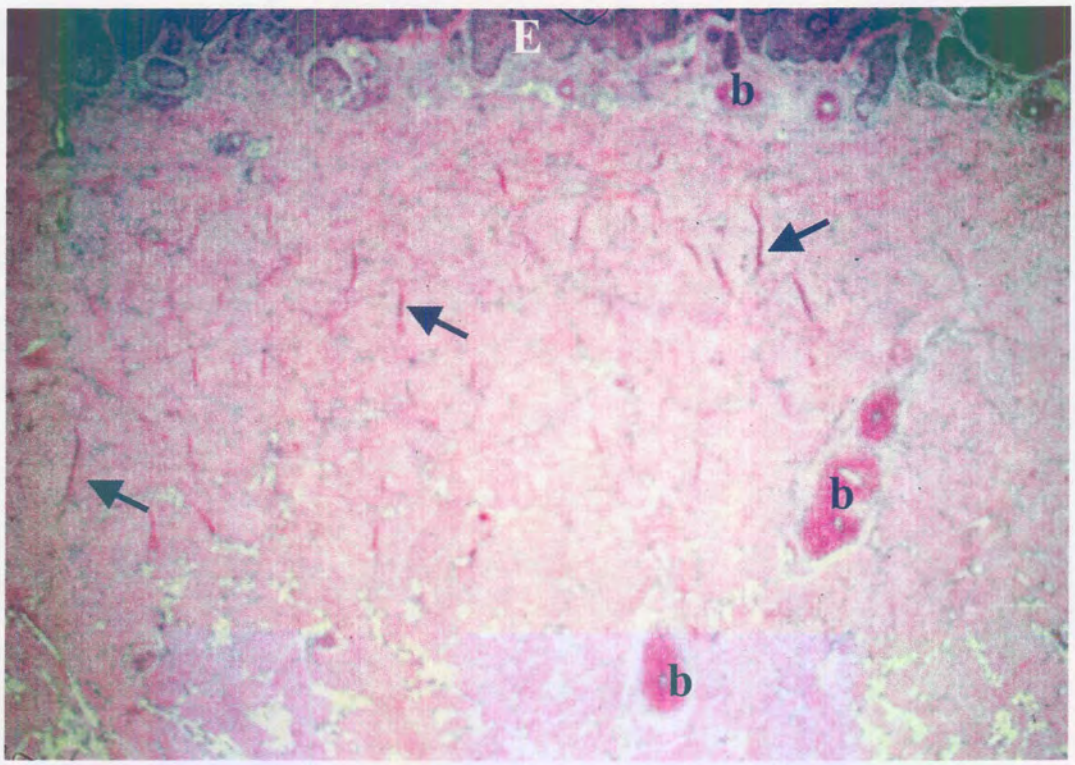

Plate 34: A skin sample from a neonatal southern right whale (00/10) showing the reticular dermis consisting of tightly packed collagen bundles (arrows) forming a "fat-free" zone. Blood vessels (b), shown in irregular cross-section here, course through this layer. Epidermis (E). (H/E, Mag 25X).

adipocytes seemed to increase ersely proportional to the connective tissue (Plate 23a).

The collagen fibres began to form small bundles, arranged in various orientations, which were completely surrounded by large groups of adipose tissue (Plate 35 and 36). This layer formed the majority of the southern right whale integument. A thin layer of tissue (superficial fascia) connected the hypodermis to the underlying muscular layers (Plates 23a and 23b).

Staining revealed that the white connective tissue in both the papillary and reticular dermis as well as in the flippers and flukes, consisted almost entirely of collagen fibres infused with small amounts of elastin fibres.

2.3.2.4 Histological structure of hair follicles and callosity "stalks"

The integumentary layers forming callosities were not found to be structurally different from the integumentary structure found along the bodies of southern right whales. The hairs associated with the callosities and present on the lower lip projected about 1-2 cm above the epidermis and arose from specialised follicles that extended about 1.1 cm into the blubber, deep to the epidermis and papillary dermis (Plate 37). The follicle was a double-walled structure that contained blood sinuses between inner and outer dermal connective tissue sheaths (Plate 38).

The pigmented epidermal projections ("stalks") that occurred within callosity formations (Page 19) were examined in a stranded juvenile and consisted of nucleated and viable stratum spinosal cells that were not extensively flattened (Plate 39). No true stratum corneum was discernible.

2.3.3 Blubber thickness

Neonates are the only age class for which sufficient data exist to examine trends in blubber thickness over the body in any detail. Dorsal blubber showed little change in thickness from position 1 to position 2, but then a gradually increasing trend with

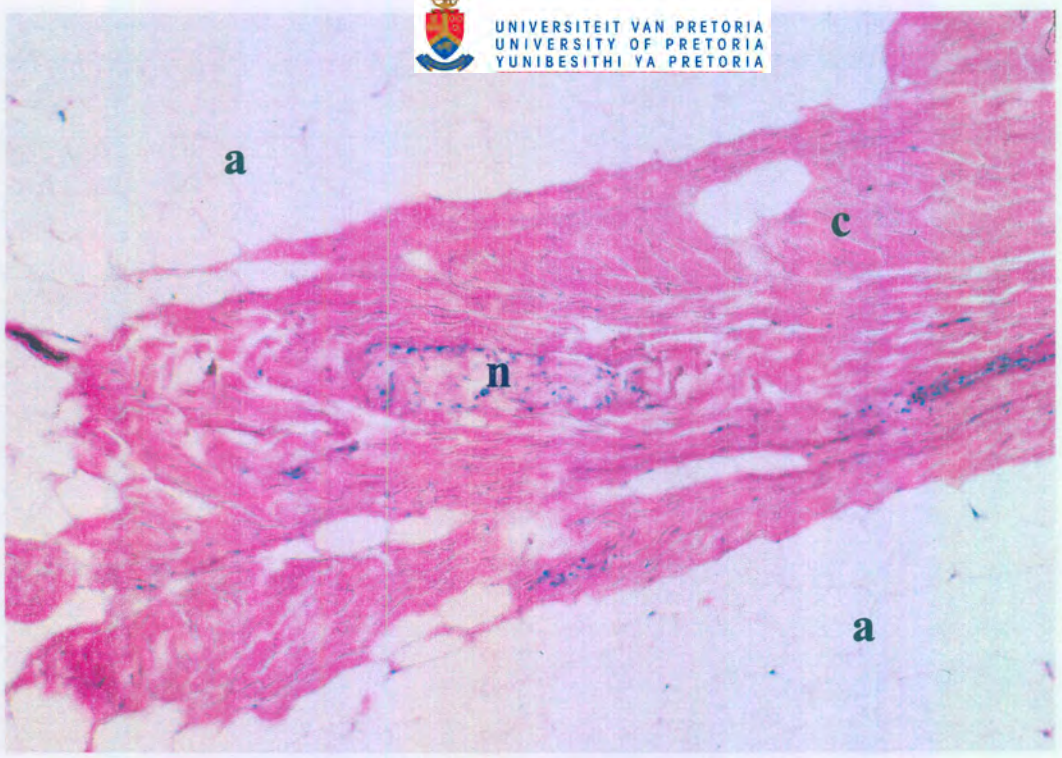

Plate 35: A nerve (n) extending through a collagen fibre bundle (c), surrounded by adipocytes (a), within the hypodermis of a neonatal southern right whale (00/09). (H/E, Mag 100X).

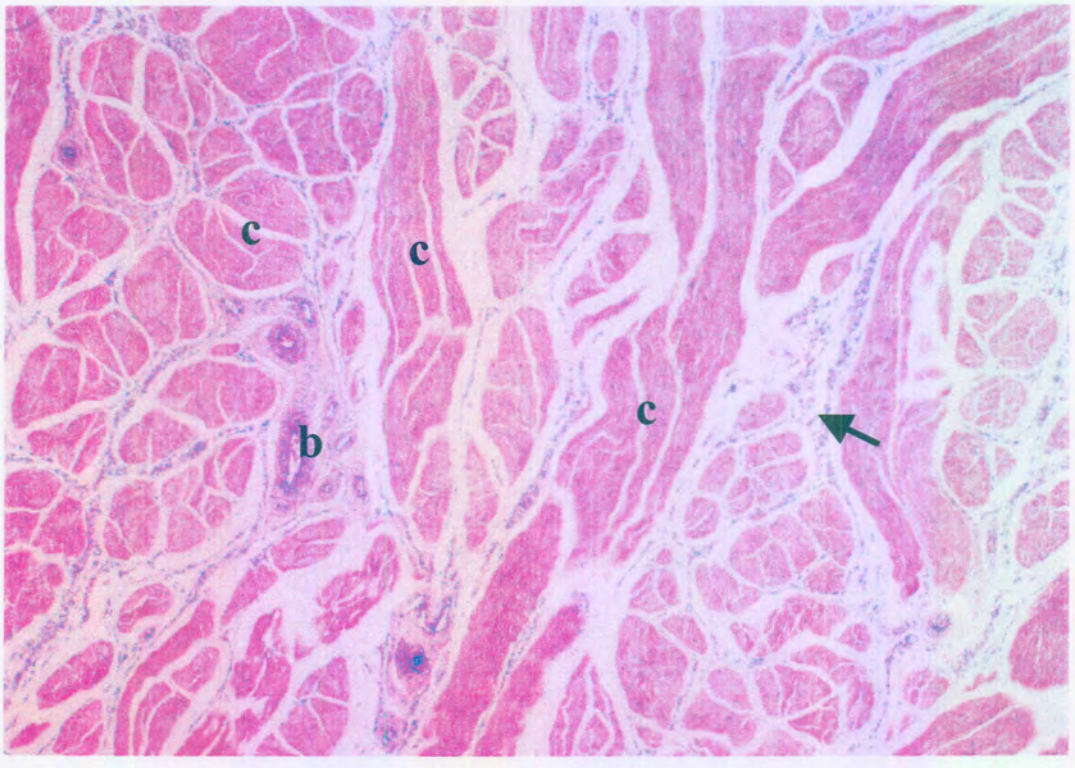

Plate 36: Vascularisation (b) is evident within collagen bundles (c) of the hypodermis of a juvenile southern right whale (00/11). Note the various orientations of the collagen bundles. Only remnants of adipocytes are visible (arrow) due to autolysis. (H/E, Mag 25X).

Plate 37: Longitudinal section through the bonnet callosity of a stranded neonate revealing a hair follicle (f), deep to the epidermis (E). (H/E, Mag 8X).

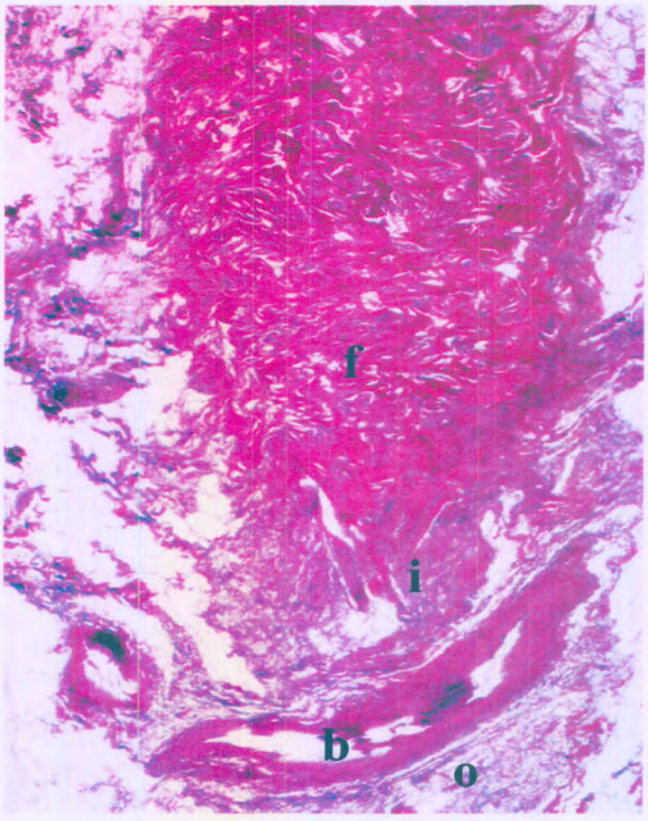

Plate 38: Photomicrograph of the base of the hair follicle (f) in Figure 37 showing the blood sinus (b) appendage between the inner (i) and outer dermal (o) connective tissue sheaths. (H/E, Mag 25X).

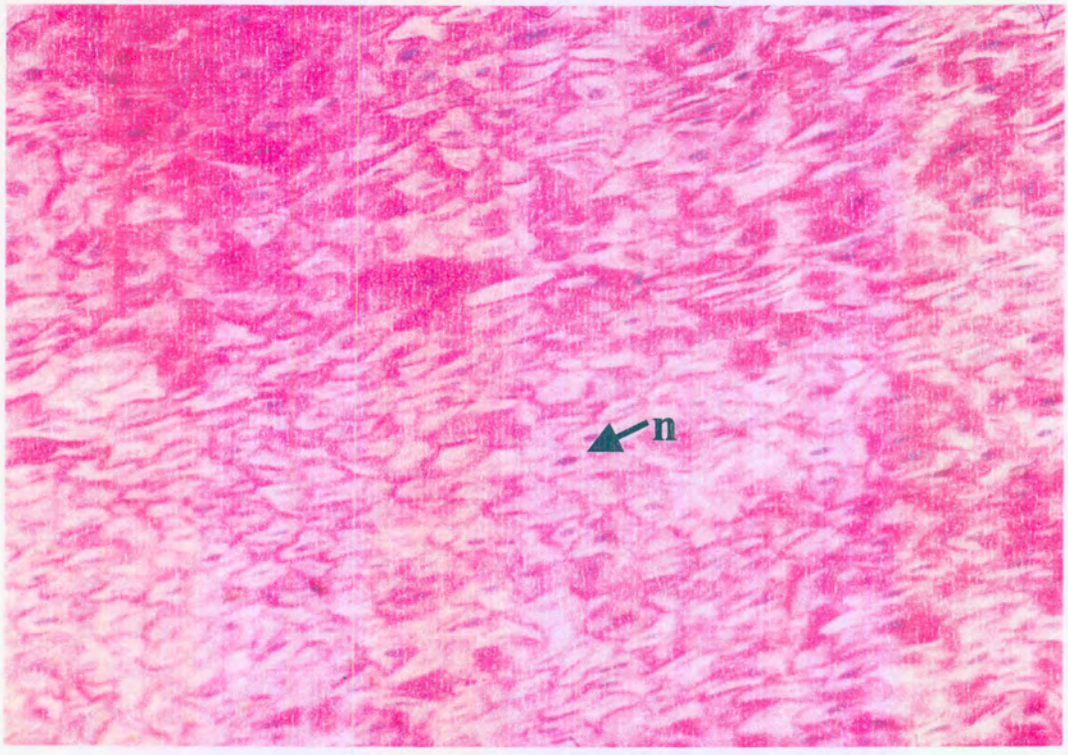

Plate 39: A longitudinal section through an epidermal "stalk", from the head of a stranded juvenile shows this structure consisting of viable, nucleated stratum spinosum cells (n). (H/E, Mag 200X).

position 5 being about twice as thick as position 2 (Figure 1). Lateral blubber was highly variable in thickness at position 1 (possibly because of the proximity to the flipper insertion) and seemed to decline from position 3 to position 5 (although one individual had atypically thick blubber at this position) (Figure 2). Ventral blubber exhibits a similar trend to that of dorsal blubber, decreasing in thickness from position 1 to position 2, but then steadily increasing in thickness, although position 5 was only about 50% thicker then position 2 (Figure 3).

Although insufficient data were available from other age classes to draw any meaningful conclusions, the differences in blubber thickness, in comparable positions, between the neonates, juvenile and subadult seem to indicate that older animals possess thicker blubber layers than younger animals (Table 1).

Biopsies were taken in the vicinities of positions 3 or 4 on the dorso-lateral plane (Plate 3). The deepest blubber sample (excluding pigmented epidermis) retrieved from an early season calf measured 9.7 cm (n = 20), 12.7 cm from a late season calf (n = 18), 17.2 cm retrieved from an early season adult (n = 13) and 21.2 cm from a late season adult (n = 9) (Table 3). None of the biopsies retrieved showed histological evidence of the superficial fascia (the innermost boundary of the integument) which may imply that full core samples were not obtained. Statistical analyses were therefore not applied to the above data as these results are more likely to reflect sampling differences (i.e. length of biopsy head, Chapter 6) than biological changes.

2.4 Discussion

2.4.1 General characteristics of southern right whale skin

Although, researchers and Eskimo captains claimed that early season calves of bowhead whales had specially thick epidermis (Eschricht & Reinhardt, 1866; Haldiman, Abdelbaki, Duffield, Henk & Henry, 1982; Haldiman, Abdelbaki, Duffield, Henk & Henry, 1984; Haldiman & Tarpley, 1993), the differences in epidermal thicknesses between seasonal and age groups of southern right whales were not significant. It is possible, however, that observers of the bowhead whale may

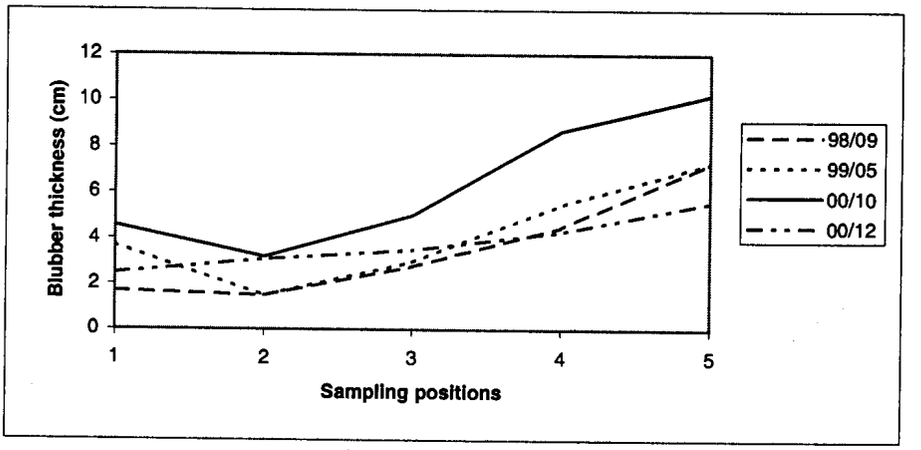

Figure 1: Blubber thickness measurements on the mid-dorsal plane along the body of neonatal southern right whales.

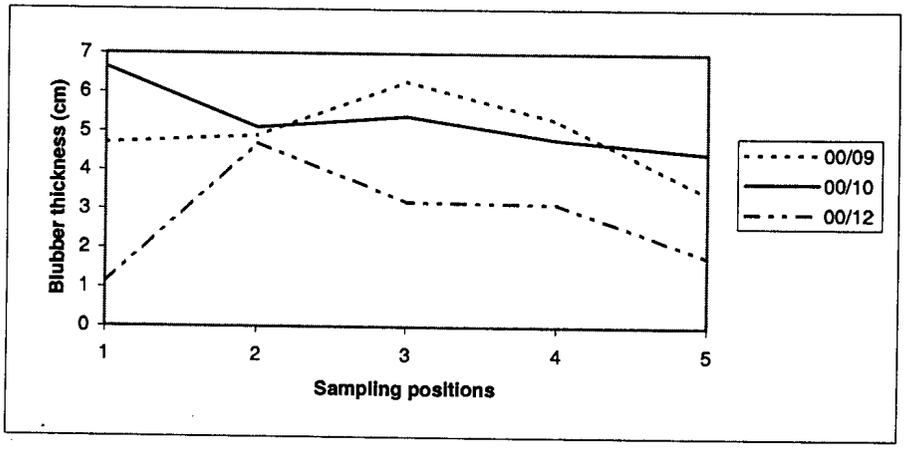

Figure 2: Blubber thickness measurements on the lateral plane along the body of neonatal southern right whales.

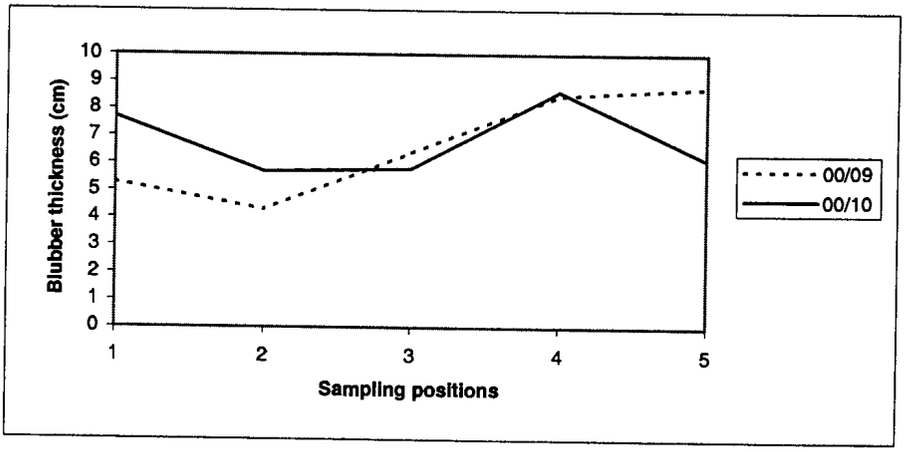

Figure 3: Blubber thickness measurements on the ventral plane along the body of neonatal southern right whales.

have been referring to the epidermis of pre-ecdysal neonates, which may be slightly thicker than in other calves (Chapter 3). No trends in the variation of epidermal thickness along the bodies of stranded animals were obvious, although individual variation was noted along all positions.

Table 3: Depths of biopsied blubber samples retrieved from southern right whale cows* and calves.

Early season calves (n = 20)	**Early season cows (n = 13)**	**Late season calves (n = 18)**	**Late season cows (n = 9)**
4.2	5.5	1.5	19.5
7.4	1.8	1.8	5.0
1.5	5.0	2.3	21.2
7.2	6.5	3.4	20.5
8.3	17.2	1.8	17.3
2.5	5.5	2.4	20.7
3.1	6.4	3.9	20.1
4.8	6.2	3.4	13.0
9.7	12.6	6.6	12.0
8.8	7.3	7.7	
1.4	2.8	12.7	
7.1	6.7	3.4	
8.9	6.9	9.7	
6.4		5.6	
8.3		4.3	
1.8		7.4	
3.3		8.4	
3.7		8.0	
6.6			
3.9			
Average = 5.4	Average = 7.0	Average = 5.2	Average = 16.6

* Based on the assumption that all adults accompanying calves were their lactating mothers

Callosities of southern right whales vary from mostly smooth in foetuses with a molded or wrinkled appearance (Lönnberg 1906; Matthews, 1938) to very rough in adults with tall, irregular epidermal projections and deep clefts. In stranded neonates, the callosities were smooth, lighter in colour than the rest of the skin and slightly raised. This observation confirms other observations that only some time after birth, do they become roughened and pitted and almost completely covered with colonies of species of amphipod crustaceans of the family Cyamidae (whale-lice) (Roussel de Vauzème, 1835; Payne *et al.* 1983; Rowntree, 1983). It is not known whether the nature of the whale-cyamid relationship is commensal, parasitic, or symbiotic, but the

cyamids contribute significantly to the appearance of the callosities (Payne *et al.*, 1983). Most of these descriptions are based on observations of animals in Argentinian waters and although they do agree with the observations made of animals in Southern African waters, the above authors make no mention of the presence of barnacles on the callosities. Lamarck (1802, in Darwin, 1854) first described the barnacles found on the callosities of southern right whales as *Tubicinella* sp. Pilsbry (1916) stated that almost all the recorded incidences of *Tubicinella* were from Southern Hemisphere right whales and that only one record of this barnacle had been made in the Northern Hemisphere (in 1650). It is therefore curious that these barnacles have not been recorded on Argentinian and Australian right whales. This is possibly due to the lack of boat-based field-work with these populations and/or the difficulties involved with attending strandings in these areas. The distribution of callosities varies with each whale and provides, together with scars and pigmentation, a means of identifying individuals (Payne *et al.*, 1983; Kraus, Moore, Price, Crone, Watkins, Winn & Prescott, 1986; Best, 1990).

The stiff hairs and hair follicles found on the heads of southern right whales are comparable to those described in the sei whale by Nakai & Shida (1948). The innervated nature and presence of "blood sinuses"possibly re-affirm Slijper's (1962) opinion that these structures are not hairs at all, but tactile organs analogous to vibrissae found in terrestrial animals. Distinct nerve nets and blood sinus appendages have been indentified in bowhead whales (Haldiman, Abdelbaki, Al-Bagdadi, Duffield, Henk & Henry, 1981; Haldiman *et al.*, 1985; Haldiman & Tarpley, 1993) and may indicate a tactile function, although the use or application of these structures in cetaceans is purely speculative.

2.4.2 Microscopic characteristics of southern right whale skin

2.4.2.1 Superficial epidermal features

The absence of diatomaceous concentrations, and the presence of various microbes, on the surface of the skin of southern right whales examined in this study is discussed in more detail in Chapter 4.

The observations of epidermal sloughing in animals sampled in South African coastal waters during winter months, coupled with histological evidence from Antarctic waters during mid-summer, suggest that sloughing continues year-round. Once evidence of moult was detected on the surface of the skin of the Antarctic animals, the question of possible differences in cellular proliferation rates was addressed. Unfortunately the PCNA staining technique applied was unsuccessful, probably due to incompatibility with the gluteraldehyde fixation (*H. McCleod, pers. comm.). The honeycomb pattern formed by flaking, superficial epidermal cells and smooth patches of undisturbed skin was seen on the skin surface of smooth-skinned calves. The presence of both skin "patterns" (i.e. honeycomb and smooth) possibly represents different stages of the epidermal sloughing cycle of the calf's skin, with the smooth patches not having been shed yet. The "unpitted" nature of the smooth patches further suggests that such calves may be undergoing their first "adult-like" epidermal exfoliation, following neonatal ecdysis (Chapter 3).

2.4.2.2 Histological and ultrastructural epidermal features

The bowhead whale has the thickest epidermis of any cetacean studied (1-25 mm) (Tomilin, 1957; Albert *et al.*, 1980; Durham, 1980; Haldiman *et al.*, 1981; 1985; Haldiman & Tarpley, 1993). Concentrations of epidermal rods arising from the stratum basale cells around the tips of the dermal papillae are characteristic of thick, hypertrophied (enlargement of tissue as a result of an increase in size, rather than in the number of constituent cells) and acanthotic (thickening of the stratum spinosum) epidermal regions of the parakeratotic (persistence of the nuclei in the cells of the stratum corneum) stratum corneum of the bowhead whale (Haldiman *et al.*, 1981; 1985; Haldiman & Tarpley, 1993). This integumentary specialisation is thought to function in holding together the thick epidermis. Besides perhaps providing insulation in cold, Arctic waters, the thickness of the bowhead epidermis as well as the presence of epidermal rods, possibly act as barriers against mechanical injury. Bowhead whales routinely break new ice at least 18 cm thick, and Inuit hunters have reported that bowheads have been known to break ice up to 60 cm thick in order to breathe (George, Clark, Carroll & Ellison, 1989). Although the southern right whale habitat and consequent behaviour is quite different to that of the bowheads, their epidermal

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thickness is second only to the bowheads. The presence of epidermal rods, the extensive interdigitation seen between stratum spinosum and stratum basale cells, the high concentrations of desmosomes and the striking association between the rete pegs and dermal papillae may all contribute to the mechanical stability required for this species to possess a thick epidermis. Perhaps such mechanical stability is an advantage for a species that seasonally inhabits the extreme near-shore region, constantly coming into close proximity with the sea floor and/or sea floor structures (e.g. rocks, reefs). The far-reaching dermal papillae may also make it possible for nutrients to reach the uppermost layers of the epidermis.

The extensive interdigitation of the dermis and epidermis is a striking feature of the integument of this species and other cetaceans. It has been suggested (Ling, 1984) that since cetaceans do not have a pelage, the ability of the epidermis to take over the role of friction reduction is aided by the greatly folded nature of the junction between the epidermis and the dermis. This may have a protective function against the hydrodynamic friction of swimming (Giacometti, 1967). Papillae are also penetrated by blood vessels and vascularisation is thus brought very close to the skin surface (Slijper, 1962; Yablokov *et al.*, 1974), which may maintain tissue temperatures at an optimum level for the rapid rate of mitosis (Ling, 1974) evident in the epidermis in some cetaceans (Palmer & Weddell, 1964; Brown, Geraci, Hicks, St. Aubin & Schroeder, 1983; Hicks *et al.*, 1985; St. Aubin *et al.*, 1990). Elaboration of dermal papillae may also increase body surface area, with consequences for thermoregulation.

A recognisable division of the epidermis into a stratum basale, stratum spinosum, and a parakeratotic stratum corneum is supported in this species, although only a stratum basale and externum have been previously recognised by other researchers (Pfeiffer & Rowntree 1996). The accumulation of melanin granules (in pigmented areas) and basophilic nuclear remnants (including pyknotic nuclei, i.e nuclei in a degenerative state) (Page 36) present in the stratum corneum give this layer a true (non-pathological) parakeratotic nature. This condition has been described in other cetacean species (Simpson & Gardner, 1972; Spearman, 1972; 1973; Ling, 1974; Menon, Grayson, Brown & Elias, 1986; Elias, Menon, Grayson, Brown & Rehfeld, 1987; Haldiman *et al.*, 1985; Haldiman & Tarpley, 1993).

The polyhedral cells of the stratum spinosum and cells of the stratum basale contained mitochondria and melanin. Keratohyalin granules were present in spinosal cell cytoplasm and abundant tonofilaments were associated with desmosomes. Lipid droplets were also detected as cellular components, essentially conforming to the epidermal studies described for other cetacean species (Ling, 1974; Menon *et al.* 1986; Elias *et al.* 1987; Haldiman & Tarpley, 1993; Haldiman *et al.*, 1985).

Rosettes formed by keratinocytes above epidermal rods were not reported on the surface of the skin in southern right whale calves by Pfeiffer & Rowntree (1996). In this study, however, using SEM, the rosettes were detected in all age groups, and for all the samples from various positions on the body on the surface of the skin (except in some taken from rough-skinned calves). These structures have been described on the surface of adult bowhead whale skin (Haldiman *et al.*, 1985), also seen superficially using light microscopy as "dots" (Chapter 3).

The high prevalence of cytoplasmic lipid droplets in lipokeratinocytes is a common feature of both the southern right whale and all other cetacean species reported thus far (Stromberg, 1985; Menon *et al.*, 1986; Elias *et al.*, 1987; Pfeiffer & Jones, 1993; Pfeiffer & Rowntree, 1996). The integumentary epidermal cells of the cetacean stratum corneum and stratum spinosum are thus properly termed, 'lipokeratinocytes' (Elias *et al.*, 1987). An important and unique finding in the right whale lipokeratinocyte is the frequent intimate association of lipid droplets with the nucleus that is thought to facilitate the energetics of nuclear metabolism (Pfeiffer & Rowntree, 1996). Lipokeratinocyte lipid storage droplets in other cetacean species studied (Pfeiffer & Jones, 1993) have also been thought to support cellular metabolism rather than functions related to insulation or secretion.

A distinctive "fat-free" zone consisting of collagen fibres makes up the reticular dermal layer, which corresponds to previous findings for other members of the Balaenidae (W. Sokolov, 1960; Sokolov, 1962; Sokolov, 1982). W. Sokolov (1960) found that the North Pacific right whale (*E. japonica*) possessed well-developed, elastic fibre networks within the dermal and hypodermal integumentary layers whereas only a few elastic fibres were detected in *E. australis* using Weigert's-

Resorcin stain. Both of the last-mentioned traits are more similar to odontocete integumentary structure than to balaenopterids (Yablokov *et al.*, 1974).

In the bowhead whale, Haldiman & Tarpley (1993) describe the innermost (deepest) aspect of the blubber layer as being bounded by a 1-2 mm thick layer of two highly tendinous connective tissue sheets. The fibres that make up these sheets are arranged perpendicular to each other (Haldiman *et al.*, 1982). These authors state that a true hypodermis extends between the innermost tendinous layer and the underlying muscles and other organs. This thin connective tissue layer was not found in the histological sections used in this study. Similarly, histological inspection did not reveal the presence of any discernible collagenous layer interrupting the hypodermis, as has been described in Nova Scotian sei whales (Ackman *et al.*, 1975b). In this study, southern right whales possessed uninterrupted collagen bundles surrounded by large amounts of adipose tissue that occurred below the dermis. This collagen bundle and adipocyte arrangement is considered, here, to compose the hypodermis. It is however acknowledged that the varying amounts of adipose tissue contained within the hypodermis depend upon the animal's age and nutritional status (Haldiman & Tarpley, 1993). It may therefore be possible that this innermost layer only becomes evident during the fattening/feeding stages of the animal's nutritional cycle, being comparable to the "isterlag" or "leaf fat" described by Heyerdahl (1932), Tveraaen (1935) and Pedersen (1950) in very fat Antarctic baleen whales. Ackman *et al.* (1975b) have likened the layer of collagenous and elastic fibres, which interrupts the hypodermal layer of the integument of Nova Scotian sei whales (also caught during the summer feeding season), to this layer.

2.4.3 Blubber thickness

The thick blubber layer found in right whales (Slijper, 1960; Omura, 1969) is second only to that found in bowhead whales (Haldiman & Tarpley, 1995). However, unlike the bowhead whales, right whales do not spend their lives in polar waters. Right whales are also not the only migratory mysticetes enduring months of low food intake in lower latitudes during mating and calving. The question therefore arises why these members of the Balaenidae should possess such a thick integument. It is here

suggested that this feature is one linked to the evolutionary origins of mysticetes. Fordyce (1980) states that the earliest known mysticetes and odontocetes are New Zealand Early Oligocene forms. Assembled evidence indicates that the evolution of mysticetes was probably induced by plankton productivity changes (and consequent increases in zooplankton availability) associated with the initiation of the psychrosphere during the Early Oligocene and the Circum-Antarctic Current (CAC) in Mid-Oligocene times (Fordyce, 1977). Glaciation in Antarctica during the Late Eocene affected temperature regimes, nutrient availability and hence productivity in Antarctic and Subantarctic waters (Kennett, Houtz, Andrews, Edwards, Gostin, Hajos, Hampton, Jenkins, Margolis, Ovenshine & Perch-Nielsen, 1975; Hayes & Frakes, 1975). The establishment of the CAC meant that, from mid-Oligocene times onward, areas such as the Campbell Plateau (which border the Sub-Antarctic region) were affected by these climatic changes. Shackleton and Kennett (1975) stated that temperatures on the Campbell Plateau dropped from 19 °C in the Early Eocene to 11 °C in the Late Eocene and to 7 °C in the Oligocene. The Balaenidae are thought to be the oldest of the modern mysticete families, evident in the fossil record during the Mid-Oligocene (Fordyce, 1980). This time period corresponds with the period that the cold, Antarctic-derived current (i.e. CAC) began to flow. Presumably bulky, slow-moving, filter-feeding mammals would need to develop a thick insulatory integument while living in such a cold-water environment.

The blubber thickness results obtained from a small number of stranded animals cannot be used to make any general inferences about this character in free-swimming animals. However, it is interesting to note that, along the dorsal plane, the blubber thickness was similar at position 1 and position 2. This area between positions 1 and 2 corresponds to the "fat roll" seen on adult female southern right whales during this study as well as during studies conducted by other researchers (*P. Best, C. Miller, pers. comm.), indicating that such "fat rolls" may indeed be temporary structures associated with nutritional status. In the neonates sampled (Table 2), there is a general trend for the mid-dorsal blubber to increase in thickness in a cranio-caudal pattern. This pattern has been described previously in other mysticetes (Lockyer, McConnell & Waters, 1985). The lateral samples include measurements taken from a juvenile and the marked decrease in blubber thickness in a cranio-caudal direction,

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especially in Position 5, helps create the laterally compressed, stream-lined tailstock. Unfortunately the lateral location of these samples makes them incomparable to results from other investigations (Ackman *et al.*, 1975b; Lockyer *et al.*, 1985).

The deep-core sampling technique was a successful first attempt at obtaining representative integument samples from a free-swimming balaenid. Histological analysis, as well as ultra-sound blubber thickness measurements (C. Miller, unpubl. data), indicate that complete cores were not retrieved from animals, adults in particular. However, on a structural level, samples from stranded animals have been used to supplement the biopsies. The large difference between blubber thickness of early and late season cows should only be used as a gauge of the increased operator efficiency of the biopsy system, and not interpreted as seasonal variation in blubber thickness.

2.5 Conclusion

The general structure of the integument of southern right whales seems to be comparable with that found in other cetaceans. This study supports previous findings that the integument of this genus is more like that of odontocetes, with a fibrous and essentially adipocyte-free dermis, than that described for balaenopterids. Three epidermal layers are present, with the stratum corneum being parakeratotic in nature. Similar in structure to bowhead whales, of the same family, southern right whales possess an acanthotic epidermis and a notably thick hypodermis. Epidermal rods and extensive papillomatosis support these characteristics, the former increasing the surface area of the germinal layer. However, unlike bowhead whales, southern right whales possess an uninterrupted hypodermal layer. Superficial moulting occurs in coastal waters throughout the austral winter and apparently in Antarctic waters in mid-summer.

CHAPTER 3

POST-NATAL ECDYSIS IN SOUTHERN RIGHT WHALES, *EUBALAENA AUSTRALIS*.

3.1 Introduction

Shedding of individual cells and small clumps of cells (exfoliation), which is necessary for maintenance of epidermal integrity, is clearly shown in many cetacean species (Palmer & Weddell 1964; Sokolov, Bulina & Rodionov, 1969; Sokolov & Kalashnikova, 1971; Brown, Geraci, Hicks, St. Aubin & Schroeder, 1983; Haldiman, Henk, Henry, Albert, Abdelbaki & Duffield, 1985; Hicks, St. Aubin, Geraci, J.R. & Brown, 1985; Geraci, St. Aubin & Hicks. 1986). In odontocetes this sloughing rate occurs nine times faster than in humans (Bergstresser & Taylor, 1977).

As in terrestrial mammals, growth and replacement of the epidermis are generally viewed as continuous processes in cetaceans (Ling, 1974; 1984). However, species occupying different environments seasonally might be expected to undergo additional (cyclical) changes in the epidermis. The beluga whale, *Delphinapterus leucas*, undergoes a unique three-phase, seasonal moult (St. Aubin, Smith & Geraci, 1990). There are no known studies of seasonal histological changes in the skins of migrating mysticetes. Whether epidermal mitotic activity accelerates on the breeding grounds (Bullough, 1962) is not known, although Whitehead, Gordon, Mathews & Richard (1990) discuss the more frequent sloughing of sperm (*Physeter macrocephalus*), humpback (*Megaptera novaeangliae*) and gray (*Eschrichtius robustus*) whales observed in warmer rather than colder waters.

Von Schumacher and Van Utrecht (1931; 1958, both in Naaktgeboren, 1960) found that a "well-developed epitrichium" is often present in toothed whales. Naaktgeboren (1960) describes an "epitrichium", consisting of several cell layers (32 μm thick) with nuclei that are flattened discs, which forms a tight-fitting covering around the entire foetal body of a fin whale. According to these authors, the "epitrichium" is lost *en utero*.

In the beluga foetus, the stratum corneum makes up approximately half the epidermis (Yablokov, Bel'kovich & Borisov, 1974). In the neonate beluga, the epidermis is about twice as thick as in the foetus, but there is almost no change in the proportion between the rest of the integumentary layers (dermis and hypodermis) and the stratum corneum. The superficial layers of this well developed epidermis, approximately half of which, is superficial to the dermal papillae, begin to moult off in young belugas. This moulting results in removal of part of the foetal epidermis. Once moulting has occurred, the stratum corneum of neonates thickens by a factor of 3 or 4 (Yablokov *et al.*, 1974). Possibly the thick stratum corneum provides insulation for the newborn and the thinner nature of the stratum corneum of more active neonates and adult animals (compared to foetuses) thus has thermoregulatory significance (Bel'kovich, 1962).

It was apparent to earlier investigators (Eschricht & Reinhardt, 1866) that the black epidermal portion of the skin was thicker in the newborn and suckling bowhead whale (*Balaena mysticetus*) than in the adult. Anecdotal evidence that young bowhead whales slough large masses of "skin" has been presented informally by some Eskimo captains and scientists (Haldiman & Tarpley, 1993). Haldiman, Abdelbaki, Duffield, Henk & Henry, 1982; 1984) have presented evidence from gross and microscopic studies, of adult bowhead skin, that some epidermal modifications take place in the skin. These modifications are generally associated with previously wounded areas of the skin. These authors propose that ecdysis (rapid loss of multiple layers of epidermis) or an ecdysis-like process also may be involved in the apparent healing of some modified areas. Haldiman & Tarpley (1993) also describe histological evidence of an ecdysal process in very young bowhead whales.

In this thesis, a similar process of rapid loss of multiple layers of epidermis is described, for the first time, for the southern right whale calf. The process is here termed ecdysis, to distinguish it from the general and continual loss of single cells (exfoliation/desquamation) or seasonal loss of epidermal layers, referred to here as moult or slough.

3.2 Materials and Methods

3.2.1 Study area

Samples of integument (skin and blubber) from living southern right whales were collected by biopsy during the August and October field seasons of 1998 and 1999, as well as during early November 2000. The study area included Walker Bay (Gansbaai), Struisbaai, De Hoop Marine Reserve and St. Sebastian Bay, all on the south coast of Southern Africa (Plate 1). Samples were also taken from stranded animals in the above areas as well as in the Cape Peninsula, Dwarskersbos and Elands Bay, along the west coast of Southern Africa (Plate 1).

3.2.2 Sample collection

3.2.2.1 Biopsies

Integumentary samples from free-swimming southern right whales (35 cows and 63 calves) were collected-along the South African coast, using a specially designed, hand-held deep-core biopsy system (Chapter 6).

Each animal was approached perpendicular to its long axis and sampled by inserting the biopsy head (on the end of a 9 m aluminium pole) into the dorso-lateral surface of the whale and immediately retracting it (Plate 2). Once a successful biopsy attempt was made, the sample was removed from the biopsy head, placed in foil and into a labelled plastic bag and then put into a cooler box with "blue ice". The biopsy heads were cleaned in 99% chloroform between samples, and the barbs reset or, if necessary, replaced. Back on land, the samples were measured, noting epidermal and blubber thicknesses. The pigmented skin was cut away from blubber samples (the cut was made on the blubber side of the intersection between the epidermis and dermis) using a sterile scalpel and the skin was immediately placed in a separate, labelled specimen bottle containing gluteraldehyde. The skin samples were left in the gluteraldehyde for a minimum of 3 days and a maximum of 6 days when they were placed in buffer (25%

gluteraldehyde + sodium dihydrogen orthophosphate + disodiumhydrogen orthophosphate anhydrous = water) until analysed.

3.2.2.2 Stranded animals

Total body length as well as blubber thickness measurements were taken from fresh/recently stranded animals (Table 1) and full core samples were placed in foil and frozen at -20 °C within a few hours of collection. Epidermal thickness measurements were taken from 5 positions along the mid-dorsal, lateral and mid-ventral surfaces from animals that stranded from 1998 onwards (Plate 3). Full core samples were taken from the same positions. Samples for histological analysis were subsequently fixed in 10 % buffered formalin and subsamples of the pigmented skin for EM analysis were fixed in gluteraldehyde (same procedure as for biopsy samples). In most instances, the positioning of the animal prohibited the collection of samples from both the mid-dorsal and mid-ventral surfaces and in other instances the location of the animal made it impossible to take measurements and collect samples from all positions along the various surfaces. On occasion, skin samples from other structures, e.g. callosities, flippers and flukes were opportunistically taken (Table 2).

3.2.3 Aerial and boat survey data used to describe skin condition: determination of an ecdysal time scale

Monthly aerial surveys were conducted by Dr Peter B. Best from a Bell Jet Ranger helicopter from August to November 1988 and July to November 1989 as part of a photogrammetric study of southern right whales (Best & Rüther, 1992). Slides (35 mm) of calves taken on the survey were examined using a microfiche reader (C.O.M. 200, Micro Design Inc., Hartford, Wisconsin, U.S.A.) that magnified the images by 24X, in order to describe their skin condition. A total of 195 photographs were analysed, including 97 animals.

Boat surveys to collect biopsies were conducted from a 6 m inflatable boat from August to October 1998 and from August to November 1999, for a total of 16 days at

sea. During these surveys, a total of 121 calves were encountered, and a visual assessment of their skin condition made (Table 4).

Table 4: Skin condition of calves seen at sea during biopsy sampling.

Month of sampling	N	"Unknown"	Smooth	Rough
28-30 August 1998	46	8	23	15
27-31 October 1998	16	1	14	1
28-31 August/ 5 September 1999	34	1	18	15
27-31 October/ 1-3 November 1999	25	0	25	0

In both aerial and boat surveys, southern right whale calves were grouped similarly according to the appearance of their skin. The skin of some calves was noticeably uneven, spongy, broken and often light grey in colour. These calves were referred to as "rough-skinned" (Plate 18). Other calves possessed very smooth and uniform, black-coloured skin and were termed "smooth-skinned" (Plate 17). Some calves were generally small in size with very little evidence of superficial exfoliation, being lighter grey in colour, and were defined as "pre-moult" (PreM). Similarly other calves, which showed extensive exfoliation and were generally large in size, were termed "probably smooth". The skin condition of some evasive calves could not be determined and some photographs were unclear, these animals were therefore described as "unknown".

A time-scale to describe the ecdysal process of southern right whale neonates was constructed using the formulae presented by DeMaster (1978) for calculating the average age of sexual maturity in marine mammals. This method lends itself well to age-specific data and incorporates a variance estimate to facilitate comparisons between populations.

3.2.4 Histological preparations

Skin samples were prepared, embedded and stained according to standard histological procedures at the Department of Anatomical Pathology, Groote Schuur Hospital. A

Leica "Jung Histokinette 2000" tissue processor was used, sections of 4-5 μ were cut on a microtome and adhered to APES coated slides. Mayer's Haematoxylin and Eosin stains were used to accentuate general histological structure. Weigert's Resourcin stain was used to stain for the presence of collagen and elastin fibres and Ayoub-Sklar to reveal keratin.

Samples for TEM were prepared at the Department of Anatomical Pathology, Groote Schuur Hospital and electron micrographs were taken using an Hitachi H600 Transmission Electron Microscope at various magnifications, operating at 75KV.

3.3 Results

3.3.1 Macroscopic appearance of neonatal southern right whale skin

During August/September in 1998 and 1999 (peak calving season) "rough-skinned" calves formed 32.6% and 44% of the calves seen (n= 46 and n=34 respectively) during biopsy trips at sea (Table 4). By October of the same years, this proportion had fallen to 6.2% and 0%, respectively (n=16 and n=25). Aerial photographs of cow/calf pairs taken from August-November 1988 and July-November 1989 were analysed to describe the skin condition of the calves seen. Most of the animals were seen at least twice during the different monthly surveys, providing sequentially comparable data. Data collected from both biopsy trips and aerial surveys are summarised in Figure 4. The aerial survey data support the biopsy data and, together, show a marked decrease in the number of "rough-skinned" calves from early to late season and the converse for "smooth" calves. The average date at which 50% of the calves have smooth skins was determined (DeMaster, 1978). This stage was reached at 91.9 days (95% Confidence Interval = $\pm$ 1.1 days) from the first day of June, or 30/31st August (Figure 5).

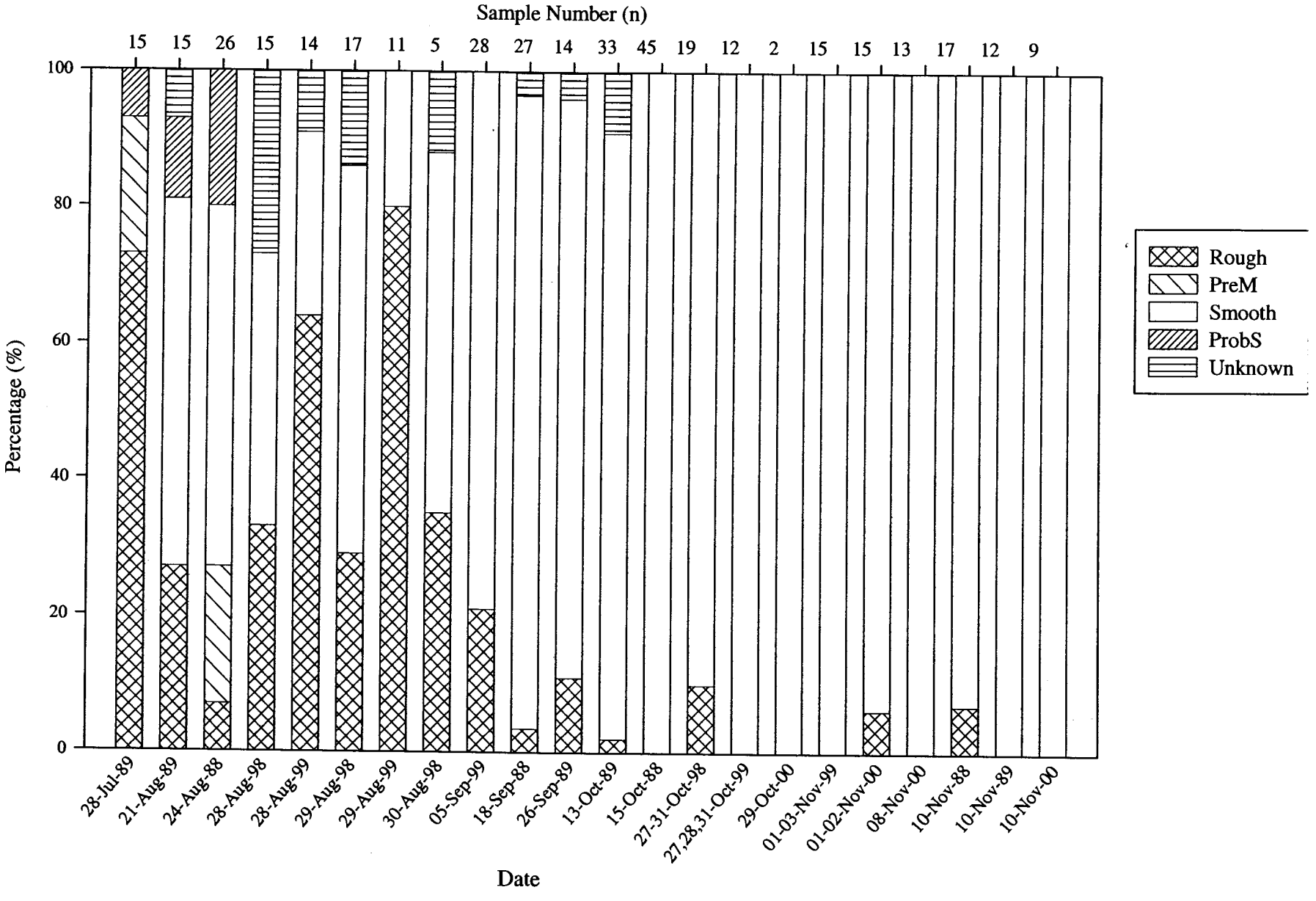

Figure 4: Frequency of different skin conditions seen in southern right whale calves during biopsy sampling and aerial surveys, South Africa 1988-1999

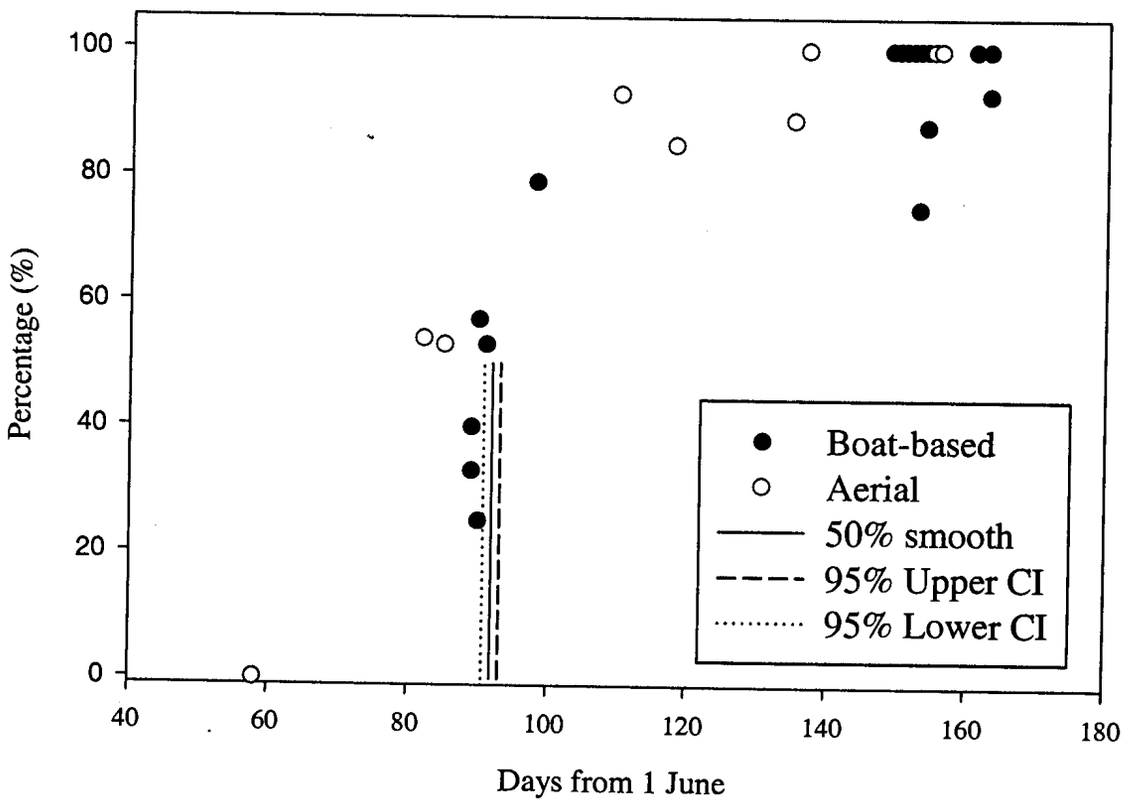

Figure 5: Percentage of smooth-skinned calves observed in boat-based and aerial surveys, South Africa 1988-1999 as a function of the number of days from 1 June (excluding "PreM, ProbS, Unknown" values). Solid vertical line represents the mean day at which 50% of the calves were smooth-skinned, broken lines indicate S.E.

3.3.2 Microscopic appearance of neonatal southern right whale skin

In stranded neonates (n=8) a distinct "line" or "plane" occurred superficial to the tips of the dermal papillae and ran in a tangential plane (Plate 40). This plane effectively delineated two separate epidermal regions. Lighter-coloured layers of epidermis occurred superficial to the "plane" and layers of highly pigmented epidermis occurred deep to the plane. This "plane" could not be distinguished in the epidermis of dark, "smooth-skinned" (and presumably older) calves (n=35) (Plate 41) and non-calves (n= 49, including Antarctic samples) (Plate 42). A lateral sample from a stranded neonate (99/05) revealed that the outer epidermal layer above the plane measured 1.2 cm in thickness, while below the "plane" the epidermis was 1 cm thick (Plate 40). In neonate 98/09, the thickness of the outer epidermal layer was reduced over the rostral callosity (5 mm) in comparison to the epidermal thickness between the callosities (13 mm) (Plate 43).

Microscopically, the distinct "plane" observed in the "neonatal epidermis" consisted of flattened, stratum corneum-like cells bordered on both sides by larger, spindle-shaped cells of the stratum spinosum within the epidermis (Plate 44). The plane was situated approximately halfway between the epidermal/dermal junction and the outer surface of the stratum corneum (Plate 45). As the ecdysal process developed, the plane became conspicuously porous in nature (Plate 45). Intercellular oedema occurred above the most superficial tips of the dermal papillae and the spinosal cells on either side of the plane possessed pyknotic nuclei (i.e nuclei in degenerative state) due to the accumulation of keratin (Plate 44). The cells at the tips of the dermal papillae seemed to be necrotic in nature (Plate 46). TEM studies of skin from rough-skinned animals revealed that the layers peripheral to the cleavage plane had reduced numbers of desmosomes and intracellular filaments, as well as lower concentrations of melanin granules compared to the proximally occurring layers (Pers. obs.). However, high concentrations of melanosomes and melanin granules were present in the basal layer of the epidermis in both rough and smooth-skinned calves (Pers. obs.).

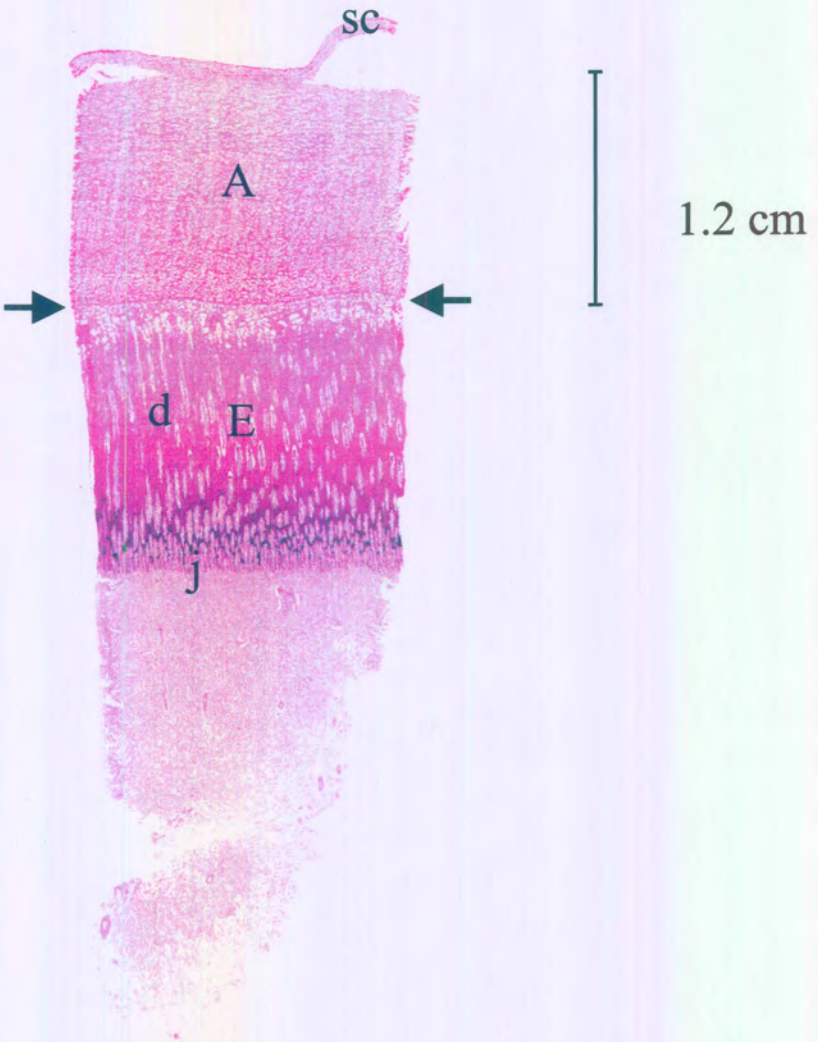

Plate 40: Longitudinal section through the mid-dorsal integument of a stranded southern right whale calf (98/09) showing a separate epidermal region (A) above the epidermis (E) and the distinct plane separating these regions (arrows). Note stratum corneum (sc) cells tearing away from the rest of the integument, dermal papillae (d), epidermal/dermal junction (j). (H/E, whole mount).

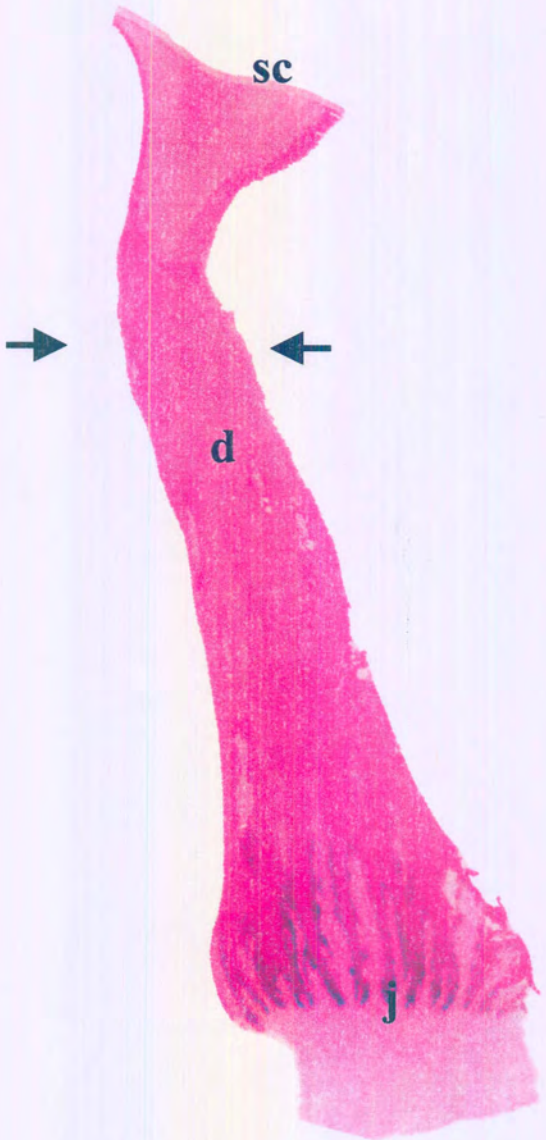

Plate 41: Longitudinal section through the integument of a "smooth-skinned" southern right whale calf. Note the absence of a second epidermal layer as well as the absence of a distinctive plane (arrows) above the dorsal tips of the dermal papillae (d). Epidermal/dermal junction (j), stratum corneum (sc). (H/E, 12.3X).

Plate 42: Longitudinal section through the integument of an adult southern right whale sampled in Antarctic waters. Note the absence of a second epidermal layer as well as the absence of a distinctive plane (arrows) above the dorsal tips of the dermal papillae (d). Epidermal/dermal junction (j), stratum corneum (sc). (H/E, 9X).

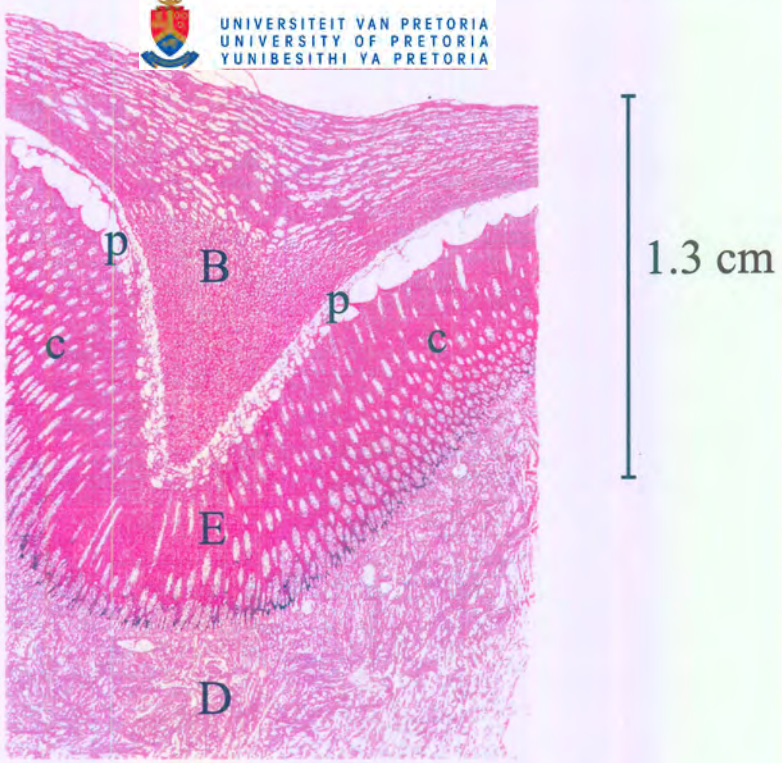

Plate 43: Longitudinal section through the integumentary layers between two rostral callosities (c) from a stranded southern right whale calf (98/09). Note the thickness of the outer epidermal layer (B) between the callosities (c), separation of the "baby skin" along the plane (p), epidermis (E), dermis (D). (H/E, whole mount).

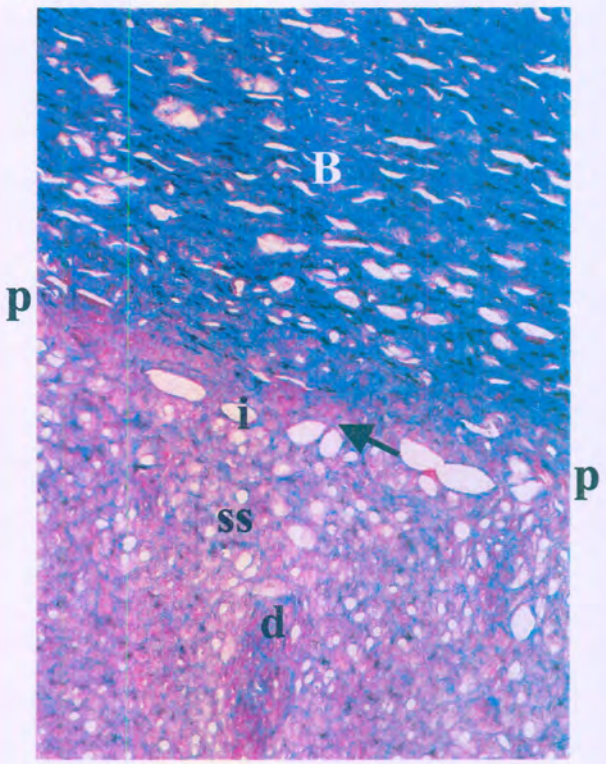

Plate 44: Longitudinal section through the epidermis of a mid-dorsal sample (Pos 4) from a stranded southern right whale calf (98/09). Note the stratum corneum cells (arrow) within the stratum spinosum (ss), inter-cellular oedema (i), "baby skin" (B) and cyto-keratin bodies (stained red) The plane (p) occurs distal to the tips of the dermal papillae (d). (Ayoub-Shklar, Mag 100X).

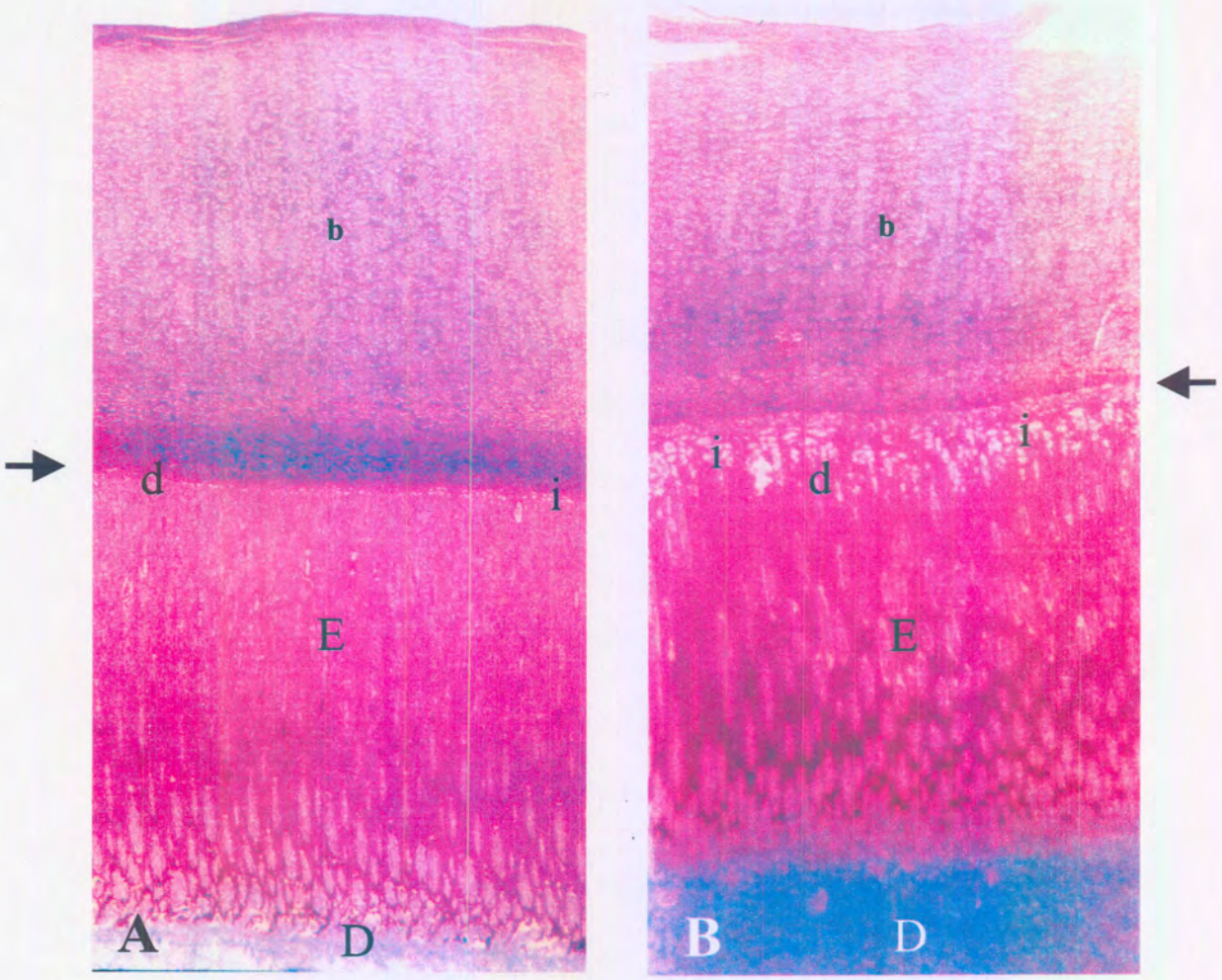

Plate 45: Longitudinal section through the integumentary layers of mid-dorsal samples from Pos 1 (A) and Pos 4 (B) along the body of a stranded southern right whale calf (98/09). Note the conspicuously porous plane (arrows) in B compared to A. Dermal papillae (d), intercellular oedema (i), "baby skin" (b), epidermis (E), dermis (D). (Ayoub-Shklar, Mag A 9X, Mag B 7X).

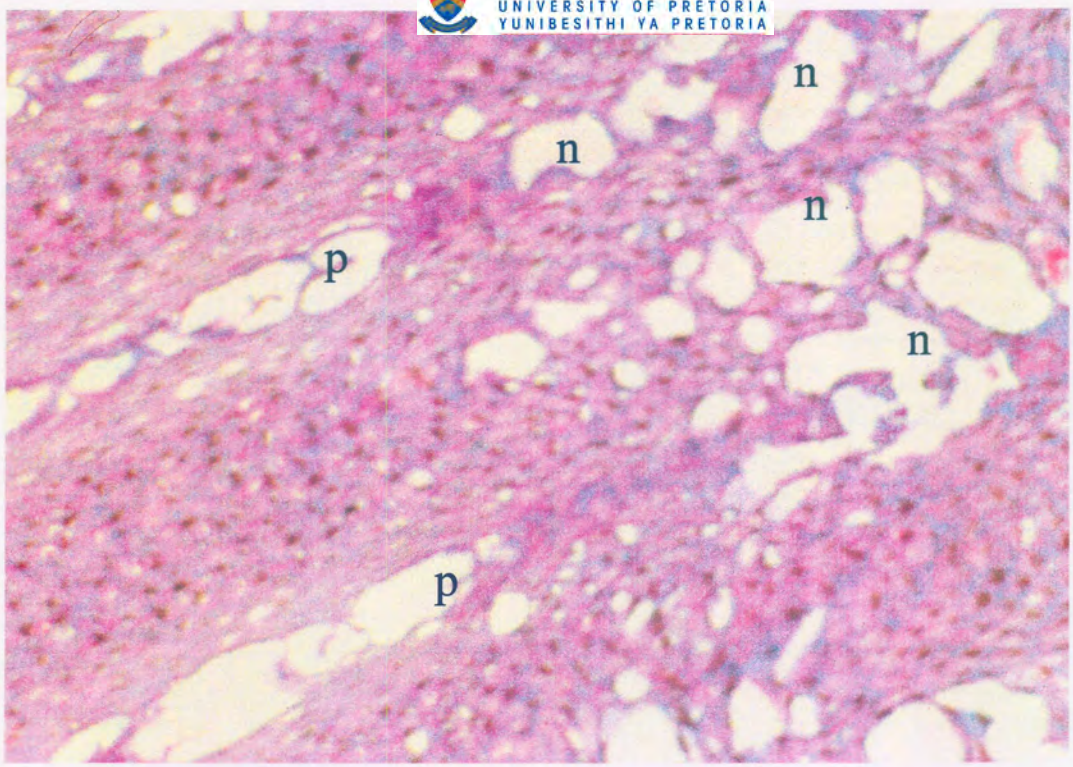

Plate 46: A (rotated) longitudinal section through the stratum spinosum of a mid-dorsal sample (Pos 1) taken from a stranded southern right whale calf (98/09), showing necrotic cells (n) at the distal tips of the dermal papillae (p). (Ayoub-Shklar, Mag 100X).

Plate 47: A neonatal southern right whale (34°2745S, 20°4212 E) with distinctly grey-coloured, "roughened" skin.

During a project collecting superficial skin biopsies from southern right whales, along the Cape south coast (34°2745S 20°4212E), researchers came across a seemingly abandoned neonate. It was distinctly grey-coloured, with "rough-looking" skin (Plate 47). As it approached the research inflatable, it gently nudged the starboard pontoon. At this point, a sizeable piece of skin came away from the lower region of the neonate's back and floated on the sea surface. The piece of sloughed skin recovered at sea was approximately 6 mm thick, measuring 27 cm in length and 14 cm in width (Plate 48). This presumably represented an extreme example of the ecdysal process. Light microscopy revealed these cells to be mid-stratum spinosal in nature, but none of the cells possessed any viable nuclei (Plate 49). Transmission electron microscopic examination revealed the presence of ghost nuclei within these cells.

Although most samples from "smooth-skinned" calves exhibited adult-like characteristics and therefore showed no evidence of a distinct plane nor of a vacuolated layer in the stratum spinosum (Plate 41), histological analysis of some calves described upon gross examination as "smooth-skinned" (Plate 50) revealed the presence of a distinct plane in the stratum spinosum as described for "rough-skinned" animals (Plate 40). Likewise, it was found that some samples from characteristically "rough-skinned" calves did not possess any evidence of the cleavage plane (Plate 51).

3.4 Discussion

This is the first documentation of the process of ecdysis in right whales. "Rough-skinned" calves have not been reported in Australian or Argentinian waters, possibly due to the fact that research in these areas is predominantly conducted from land-based stations or aircraft that do not allow for close interactions with the whales. Payne, Brazier, Dorsey, Perkins, Rowntree & Titus (1983), however, did note that "when small calves are first seen, they are lighter grey than their mothers and become darker with age". This agrees with the findings of this study, in that the layers superficial to the cleavage plane have lower concentrations of melanin compared to the proximally occurring layers.

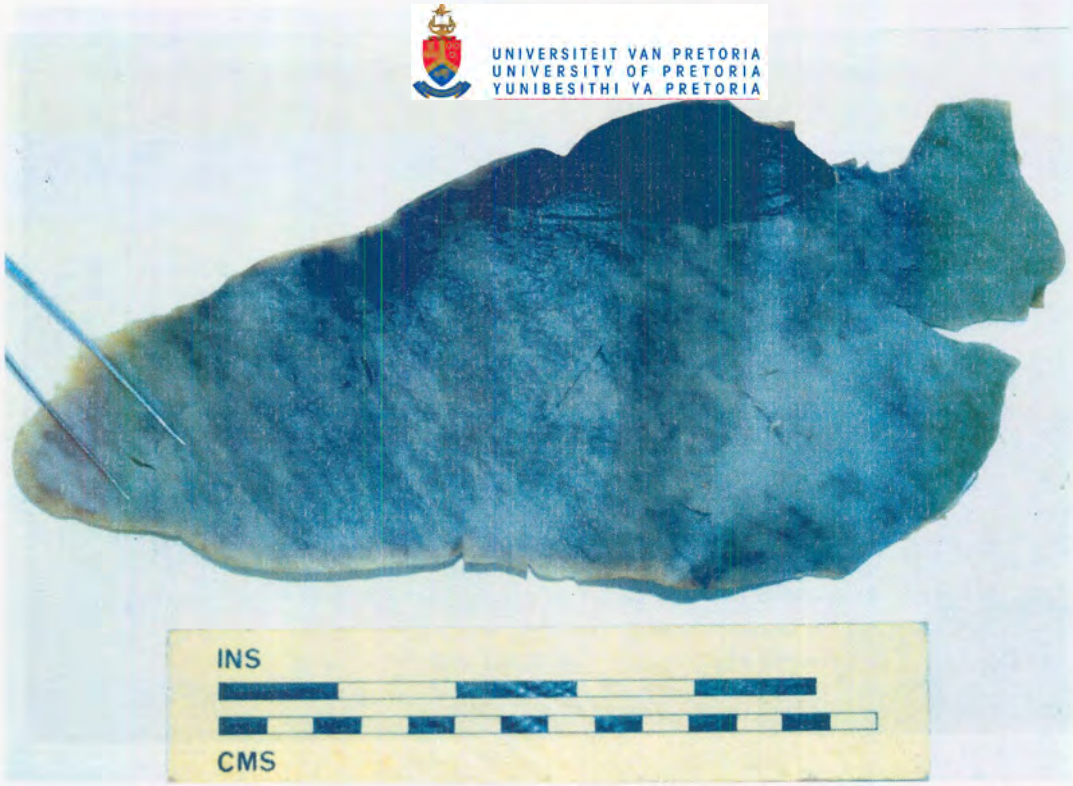

Plate 48: A piece of sloughed skin recovered at sea from an apparently abandoned neonatal southern right whale.

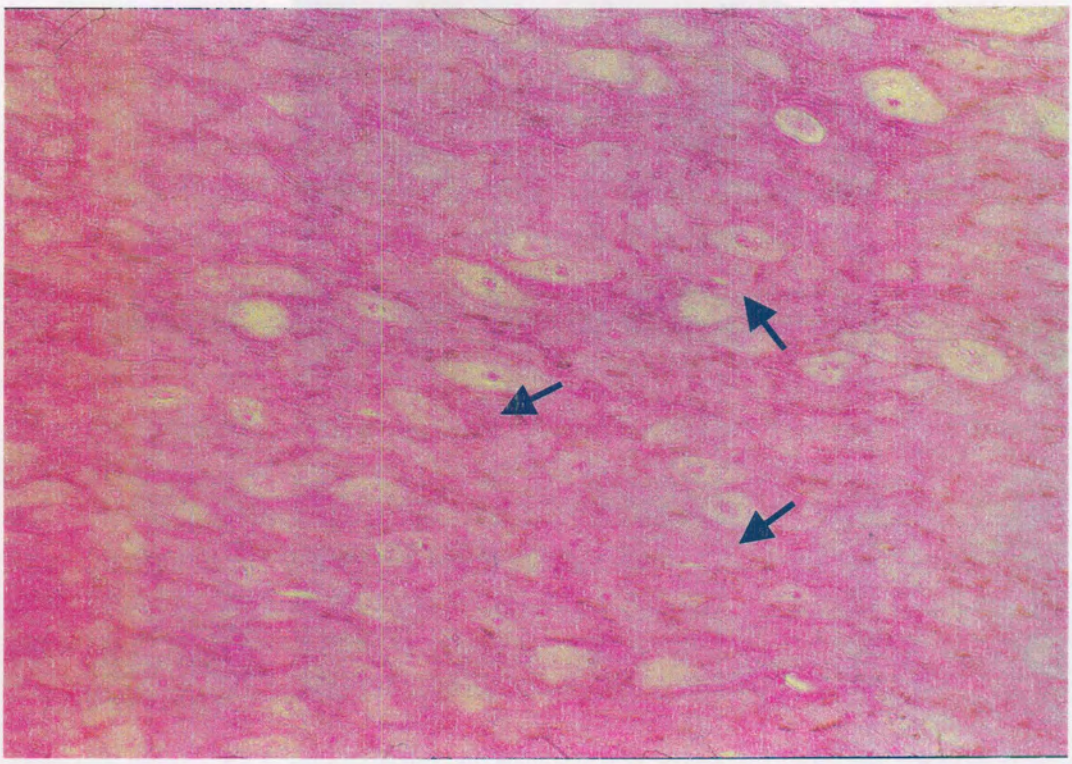

Plate 49: Longitudinal section through a piece of sloughed skin recovered at sea from a neonatal southern right whale. Note the spindle-shaped spinsosal cells possessing only ghost nuclei (arrows). (H/E, Mag 200X).

Plate 50: A stranded neonatal southern right whale (98/09). The dark skin colour and smooth appearance of the skin initially defined this animal as "smooth-skinned", but it possessed an outer epidermal layer and "plane" (refer to Plates 43-45).

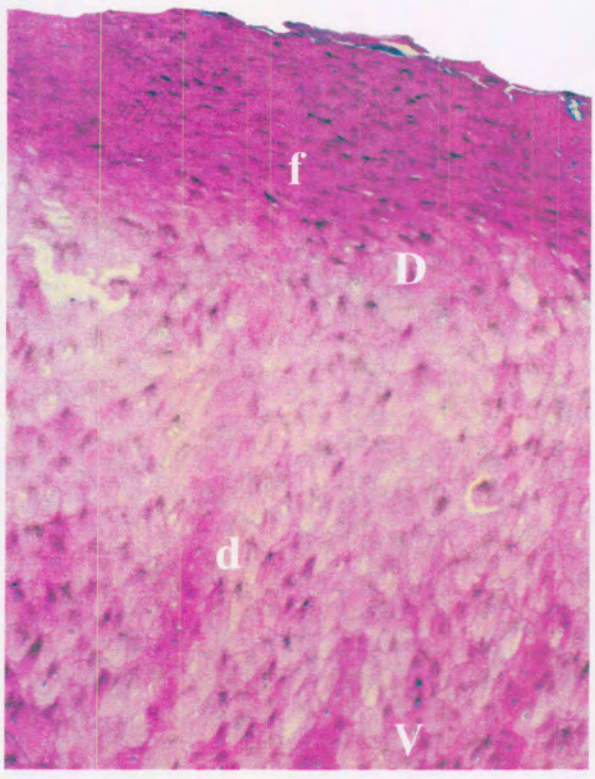

Plate 51: Longitudinal section through the upper epidermal layers of an apparently "rough-skinned" southern right whale calf (00/09). Note the flattened stratum spinosal cells (f) replacing a true stratum corneum and the absence of an ecdysal plane at the distal tips of the dermal papillae (d). (H/E, Mag 100X).

Given this ecdysis, the minimal differences between the epidermal thickness of calves sampled early in the season and those sampled later in the season is surprising. However, samples from a stranded neonate (99/05) showed the outer epidermal layer to be slightly thicker than the deep "adult-like" epidermal layers. It is possible that during sampling a majority of the outer epidermal skin layers are lost, or, because the ecdysal process proceeds in an uneven or non-uniform manner, the outer epidermal skin layer may have already separated from the epidermis at the sampling position. This also explains the reason why samples from some rough-skinned calves did not show the presence of cleavage planes when analysed histologically. On the other hand, skin samples analysed from seemingly smooth-skinned neonates show the presence of distinct, although non-vacuolated, cleavage planes. This situation is probably indicative of the "pre-ecdysal" stage and shows that the skin of neonates starts to "break up" visibly after birth. Best (1994) estimated that the mean date of birth of right whale calves on the South African coastline was 24th August. The date on which 50% of the calves lose their rough-skinned status has been estimated above as 30/31st August. From this it could be deduced that on average the ecdysal process is completed about one week after birth.

The variable thickness of the outer epidermal layer over the rostral callosity of another stranded neonate (98/09) in comparison to the rest of the samples taken along the body, indicates that once ecdysis has taken place the callosities are likely to become more defined and thus more favourable for colonisation by cyamid species such as *Cyamus ovalis* and *C. gracilis*, which seem to favour areas of reduced waterflow. The high incidence of *C. erraticus* on neonates may also be associated with the process of ecdysis, as this seems to be a species associated with wounds and other areas of high epidermal activity (Payne *et al.*, 1983).

The ghost nuclei present in the large sample of skin obtained from the presumably abandoned, "rough-skinned", calf implies that the tissue was dead at the time of sloughing. However, the large size of the ghost nuclei indicated that the cells were mid-epithelial in depth and that they did not die due to keratinisation (M. Duffield, pers. comm.). Hence a process other than keratinisation was probably responsible for the ecdysal activity. I hereby propose that focal oedema develops in between the cells

and forms the "cleavage plane", which will eventually lead to separation of the outer epidermal cell layer. Accumulation of fluid between epidermal cells causes gaps to appear which may coalesce to form fluid-filled vesicles. The necrotic nature of the tips of the dermal papillae seen in a pre-ecdysal calf (98/09) could possibly be the source of the intercellular fluid, which may be the catalyst for the ecdysal process. The mechanical integrity of this layer is further hampered by the lower concentration of desmosomes and intracellular filaments. The movement from the intra-uterine to the oceanic milieu, and the osmo-regulatory consequences thereof, may also be a catalytic factor for this process to occur. This condition/process closely emulates the process described in humans as "spongiosis" (M. Duffield, pers. comm.), where it is noted to occur particularly in the stratum spinosum (Stevens, Wheater & Lowe, 1989). Histologically, in humans, spongiosis manifests as epidermal intercellular oedema with exocytosis of numerous eosinophils and mononuclear cells both within the epidermis as well as in spongiotic foci (Machado-Pinto, McCalmont & Golitz, 1996). Although this is often a genetically-linked, pathological condition (e.g. Incontinentia pigmenti (Machado-Pinto & Golitz, 1996)) in humans, it does not appear to be so in southern right whales.

Haldiman & Tarpley (1993) describe histological evidence of ecdysis in very young bowhead whales that seems to occur in the same manner as described here for southern right whales. However, these authors indicate that keratinisation is responsible for this process whereas the evidence in this paper would suggest that spongiosis and not simply keratinisation, might be responsible.

Post-natal ecdysis has not been described for any other mysticete species, so it may be a characteristic of balaenids. The epitrichium described in toothed whales (Von Schumacher, 1931; Van Utrecht, 1958, both in Naaktgeboren, 1960) and in fin whales (Naaktgeboren, 1960) is a thin covering that is apparently lost *en utero*. Balaenopterid foetuses show an accelerated growth in body weight, from approximately 5-7 months after conception until birth (Frazer & Huggett, 1973; Lockyer, 1981), whereas balaenids show consistent and linear foetal growth (Philo, George, Tarpley, Zeh & Albert, 1992; Best, 1994). The accelerated foetal growth in balaenopterids leads to the conclusion that certain physiological processes may proceed at a more rapid rate in

this group of cetaceans than in balaenid whales. Hence the "epitrichium" (Von Schumacher, 1931; Van Utrecht, 1958, both in Naaktgeboren 1960) lost *en utero* in balaenopterids may be a vestige of the neonatal, outer epidermal layer of balaenids.

The phocid 'embryonic coat' or lanugo (Ling & Button, 1975) is thought to protect pups against thermal losses in the harsh environments into which they are often born (Elsner, Hammond, Denison & Wyburn, 1977). Oftedal, Bowen, Widdowson and Boness (1991) describe hooded and harbor seals as exceptions in the phocid family as the pups of these two species lose their lanugos *en utero*. These authors argue that fetal shedding, like prenatal blubber deposition, is an adaptation enabling newborn pups to enter cold water without adverse consequences, seeing as though the lanugo provides insulation only while dry. This ability therefore allows the use of pupping substrates that are unstable or regularly inundated with water (Oftedal *et al.*, 1991). Considering these arguments, the thickened epidermis may originally have provided insulation or protection for neonatal balaenids that existed when the cold, Antarctic-derived current began to flow (Fordyce, 1980), but is now an inherited character which, in the more modern balaenopterids, is vestigial.

3.5 Conclusion

Neonatal southern right whale calves exhibit a form of epidermal ecdysis approximately one week after birth, apparently caused by a process that resembles spongiosis as described in humans. This may be an inherited character in balaenids, vestigial evidence of which is found in the more modern balaenopterids.

CHAPTER 4

OBSERVATIONS OF MICROBIAL ASSOCIATIONS ON THE SKIN OF SOUTHERN RIGHT WHALES, *EUBALAENA AUSTRALIS*.

4.1 Introduction

A diverse range of micro-organisms, including bacteria, filamentous fungi, yeast, micro-algae (i.e. diatoms) and protozoa, are regarded as true inhabitants of the marine environment. Representatives of >100 prokaryotic genera (organisms that consist of cells in which genetic material is organised in single filaments of DNA, and not enclosed in a nucleus) may be described as marine inhabitants (Austin, 1988). Although yeast populations decrease in number with distance from land (Van Uden & Fell, 1968), diminishing more rapidly than the bacteria (Hoppe, 1972a; 1972b, in Sieburth, 1979), they are still the dominant fungi in the open ocean (Van Uden & Fell, 1968; Bahnweg & Sparrow, 1971). Microbial life carpets all types of surfaces, providing food for grazing animals.

Many diatom species have been recorded on the surface of the skin of cetaceans (Bennett, 1920; Hart, 1935; Hustedt, 1952; Nemoto, 1956; 1958; Nemoto, Brownell & Ishimaru, 1977; Haldiman, Abdelbaki, Al-Bagdadi, Duffield, Henk & Henry, 1981; Haldiman, Henk, Henry, Albert, Abdelbhaki & Duffield, 1985; Nagasawa, Holmes & Nemoto, 1990). Diatom films on the skin of whales have been mostly found in the waters of high latitudes, and they have generally been considered common in the colder waters of both hemispheres (Bennett, 1920; Omura, 1950; Okuno, 1954; Nemoto, Best, Ishimaru & Takano, 1980). A few whales caught or stranded on the coast of South Africa (Mackintosh & Wheeler, 1929; Best, 1969; Nemoto *et al.*, 1980) have been observed to possess diatom films on their skin, mainly *Bennettella ceticola* and its related forms (previously known as *Cocconeis ceticola* (Holmes, 1985)). However, *Bennettella* infestation on whales in lower latitudes is usually the exception rather than the rule (Mackintosh & Wheeler, 1929; Best, 1969; Nemoto *et al.*, 1980). Substantial numbers of diatoms and bacteria occur in minute cracks and crevices on the surface of the skin of cetacean species, but greater numbers occur in areas of

damaged epidermis (Haldiman *et al.*, 1981; 1985; Heckmann, 1981; Heckmann, Jensen, Warnock, & Coleman, 1987; Henk & Mullan, 1996). Shotts, Albert, Wooley & Brown (1990) found that certain species of bacteria and yeasts occur preferentially or exclusively on lesioned skin in bowhead whales. In cetaceans, superficial bacterial infections are generally secondary to a disruption of the skin (Greenwood, Harrison & Whitting, 1974; Howard, Britt, Matsumoto, Itahara & Nagano, 1983). However, Shotts *et al.* (1990) and Davis (1984, in Henk & Mullan, 1996) identified several erosive enzymes in the bacteria and yeasts from bowhead whale skin and suggested they might be pathogenic. In addition, some species isolated by Shotts *et al.* (1990) are known pathogens of other mammals.

There are several mechanisms that protect the mammalian skin against colonisation by pathogenic micro-organisms and their subsequent invasion into the epidermis or dermis. These include the physical barrier provided by the epidermis, desquamation, the humidity of the skin, pH, niche filling by the resident microflora, and the presence of inhibitory substances on the skin surface (Cove, Eady, Tipper & Cunliffe, 1992).

As with most glandular membranes, human skin is colonised by enormous numbers of microbes. Resident bacteria and fungi are present in the hair follicles and on the surface of normal adults in such quantities that they outnumber human cells (Gebhart & Kersten, 1992). Perhaps the most important benefit conferred by the resident microflora is as an integral part of cutaneous host-defence systems, where antigenic material of any kind can be prevented from invading tissues as rapidly as possible, i.e. at the very surface of the skin (Gebhart & Kersten, 1992). Cellular immune mechanisms are not appropriate tools for mediating surface immunity. Living and functionally immunocompetent cells will usually not be able to penetrate up to the outer body surface. However, mediators of specific humoral immunity can reach these areas via the typical cutaneous products of sebum and sweat (Gebhart & Kersten, 1992). A typical feature of the external body of cetaceans, however, is the total absence of hair, though there are individual vibrissae on the heads of mysticetes, as well as the absence of sebaceous and sweat glands (Parry, 1949; Yablokov, Bel'kovich & Borisov, 1974; Sokolov, 1982). Consequently these mechanisms for host-defence are presumably not available for cetaceans.

As may be expected, the resident microflora varies between different mammalian species and between different body sites (Cove *et al.*, 1992). A combination of host and external environmental factors plays a key role in the microbial colonisation of mammalian skin, a change in either of which may perturb this association (Cove *et al.*, 1992).

Bacteria and fungi, both the filamentous and yeast-like forms, are frequently isolated from the surface of marine mammal skin (Migaki & Jones, 1983; Buck, 1984; Haldiman *et al.*, 1985; Henk & Mullan, 1996) and bacterial and fungal diseases are known to cause death in captive and beach-cast marine mammals (Sweeney & Ridgway, 1975; Sweeney, Migaki, Vainik & Conklin, 1976; Stroud & Roffe, 1979; Tangredi & Medway, 1980; Buck, Shepard & Spotte, 1987). However little information is available on the types of micro-organisms associated with healthy free-swimming cetaceans to compare with data from debilitated animals. This study describes the presence of microbes on the skin of free-swimming southern right whales, and investigates the possible contribution of a mycological infection to the death of a stranded neonatal southern right whale calf.

4.2 Materials and Methods

4.2.1 Sample collection

4.2.1.1 Study area

Samples of integument (skin and blubber) from living southern right whales were collected by biopsy during the August and October field seasons of 1998 and 1999, as well as during early November 2000. The study area included Walker Bay (Gansbaai), Struisbaai, De Hoop Marine Reserve and St. Sebastian Bay, all on the south coast of Southern Africa (Plate 1). Samples were taken from stranded animals in the above areas as well as in the Cape Peninsula, Dwarskersbos and Elands Bay, along the west coast of Southern Africa (Plate 1).

4.2.1.2 Sampling

It should be stressed that the principal objectives of the biopsy sampling were to examine the anatomy of the skin and the lipid composition of the blubber layers, so the protocols described below were not designed for sampling microbial associations *per se*.

4.2.1.2.1 Biopsies

The free-swimming animals off South Africa were sampled using a specially designed, hand-held biopsy system (Chapter 6). These biopsy heads allowed rectangular skin samples (3-7 mm^2 in surface area) to be obtained with low impact. Such samples as well as the samples collected from stranded animals, were well-suited for microbial analysis of the skin surface (Table 5). Once a successful biopsy attempt was made, the sample was removed from the biopsy head, placed in foil and into a labelled plastic bag and then put into a cooler box with "blue ice". The biopsy heads were cleaned in 99% chloroform between samples, and the barbs reset or, if necessary, replaced. Back on land, the samples were measured, noting skin and blubber thickness. The pigmented skin was cut away from blubber samples (the cut was made on the blubber side of the intersection between the epidermis and dermis) using a sterile scalpel and the skin was im4mediately placed in a separate, labelled specimen bottle containing gluteraldehyde. The skin samples were left in the gluteraldehyde for a minimum of 3 days and a maximum of 6 days when they were placed in buffer (25% gluteraldehyde + sodium dihydrogen orthophosphate + disodiumhydrogen orthophosphate anhydrous = water) until analysed.

Samples from the Antarctic were collected using various techniques including crossbow, Paxarms biopsy gun and Japanese air gun. These samples were fixed as described for the above skin samples and exported from Japan under CITES permit number T-AG 99-100172(W).

The sample of shed skin was obtained at sea from an apparently abandoned live calf (at 34°2745S 20°4212E) and stored in 10% buffered formalin (Plate 48).

Table 5: Details of southern right whales sampled for an electron microscopic study of the skin.

Sample #	Type	Date	Age	Location[1]	Length (m)	Gender
Abandoned	Shed skin	10/07/96	Neonate	Witsand	?	?
89/30	Stranding (mid-dorsal)	12/06/89	Adult	Gansbaai	14.7	Male
94/12	Stranding (mid-dorsal)	22/09/94	Juvenile	Witsand	11.23	Female
98/09	Stranding (mid-dorsal, Pos 3)	20/08/98	Neonate	Witsand	3.9	Female
99/05	Stranding (mid-dorsal, Pos 2-5)	16/09/99	Neonate	Hermanus	4.84	Male
99/05	Stranding (mid-ventral, Pos 4)	16/09/99	Neonate	Hermanus	4.84	Male
99/05	Stranding (fluke)	16/09/99	Neonate	Hermanus	4.84	Male
99/05	Stranding (callosity)	16/09/99	Neonate	Hermanus	4.84	Male
00/09	Stranding (mid-dorsal, Pos 3)	24/07/00	Neonate	Witsand	5.91	Male
00/10	Stranding (mid-lateral, Pos 2)	29/07/00	Neonate	Elands Bay	4.42	Male
00/11	Stranding (lateral, Pos 2-4)	06/09/00	Juvenile	Sea Point	9.85	Female
00/12	Stranding (mid-dorsal, Pos 4)	18/09/00	Neonate	Dwarskersbos	4.43	Male
00/14	Stranding (dorso-lateral, Pos 2,4)	13/10/00	Subadult	Cape Point	15.7	Male
11	Biopsy	30/10/98	Calf	Gansbaai	n/a	ud
12	Biopsy	30/10/98	Adult	Gansbaai	n/a	Female*
22	Biopsy	28/08/98	Calf	Witsand	n/a	ud
23	Biopsy	28/08/98	Calf	Witsand	n/a	ud
24	Biopsy	28/08/98	Calf	Witsand	n/a	ud
26	Biopsy	28/08/98	Calf	Witsand	n/a	ud
28	Biopsy	28/08/98	Calf	Witsand	n/a	ud
30	Biopsy	28/08/98	Calf	Witsand	n/a	ud
45	Biopsy	05/09/99	Calf	Witsand	n/a	ud
46	Biopsy	05/09/99	Adult	Witsand	n/a	Female*
50	Biopsy	05/09/99	Calf	Witsand	n/a	ud
51	Biopsy	05/09/99	Adult	Witsand	n/a	Female*
58	Biopsy	05/09/99	Adult	Witsand	n/a	Female*
61	Biopsy	27/10/99	Calf	Struisbaai	n/a	ud
62	Biopsy	27/10/99	Calf	Struisbaai	n/a	ud
64	Biopsy	28/10/99	Calf	Struisbaai	n/a	ud
70	Biopsy	01/11/99	Adult	Struisbaai	n/a	Female*
73	Biopsy	01/11/00	Calf	Witsand	n/a	ud
74	Biopsy	01/11/00	Adult	Witsand	n/a	Female*
77	Biopsy	01/11/00	Adult	Witsand	n/a	Female*
78	Biopsy	02/11/00	Adult	Witsand	n/a	Female*
80	Biopsy	02/11/00	Calf	Witsand	n/a	ud
84	Biopsy	08/11/00	Calf	Gansbaai	n/a	ud
85	Biopsy	08/11/00	Adult	Gansbaai	n/a	Female*
89	Biopsy	10/11/00	Adult	Gansbaai	n/a	Female*
90	Biopsy	10/11/00	Calf	Gansbaai	n/a	ud
29S	Biopsy	25/01/99	Non-calf	650335S/0884517E	n/a	ud
35S	Biopsy	26/0199	Non-calf	633292S/0912944E	n/a	ud
44S	Biopsy	29/01/99	Non-calf	624021S/0961232E	n/a	ud
46S	Biopsy	31/01/99	Non-calf	624012S/0992990E	n/a	ud

Table 5: continued

Sample #	Type	Date	Age	Location[1]	Length (m)	Gender
64S	Biopsy	14/02/99	Non-calf	622174S/1185408E	n/a	ud
140S	Biopsy	05/02/99	Non-calf	631935S/1032848E	n/a	ud
146S	Biopsy	09/02/99	Non-calf	643123S/1130600E	n/a	ud
147S	Biopsy	11/02/99	Non-calf	641172S/1171433E	n/a	ud
149S	Biopsy	14/02/99	Non-calf	641714S/1173543E	n/a	ud
150S	Biopsy	14/02/99	Non-calf	633632S/1185262E	n/a	ud
151S	Biopsy	14/02/99	Non-calf	633632S/1185262E	n/a	ud

* Based on the assumption that all adults accompanying calves were their lactating mothers
[1] See Plate 1
ud = undetermined

4.2.1.2.2 Stranded animals

All samples from stranded animals were placed in foil and frozen at –20 °C within a few hours of collection. When possible, samples were taken from five equally-spaced positions along the mid-dorsal, lateral and mid-ventral surfaces of the animals (Plate 3). Subsamples of epidermal tissue were taken from the stranded material while still partly frozen for histological analysis. This tissue was fixed in gluteraldehyde and then stored in buffer (same procedure as described above for biopsy samples). In most instances, the positioning of the animal prohibited the collection of samples from both the mid-dorsal and mid-ventral surfaces and in other instances the location of the animal made it impossible to take measurements and collect samples from all positions along the various surfaces. On occasion, skin samples from other structures, e.g. callosities, flippers and flukes were opportunistically taken (Table 1).

4.2.1.2.3 Scanning Electron Microscopy (SEM)

In total, 11 biopsies from the Antarctic, 26 biopsies from South Africa, 1 sample of shed skin and 18 frozen samples from stranded material were prepared for scanning electron microscopy (SEM) (Table 5). Both frozen and buffered samples were dehydrated through an ethanol (Merck AG EtOH) series (30%, 50%, 70%, 80%, 90%, 100%) for 2.5 hours (minimum of 2 hours) in each solution. The samples were placed in two additional washes of absolute alcohol for 2.5 hours each. The samples were

critical point dried (CPD) from 100% EtOH in CO_2, mounted and coated with gold-palladium in a sputter coater; and viewed using a JEOL JSM-5200 Scanning Microscope operating at 15kV.

4.2.2 Determination of viable microorganisms

4.2.2.1 Sampling of skin

The fungal and bacterial populations observed during scanning electron microscopy (SEM) on the various samples of whale skin (Plate 52) could have been a result of post-sampling contamination. However, these microorganisms may also occur as part of the natural microbial populations on the skin. Consequently, in an attempt to determine whether some of the micro-organisms observed (using SEM) in this study originated from post-sampling contamination, frozen skin samples were analyzed. Nine samples, consisting of epidermis attached to small amounts of blubber, collected from various positions on stranded animals from different age groups, were selected (Table 6). To remove potential microbial contaminants, the pigmented surface of each sample was first swabbed with 70 % ethanol. A sub-sample, consisting of a block (*circa* 1 cm^3) was then aseptically cut from the middle of each sample. These sub-samples were subsequently analyzed for the presence of viable microorganisms using standard culture techniques.

Table 6: Description of stranded southern right whales sampled for viable micro-organisms on the skin.

Sample #	Position of samples	Age	No. of sub-samples
89/30	Mid-dorsal, pos 3	Adult	1
94/12	Mid-dorsal, pos 3	Juvenile	1
98/09	Mid-dorsal, pos 3	Neonate	1
99/05	Mid-dorsal, pos 3, 4	Neonate	2
00/11	Left-lateral, pos 3,4	Juvenile	2
00/12	Mid-dorsal, pos 4	Neonate	1
00/14	Dorso-lateral, pos 4	Subadult	1

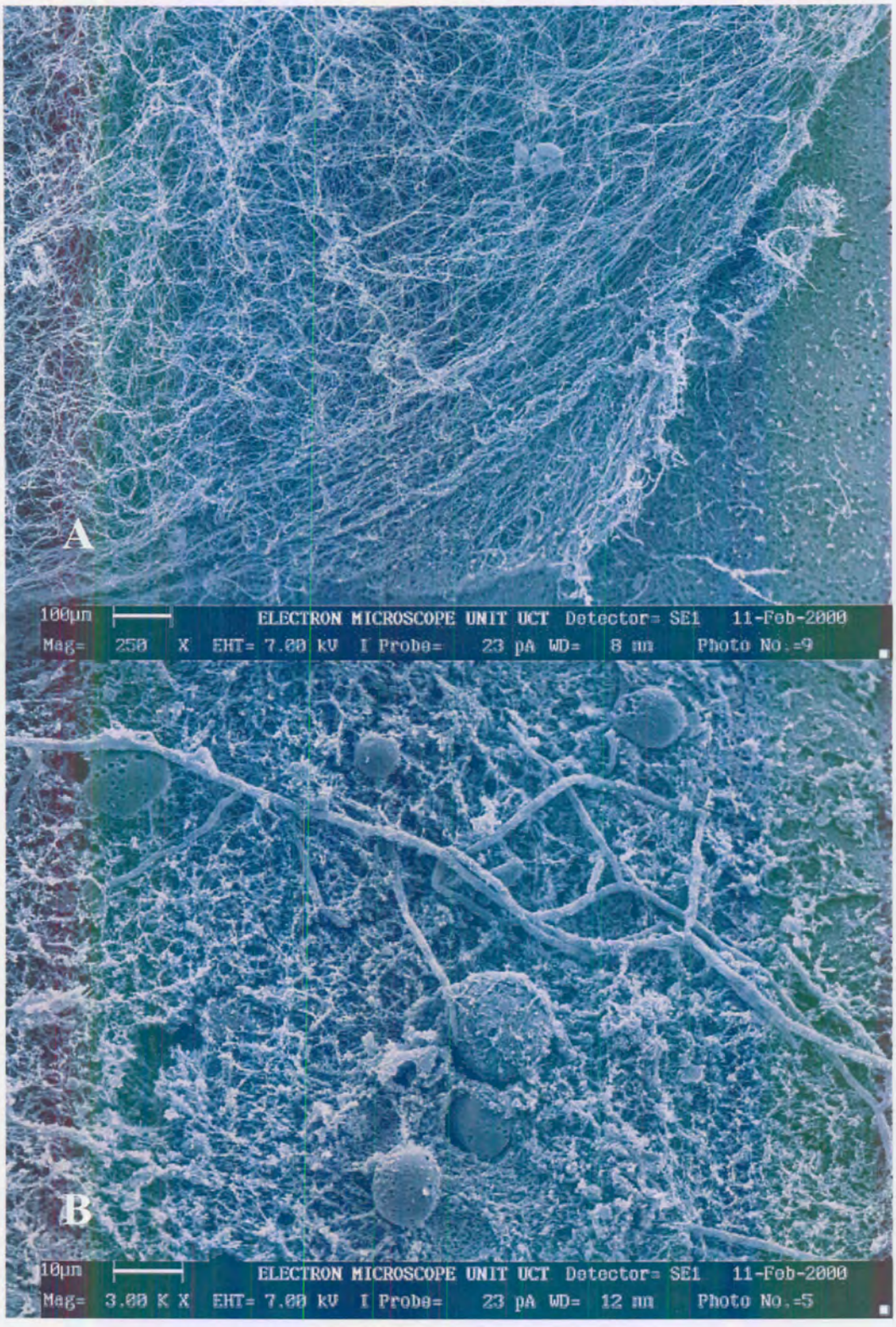

Plate 52: SEMs taken at the University of Cape Town's Microscopy Unit during random trial inspections showing microbial aggregations on the skin of a stranded neonatal southern right whale (99/05) . (Mag A 250X, Mag B 3 000X).

4.2.2.2 Culture techniques

Microorganisms embedded in skin. In a sterile laboratory, each skin sample was cut in half lengthwise. One half was cut into 3 sections, along the tangential plane, to sample the superficial, middle and deep aspects of the epidermis. A non-selective isolation medium, yeast-malt extract agar (YM) containing 1% (v/v) Tween 80, was inoculated with the different sections of skin. The plates were incubated at 22°C for a week and observed for growth. Developing colonies were isolated and further purified by successive sub-culturing on YM.

Microorganisms on surface of skin: The remaining halves of the skin samples used in the above experiment were used to detect any microorganisms on the skin surface. Nine superficial sections were individually washed in separate vials, containing 9 ml sterile distilled water, and 100 µl of each supernatant was plated onto malt extract agar (MEA). The plates were incubated at 22°C for a week and observed for growth.

4.3 Results

4.3.1 Microbial populations on the skin samples as revealed by SEM

Scanning electron microscopy revealed the presence of various "microfloral" organisms on 23 of the 56 skin samples analysed. The 23 samples included stranded animals (5 neonates, 2 juveniles) as well as live animals from different seasonal and age groups and animals sampled in the Antarctic (Table 7).

Amongst the biopsied animals, 45% of early season calves (n=9), all of the late season calves (n=7), 57% of late season adults (n=7) and 18% of the animals sampled in the Antarctic (n=11) possessed "microfloral" aggregations on the skin. No aggregations were detected on any of the early season adults (n=3).

Table 7: List of samples from southern right whales on which "microfloral" organisms and bacterial[b] growths were found on the skin, using SEM.

Sample #	Type	Location[1]	Date	Age	Sex	"Microfloral" organisms present
94/12	Stranding (mid-dorsal)	Witsand	22/09/94	Juvenile	Female	Spores
99/05	Stranding (mid-dorsal, Pos 2)	Hermanus	16/09/99	Neonate	Male	Spores
99/05	Stranding (fluke)	Hermanus	16/09/99	Neonate	Male	Spores
99/05	Stranding (callosity)	Hermanus	16/09/99	Neonate	Male	Prosthecate appendages on spores
00/09b	Stranding (mid-dorsal)	Witsand	24/07/00	Neonate	Male	Spores
00/10b	Stranding (mid-dorsal)	Elands Bay	29/07/00	Neonate	Male	Spores
00/11	Stranding (lateral, Pos 2)	Sea Point	06/09/00	Juvenile	Female	Spiky spores
11	Biopsy	Gansbaai	30/10/98	Calf	n/a	Cyanobacteria sp.
46S	Biopsy	624012S/0992990E	31/01/99	Non-calf	n/a	Spores
149S	Biopsy	641714S/1173543E	14/02/99	Non-calf	n/a	Spiky spores
22	Biopsy	Witsand	28/08/99	Calf	n/a	Spiky spores
23	Biopsy	Witsand	28/08/99	Calf	n/a	Spiky spores
28	Biopsy	Witsand	28/08/99	Calf	n/a	Spiky spores
61	Biopsy	Struisbaai	27/10/99	Calf	n/a	Spiky spores
62	Biopsy	Struisbaai	27/10/99	Calf	n/a	Spiky spores
70	Biopsy	Struisbaai	01/11/99	Adult	Female*	Spiky spores
73	Biopsy	Witsand	01/11/00	Calf	n/a	Encrypted spores
74	Biopsy	Witsand	01/11/00	Adult	Female*	Spiky spores
77	Biopsy	Witsand	01/11/00	Adult	Female*	Spiky spores
80	Biopsy	Witsand	02/11/00	Calf	n/a	Spiky spores
84	Biopsy	Gansbaai	08/11/00	Calf	n/a	Corrugated edges on spores
85	Biopsy	Gansbaai	08/11/00	Adult	Female*	Spores
90	Biopsy	Gansbaai	10/11/00	Calf	n/a	Spores

[b] Samples possessed bacterial cocci
* Based on the assumption that all adults accompanying calves were their lactating mothers
[1] (Plate 1)

In the stranded samples, 56% (n=7) of the animals possessed "microfloral" aggregations on the skin. Inspection by SEM revealed that, although very few in number, "smooth" spores were scattered all over the shed skin of the apparently abandoned neonate.

An undetermined species of cyanobacteria, 5 μm in diameter, was found on biopsies from a late season cow and two late season calves (*D.R. Du Preez & +R. Pienaar, pers. comm.) (Plate 53). On one calf, this micro-organism seemed have to secreted enzymes which dissolved the whale skin, forming crypts, in which they lay (Plate 53).

*Botany Department, University of Port Elizabeth, Port Elizabeth, South Africa.
+Department of Zoology, University of the Witwatersrand, Gauteng, South Africa.

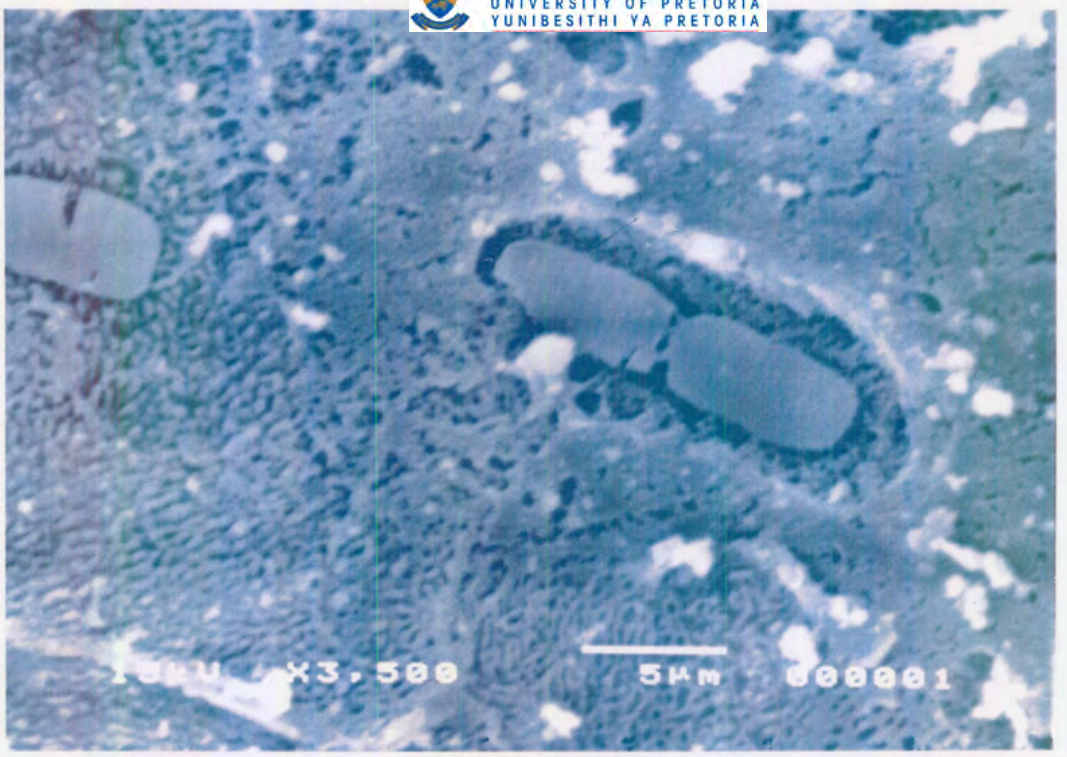

Plate 53: SEM of an undetermined species of cyanobacteria possibly forming crypts in the skin of a late season calf (# 90). (Mag 3 500X).

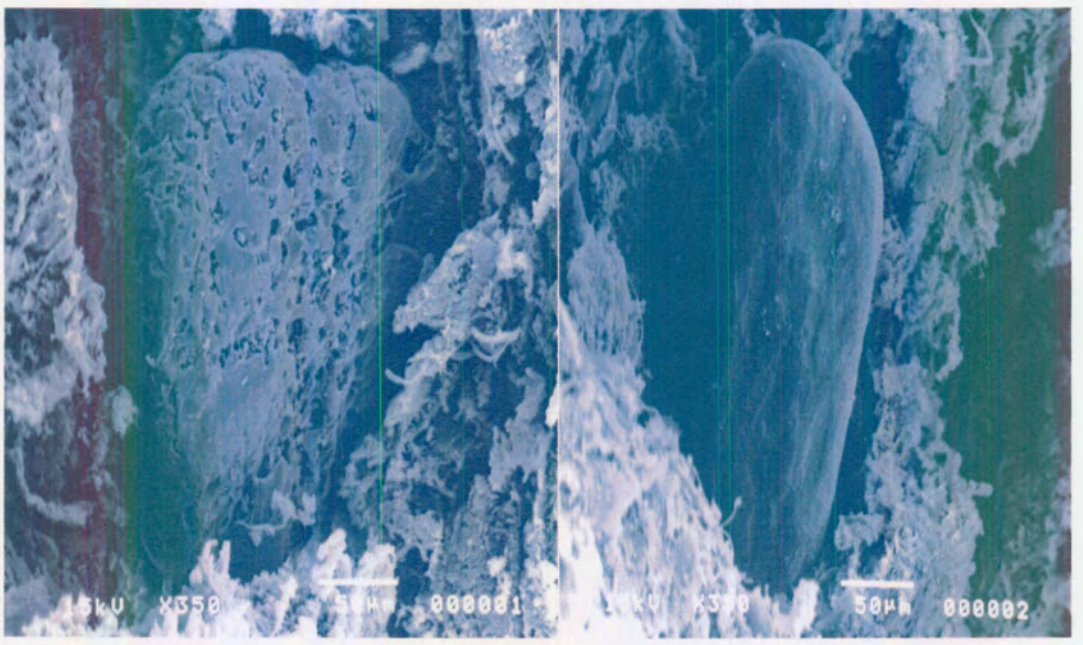

Plate 54: SEMs of "stonelike" micro-organisms deeply seated in the skin of a stranded neonatal southern right whale (99/05) (Mag 350X).

One stranded neonate (99/05) possessed large rectangularly shaped, "stone-like" micro-organisms (longest axis 180 μm) deeply-seated in the skin (Plate 54). On the same animal, spores with prosthecate appendages (cytoplasmic extrusions bounded by cell wall that are smaller than the mature cell) were found scattered on the skin surface, 5 μm in diameter (Plate 55). Different types of spherical, "smooth" spores (10 μm in diameter) were also detected on other animals (Plate 56). One late calf (# 84) possessed a unique spore with corrugated edges, 5.5 μm in diameter (Plate 57), whereas late season cows (# 70, # 77), late season calves (# 61, # 62), an early season calf (# 28) and 2 Antarctic animals (# 149S, # 46S) all possessed what seemed to be a similar type of "spiky" spore, 5 μm in diameter (Plate 58).

Fungal growths were visibly associated with some samples stored in buffer solution, approximately 6 months to 1 year after collection. Varying amounts of fungal mycelia (Plate 58) and/or yeast colonies (Plates 59 and 60) and bacteria (Plates 58 and 61) were present on the skin surface of animals in both seasonal and age groups, including animals sampled in the Antarctic and stranded animals (34 of the 56 samples analysed possessed fungal mycelia). Comparing seasonal groups, 78% of early season calves (n=9), 33% of early season adults (n=3), 43% of late season calves (n=7), 29% of late season adults (n=7) and all the animals sampled in the Antarctic (n=11) possessed fungal growth on the skin (Table 8). The amount and distribution of the fungi varied from animal to animal. Fungal mycelia were found in large patches or smaller clumps, while single mycelia were also noted scattered over the surface of the skin. Some fungal mycelia exhibited different structural characteristics and were probably different species (*J.D. Buck, pers. comm.) (Plate 62). Fungal mycelia were noted on the cut edges of two Antarctic samples (Plate 63) and one neonate mid-dorsal (99/05, Position 3) sample. Small clumps of mycelia were seen on the shed skin of a neonate (Table 8).

*Retired – Department of Zoology, University of Connecticut, Connecticut, USA.

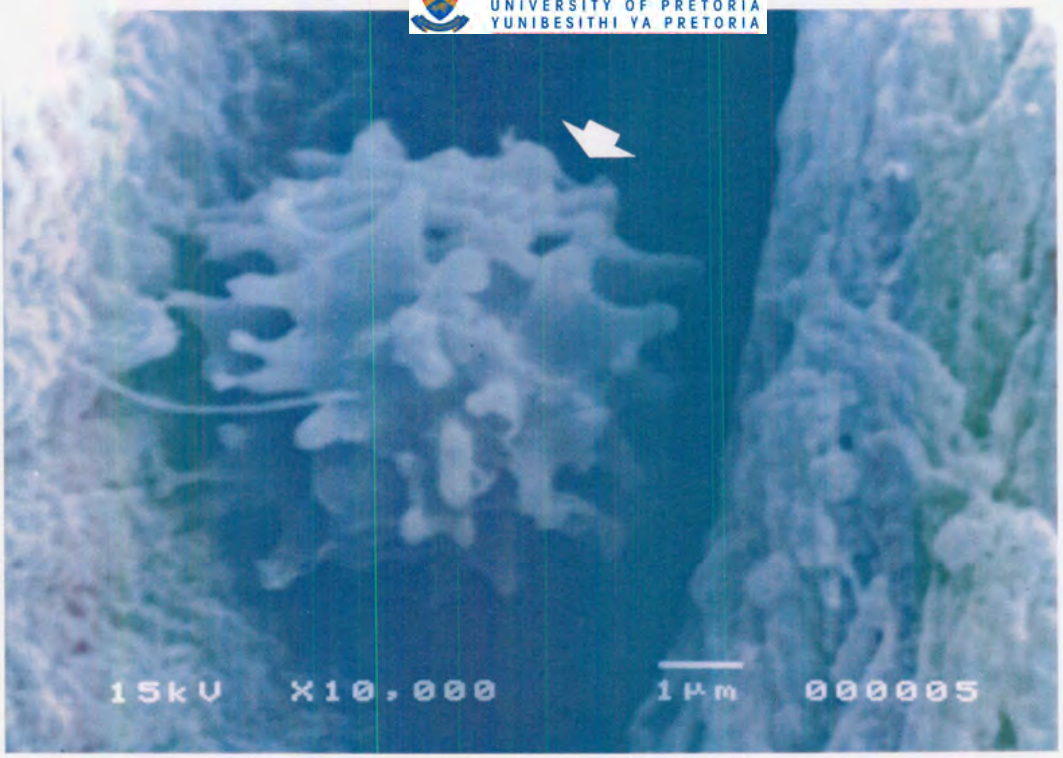

Plate 55: SEM of a spore with prosthecate appendages (arrow) found on the skin of a stranded neonatal southern right whale (99/05). (Mag 10 000X).

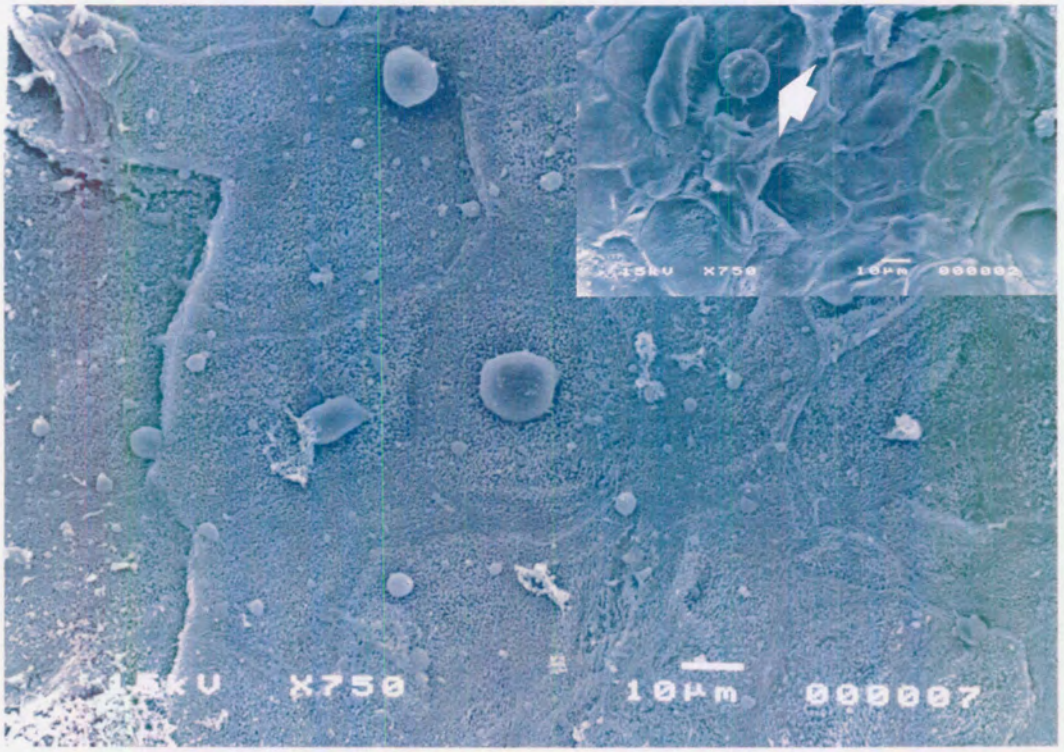

Plate56: SEM of spherical, smooth spores found on the skin of a late season southern right whale calf (# 61). (Mag 750X). Insert: SEM of smooth spore found on the skin of a stranded juvenile southern right whale (00/11) (Mag 750X).

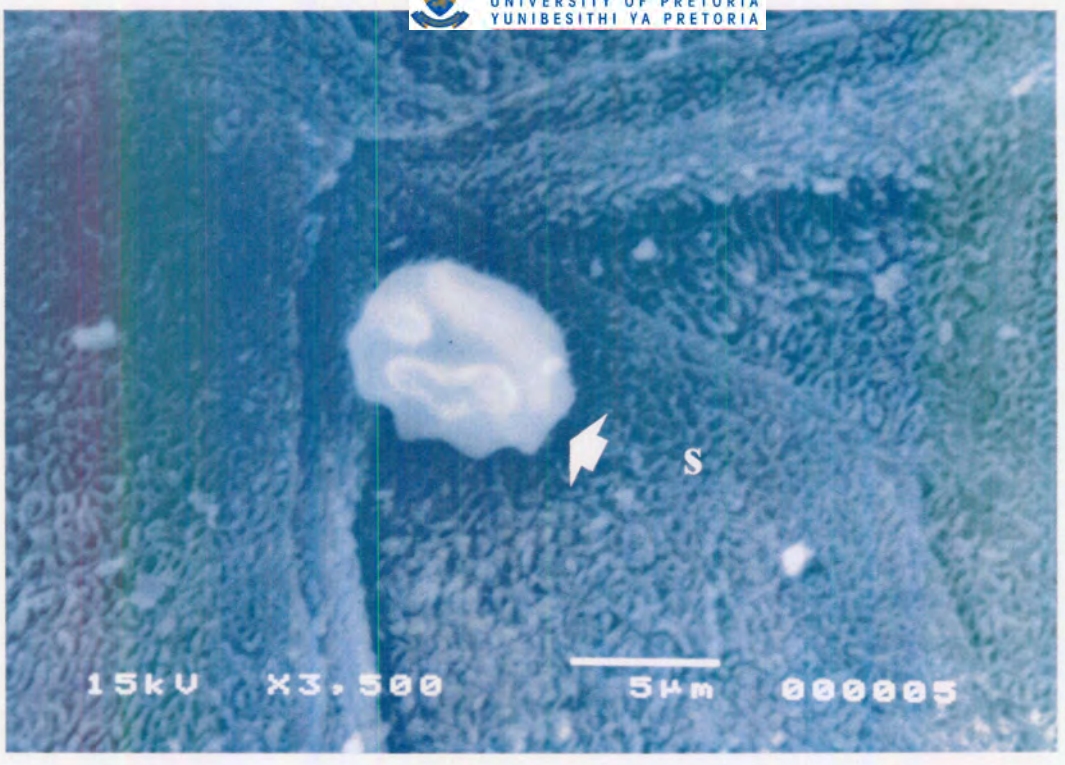

Plate 57: SEM of a unique spore, with corrugated edges (arrow), found on the exfoliated skin surface (s) of a late season southern right whale calf (# 84). Mag (3 500X).

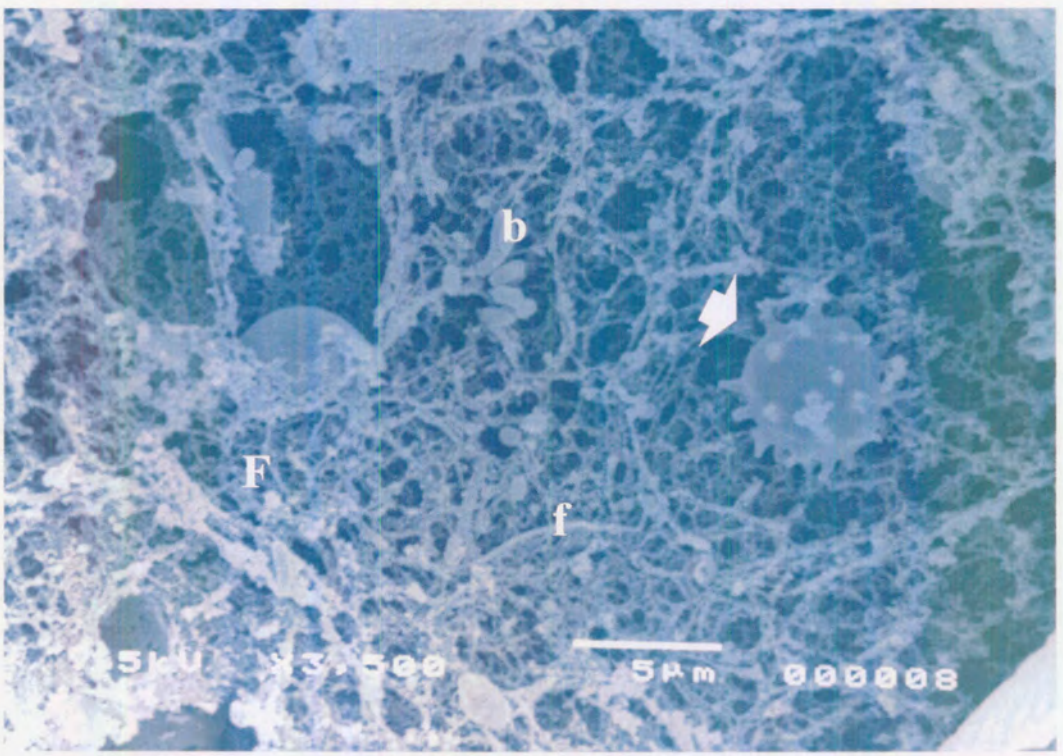

Plate 58: SEM showing a "spiky" spore (arrow) found on late season cows and calves, an early season calf and two non-calves sampled in the Antarctic. Bacterial cocci (b) and fungal mycelia (f) also present. (Mag 3 500X).

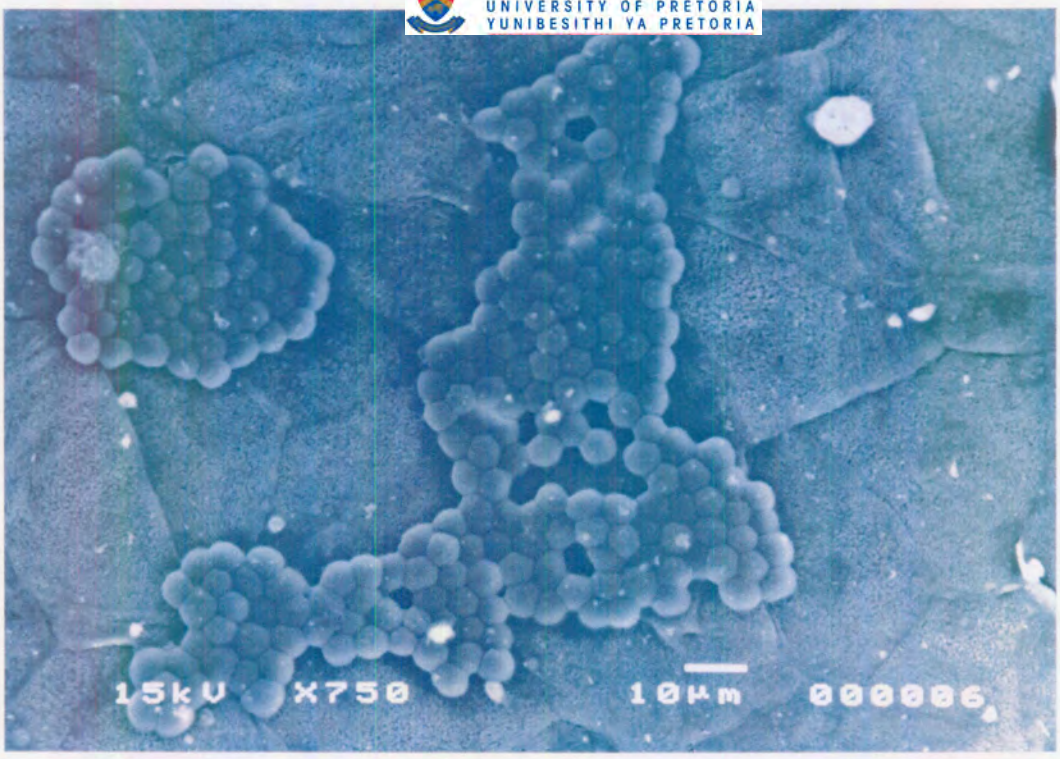

Plate 59: SEM showing yeast colonies on the surface of the skin of a late season southern right whale calf (# 73). (Mag 750X).

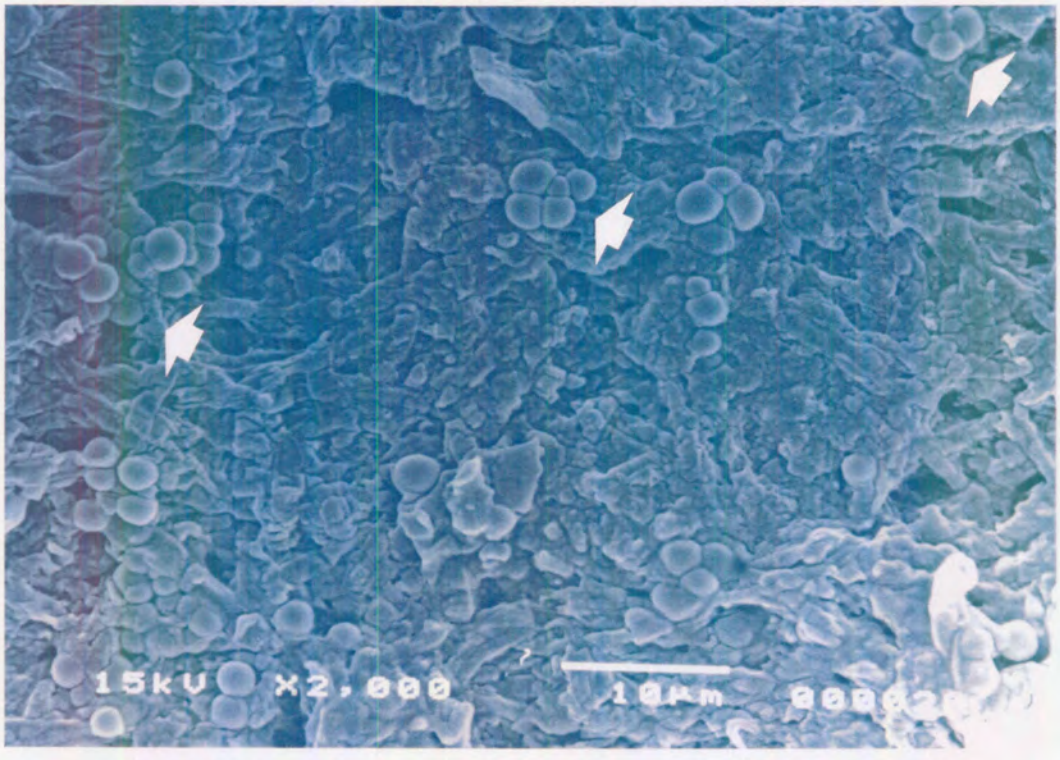

Plate 60: SEM showing actively dividing yeast cells (arrows) on the skin of a late season southern right whale calf (# 45). (Mag 2 000X).

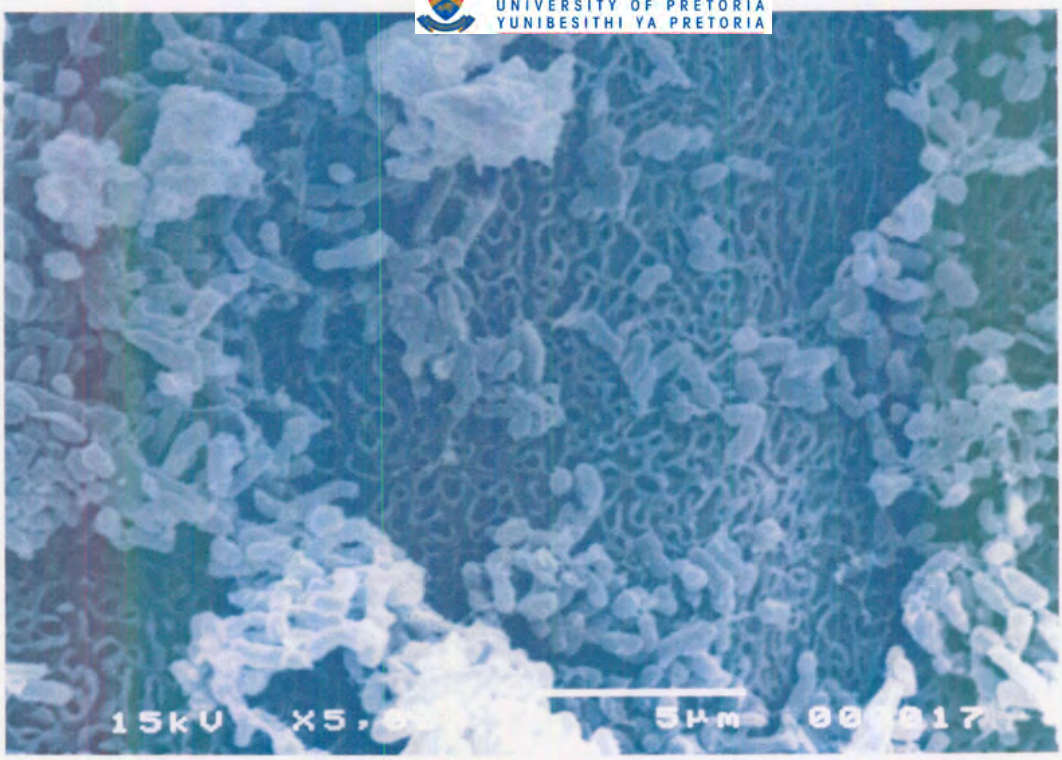

Plate 61: SEM showing the presence of bacterial cocci distributed on the sloughed surface of the skin of a non-calf southern right whale (# 140S) sampled in Antarctic waters. (Mag 5 000X).

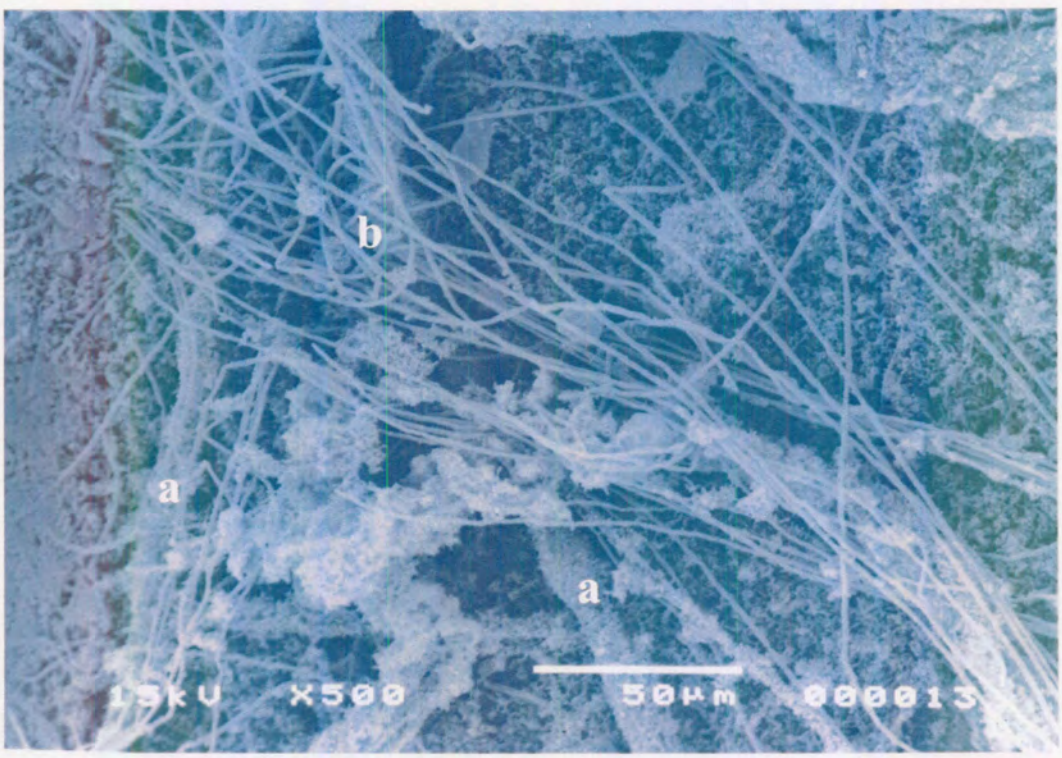

Plate 62: SEM showing the mycelia of different fungal species (a + b) on the skin of a non-calf southern right whale (# 146S) sampled in Antarctic waters. (Mag 500X).

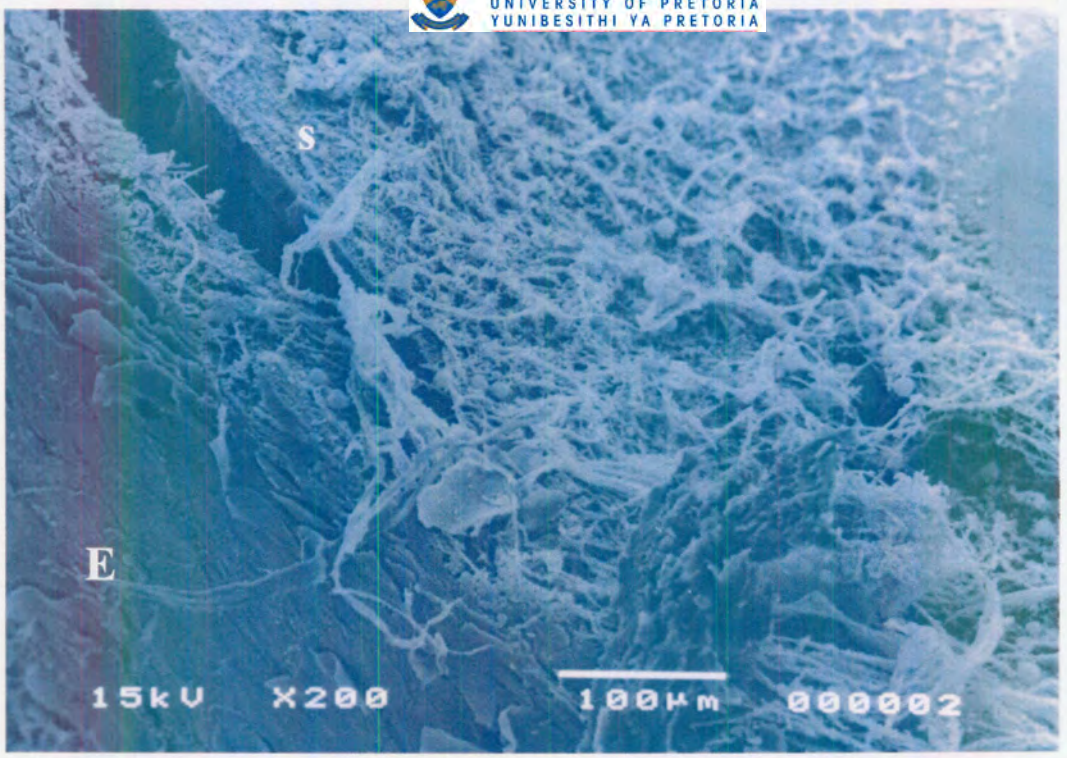

Plate 63: SEM showing the fungal aggregations on the sides (s) of a skin sample taken from a non-calf southern right whale (# 149S) in Antarctic waters. Epidermis (E). (Mag 200X).

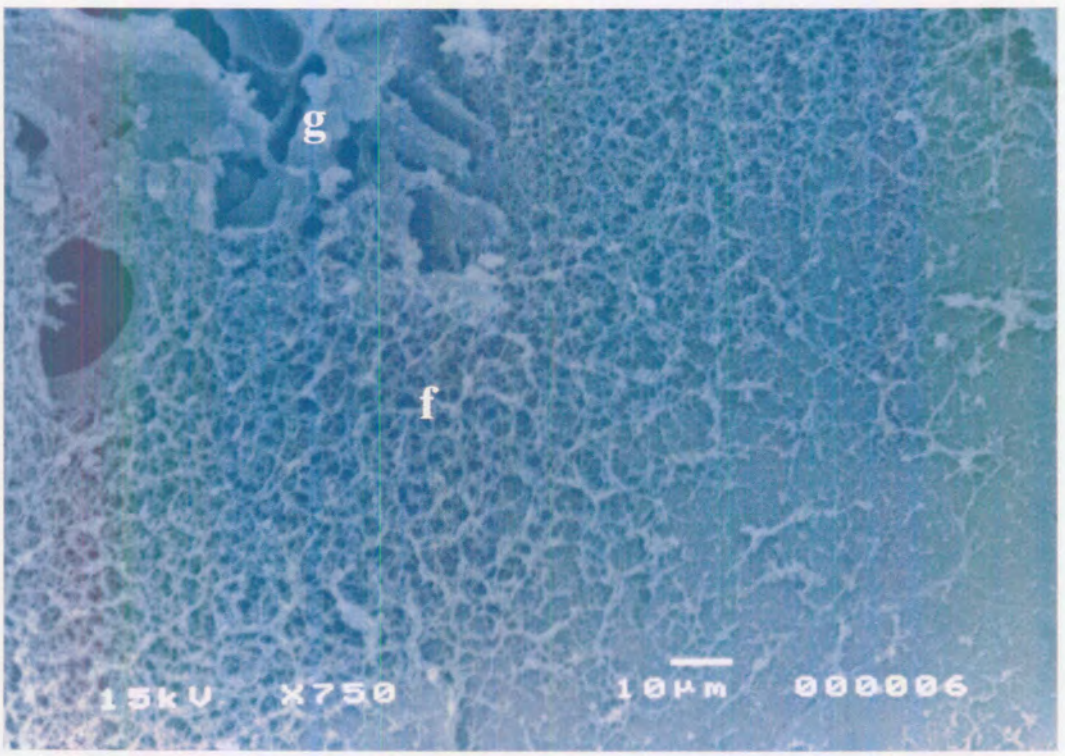

Plate 64: SEM of fungal mycelia (f) congregating around degenerating skin (g) of a stranded juvenile southern right whale (# 00/11). (Mag 750X).

Table 8: Samples from southern right whales on which fungal and/or bacterial[b] growths were found on the skin using SEM.

Sample #	Type	Location	Date	Age	Sex
Abandoned	Shed skin	Witsand	10/07/96	Neonate	?
99/05[b]	Stranding (mid-dorsal, pos 2-5)	Hermanus	16/09/99	Neonate	Male
99/05	Stranding (mid-ventral, pos 4)	Hermanus	16/09/99	Neonate	Male
99/05	Stranding (fluke)	Hermanus	16/09/99	Neonate	Male
99/05	Stranding (callosity)	Hermanus	16/09/99	Neonate	Male
00/10[b]	Stranding (mid-dorsal, pos 3)	Elands Bay	29/07/00	Neonate	Male
00/11	Stranding (dorso-lateral, pos 4)	Sea Point	06/09/00	Juvenile	Female
12	Biopsy	Gansbaai	30/10/98	La[+]	Female[*]
22	Biopsy	Witsand	28/08/98	Ec[+]	n/a
23	Biopsy	Witsand	28/08/98	Ec[+]	n/a
26[b]	Biopsy	Witsand	28/08/98	Ec[+]	n/a
28	Biopsy	Witsand	28/08/98	Ec[+]	n/a
30	Biopsy	Witsand	28/08/98	Ec[+]	n/a
45[b]	Biopsy	Witsand	05/09/99	Ec[+]	n/a
50	Biopsy	Witsand	05/09/99	Ec[+]	n/a
58	Biopsy	Witsand	05/09/99	Ea[+]	Female[*]
61	Biopsy	Struisbaai	27/10/99	Lc[+]	n/a
62	Biopsy	Struisbaai	27/10/99	Lc[+]	n/a
73	Biopsy	Witsand	01/11/00	Lc[+]	n/a
77	Biopsy	Witsand	01/11/00	La[+]	Female[*]
29S	Biopsy	650335S/0884517E	25/01/99	Non-calf	n/a
35S	Biopsy	633292S/0912944E	26/0199	Non-calf	n/a
44S	Biopsy	624021S/0961232E	29/01/99	Non-calf	n/a
46S	Biopsy	624012S/0992990E	31/01/99	Non-calf	n/a
64S	Biopsy	622174S/1185408E	14/02/99	Non-calf	n/a
140S[b]	Biopsy	631935S/1032848E	05/02/99	Non-calf	n/a
146S	Biopsy	643123S/1130600E	09/02/99	Non-calf	n/a
147S	Biopsy	641172S/1171433E	11/02/99	Non-calf	n/a
149S	Biopsy	641714S/1173543E	14/02/99	Non-calf	n/a
150S	Biopsy	633632S/1185262E	14/02/99	Non-calf	n/a
151S	Biopsy	633632S/1185262E	14/02/99	Non-calf	n/a

[b] Samples possessed bacterial cocci
[*] Based on the assumption that all adults accompanying calves were their lactating mothers
[+] Ec = early season calf, Lc = late season calf, Ea = early season adult, La = late season adult
n/a = not applicable

Samples collected from a stranded juvenile displayed fungal mycelia which grew and congregated around areas of skin that seemed to be degenerated (Plate 64). A late season calf possessed clumps of both mycelia and what seemed to be "encrypted" spores in association with one another (Plate 65). The tissue around these microbes

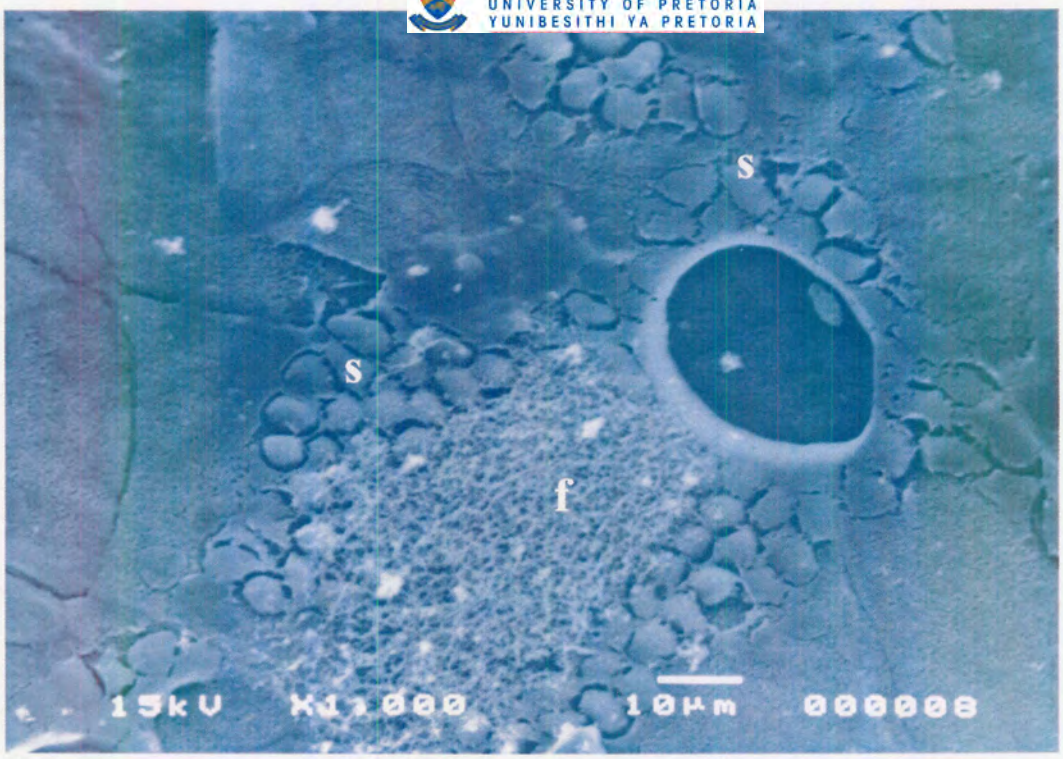

Plate 65: SEM of the skin of a late season calf (# 73) showing encrypted spores (s) in association with fungal mycelia (f). (Mag 1 000X).

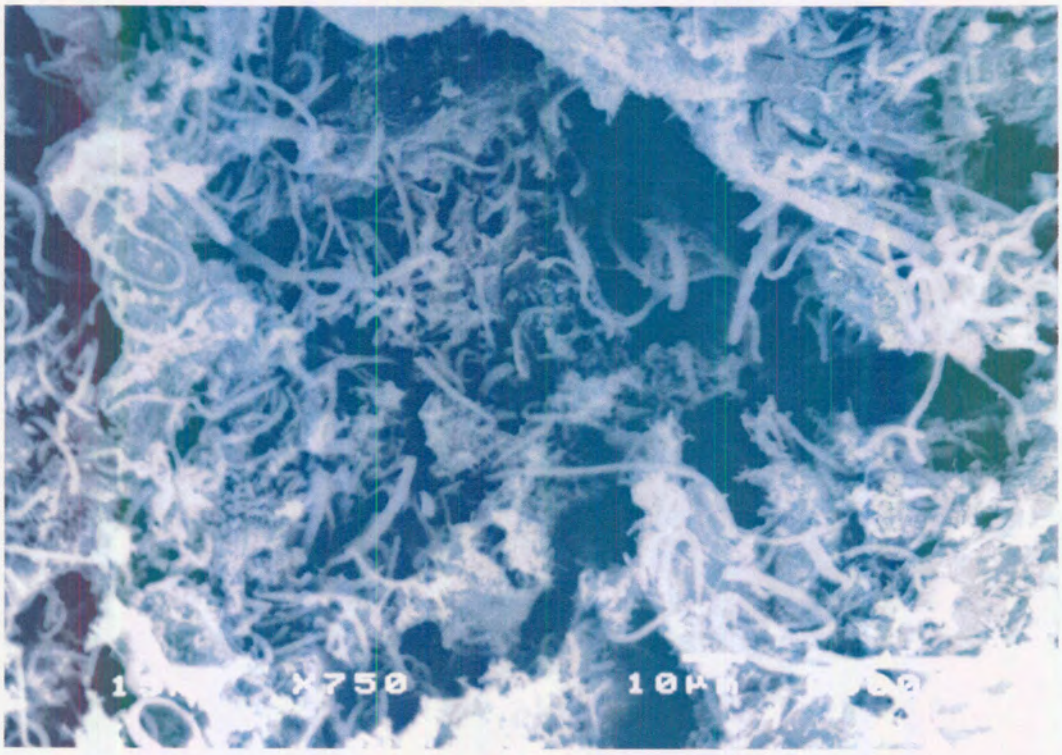

Plate 66: SEM of the skin of a stranded neonate southern right whale (99/05) showing invasive fungal growth. (Mag 750X).

appeared shrivelled and seemed to be necrotic in nature. A tremendous mat of fungal and yeast-like mycelia, as well as bacterial colonies, covered all the skin samples from a stranded neonate whose skin, on gross inspection, appeared unusually formed (see page 24) (Plate 52). On close inspection of frozen samples, the fungal mats were not as developed as in the preserved tissue, but the skin was very broken up with the fungus growing invasively (Plate 66). Single mycelial strands, as well as groups of mycelia, and stalks of budding yeast-like spores occurred on the surface of and within the skin tissue (Plate 67). A side view of the mid-dorsal (Position 3) sample revealed small, spherical spore-like structures nestled within the tissue (Plate 68).

4.3.2 Viable microbial populations as revealed by culturing

No viable microbial growth was detected on any of the plates that were innoculated with superficial epidermal washings. However, cultures of viable micro-organisms were found within 4 of the 9 plates that contained tissue samples (Table 9).

Table 9: Viable micro-organisms cultured from different skin sections, as well as from skin washings, compared to those detected in the same samples using SEM.

Samples	**Skin section (layer)**			**Skin washings**	**SEM**
	Superficial	**Middle**	**Deep**		
89/30 MD3	-	-	-	-	-
94/12 MD3	Fungi/bacteria	Bacteria	Bacteria	-	Fungi/yeast-like Structures and bacteria
98/09 MD3	-	-	Fungi/bacteria	-	-
99/05 MD3	-	-	-	-	Fungi/bacteria
99/05 MD4	-	-	-	-	Fungi/bacteria
00/11 LL3	-	-	-	-	-
00/11 LL4	-	Bacteria	Bacteria	-	Fungi
00/12 MD4	-	-	-	-	-
00/14 DL4	Yeast(D/V)	-	-	-	-

- denotes no growth
(D/V) = Yeast growth on dorsal and ventral side of skin

Although on initial inspection under the SEM, samples from a neonate (99/05), preserved in gluteraldehyde and stored in buffer, showed heavy fungal infestation (Plate 52), two frozen samples taken from the same animal did not show any signs of viable microbial growth when prepared for culture. One sample from the juvenile

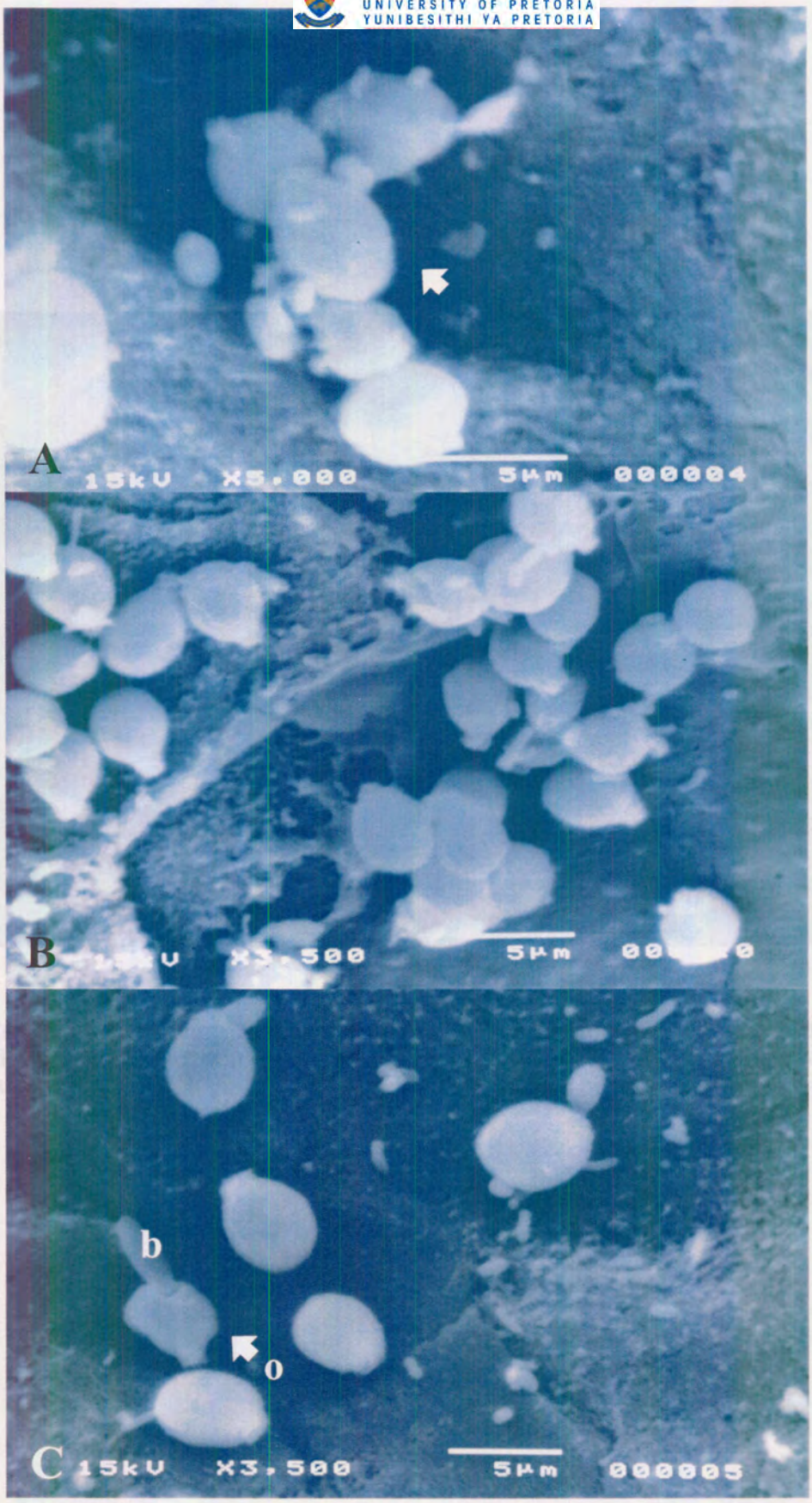

Plate 67: SEMs of yeast cells found on the surface of the skin of a stranded neonatal southern right whale (99/05). A: stalks (arrow), B: clumps and C: budding (b) yeast cells. Blastospore (o) (Mag A, 5 000X; Mag B + C, 3 500X).

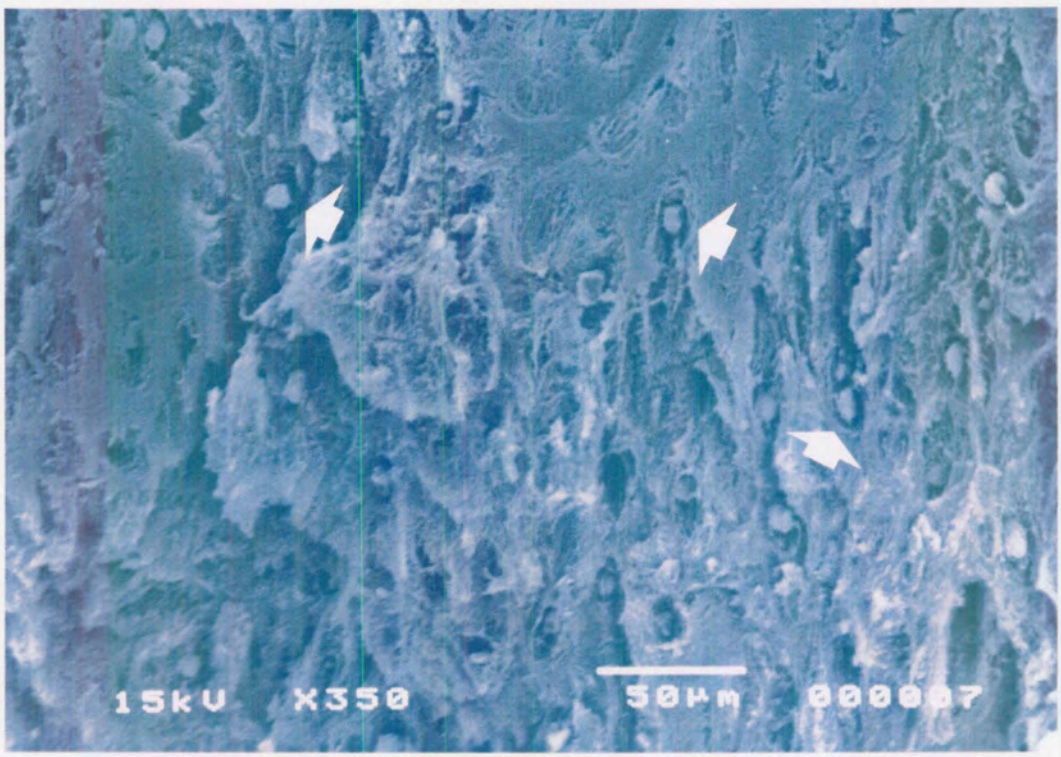

Plate 68: SEM showing small, spherical spore-like structures nestled within the epidermal tissue (arrows) of a stranded neonatal southern right whale (99/05). (Mag 350X).

(00/11), killed due to a collision with a vessel, showed viable bacterial growth in the middle and deep sections of the skin. The morphology of the yeast cells cultured on skin from the subadult (00/14) was typical of a basidiomycetous yeast (Yarrow, 1998). A small band (collarette) was detected at the site of budding by focussing the light microscope up and down.

4.4 Discussion

In his description of a southern right whale cow-calf pair that were caught off the West Coast of South Africa, Matthews (1938) makes no mention of the presence of diatom films, although the lack thereof was indicated for a southern right caught off South Georgia. Klumov (1962) reported the presence of a "greenish, very fine and thin bloom" of diatoms (unidentified) occurring in patches, mainly on the chin, lower surface of the pectoral fins and on the middle back region, of a right whale caught near the Kuril Islands during the North Pacific summer. The absence of *Bennettella* aggregations on early season adults and the low rate of detection on the samples taken from Antarctic animals is contrary to the observations for other southern hemisphere mysticetes (Mackintosh & Wheeler, 1929; Best, 1969; Nemoto *et al.*, 1980). The patchy nature of diatom films would reduce the chances of acquiring samples using biopsy techniques, which may explain the absence of diatoms on the southern right whale skin used in this study. Mackintosh & Wheeler (1929) concluded that the presence of *Cocconeis* [*Bennettella*] *ceticola* on whales in South African waters indicated that they were recent migrants from higher latitudes. In this study, however, "microfloral" organisms were found to aggregate mostly on late season cows and calves, animals that had already spent some time in warmer coastal waters, and not on early season adults, suggesting that their origin was local.

The definite sloughing of the superficial epidermal cells of the animals sampled in the Antarctic and the unique ultrastructural nuclear bodies (which provide readily accessible energy to the cells) in southern right whale stratum corneum cells (Pfeiffer & Rowntree, 1996) suggest that even at higher latititudes, diatom films may not have sufficient time to form on the skin of this species.

During SEM analysis, various species of fungal mycelia, yeast-like colonies and coccal chains of bacteria were detected on 23 of the 56 skin samples. Considerable effort was made to ascertain the nature and origin of these micro-organisms during subsequent analyses of these samples. However, the results obtained were inconclusive. Due to the fact that the skin samples were initially collected for structural description, contamination of samples during collection, during any subsequent handling or the use of contaminated preservatives, are all factors that may apply in this case. The latter factor seems highly unlikely, however, since the preservatives were acquired from the Anatomical Pathology Unit (Groote Schuur Hospital) who maintain that any contamination would be detected during their daily analyses of various human tissue or subsequently, as tissue is stored indefinitely in the buffer solution. Natural populations of micro-organisms, however, may proliferate excessively if samples are inappropriately preserved and/or stored. The presence of fungal growth on samples stored in buffer may also indicate that the nature of the gluteraldehyde and buffer were such that any microbial spores present on or in the samples, could not only survive, but proliferate. An attempt to exclude these possible sources of contamination was made by using and correctly preparing frozen samples collected from stranded animals for mycological analysis. Although the microbial culture results are difficult to interpret, they do seem to shed some light on the above questions.

It is widely understood among microbiologists that the vast majority (>90%) of prokaryotic organisms cannot be cultivated using classical methods involving agar plates and broth cultures (*R. Fulthorpe, pers. comm.). The fact, therefore, that only 4 out of 9 skin samples showed evidence of viable microbial activity in culture is not surprising. The viable microorganisms that were found, however, suggest that these organisms may be endogenously associated with the whale skin cells, especially since strict sterilization procedures were followed and precautions were taken to prevent any contamination. Scanning electron microscopy of these samples revealed the lack of any microbial presence in three of the nine samples, but did, however, identify fungal and/or bacterial microbes in 4 of the nine samples (2 of which were not detected in culture). Yeast cells (00/14 DL4), on the superficial, and bacteria (00/11 LL4), on the middle and deep layers of skin, detected in culture, were not detected

*Department of Microbiology, University of Toronto, Toronto, Canada.

using SEM. It is highly possible that some of these microorganisms (especially the fungi) that were detected using SEM, died during storage at -20 °C and were therefore not detectable using culture techniques (*A. Botha, pers. comm). The yeast cells that were not detected microscopically could possibly have been present beneath the surface layers of the stratum corneum, assessed using SEM. The juvenile specimen that was killed by colliding with a ship was presumably in better physical condition than any stranded specimens. The presence of isolated fungal colonies associated with autolytic epidermal cells (detected by SEM) on the skin of this animal could be due to post mortem colonisation (Greenwood *et al.*, 1974). The probability of this occurring, however, is questionable. The animal was intact and in a fresh condition when samples were collected (within 24 hours after washing ashore). It is therefore more likely that these micro-organisms occurred naturally on the surface of the skin and began to make use of the nutrients supplied by autolytic activities of the skin tissue. If one ignores the above-mentioned possibilities of contamination; the occurrence of fungi on the skin of animals sampled in the Antarctic might lead one to conclude that these microbes occur naturally on southern right whales in both South African and portions of Antarctic waters. It is also possible that the micro-organisms found on the Antarctic whales are remnants of their over-wintering in South African waters. Early season calves (including the apparently abandoned neonate) would not have left South African coastal waters before being biopsied and the high proportion of early season calves carrying fungal organisms on/in their skin, together with the fact that all the Antarctic samples possessed fungal growth, seems to support the suggestion of their being virtually universally present on southern right whales. The decrease in proportions of animals with fungal growths from early to late season, coupled with the results obtained from animals sampled in the Antarctic, may also indicate that these fungal growths proliferate more successfully in colder waters. This is a question that late summer sampling in the Antarctic could clarify.

In the stranded neonate (99/05) with an uncharacteristic skin appearance, due to the apparent lack of epidermal layers (Page 24, Plate 10), the nature and growth (i.e. budding) of the fungus that infested the skin all over this animals body resembled that seen in *Candida*-like infestations, with structures similar to blastospore outgrowths (fungal spores produced by budding) and pseudohyphae (fungal stalks with no true

*Department of Microbiology, University of Stellenbosch, Western Cape, South Africa.

transverse cell walls) (Odds, 1988). *Candida* infestations (also referred to as candidiasis, candidosis or moniliasis) are known to flourish in cetaceans that are immunosuppressed for unknown reasons (Werner, Halliwell & Beusse, 1979 in Medway, 1980). It is generally accepted that if mycelial forms are demonstrated to be penetrating healthy tissues, the organism is said to be pathogenic (Medway, 1980). Although the primary cause of the animal's death was not determined, its pericardium contained several litres of straw-coloured fluid, indicating a breakdown in the metabolic pathway (i.e. a uraemic condition), such as kidney failure, for example. The calf's distended bladder, filled with urine, seems to support the latter diagnosis. This uraemic condition is fatal (M. Duffield, pers. comm.). The invasive fungal infestation of the calves' skin could certainly be considered as a good indicator of the animal's poor health and possibly even as an additional factor in its demise (Sweeney *et al.*, 1976; Medway, 1980; Migaki, 1980). This supports the hypothesis that the fungus may occur naturally, in reduced numbers, on this species of whale and that it was able to proliferate due to the fatal condition of the neonate. In 2001, another stranded southern right whale neonate was found with a grossly distended bladder and straw-coloured fluid in the pericardium; histological examination of the kidney and heart revealed renal congestion and interstitial myocardial oedema. Culture of the urine from this animal produced a mixed growth of *Proteus*, yeasts and fungi (unidentified). The pathology report commented that "*Proteus* is a recognised cause of urinary tract infections in animals, however, due to the long interval between sampling and culture, it is more likely a contaminant. Yeasts and fungi should also be regarded as contaminants" (Pathcare, Cape Town, South Africa). Unfortunately the skin from this neonate was not analysed.

Tuberculate chlamydospore-like organisms ("spiky spores") were identified on some samples, resembling *Histoplasma capsulatum* (Davis, Dulbecco, Eisen, Ginsberg, 1990). This is a dimorphic fungus which exists in nature as a soil saprophyte (Kaplan, 1973) and is usually associated with fecal droppings of various birds and bats (Ajello, 1967 in Migaki & Jones, 1983). Among marine mammals, histoplasmosis, to date, has only been recorded in an adult female harp seal (Wilson, Kierstead & Long, 1974).

Many of the bacteria and fungi isolated from normal and abnormal tissues from harvested bowhead whales (Johnston & Shum, 1981; Philo, Shotts & George, 1993; Henk & Mullan 1996) have been associated with mortality in captive and free-ranging cetaceans. These organisms included the genera *Staphylococcus, Streptococcus, Clostridium, Vibrio, Pseudomonas*, and *Candida.* Members of these genera have also been obtained from, among others, dorsal muscle, blood, the left heart ventricle, thoracic and abdominal fluid of an Atlantic bottlenosed dolphin (*Tursiops truncatus*), from sanguinous fluid in the peritoneal cavity, lungs, kidneys, blood, spleen and liver of an Atlantic white-sided dolphin (*Lagenorhynchus acutus*), from the anus, blowhole, mesenteric lymph node and the gastrosplenic lymph node of a North Atlantic long-finned pilot whale (*Globicephala melas*), and from the blowhole and various skin lesions of a pygmy sperm whale (*Kogia breviceps*) (Tangredi & Medway, 1980; Buck, 1984; Buck *et al.*, 1987).

The further study of microbial populations on southern right whales would require more research adopting appropriate sampling and preservation techniques. Within the last decade, it is increasingly apparent that entire phylogenetic groups of microbial species, that have never been cultivated, are present, active and important in the environment. This is especially true of habitats such as tropical soils (Bornemen and Triplett, 1997) and marine environments (Wright, Vergin, Boyd & Giovannoni, 1997). In many cases researchers have found that the organisms that can be cultivated have very little to do with the major metabolic processes that are active in the environment (Dunbar, Takala, Barnes, Davis & Kuske, 1999). If a key organism is present, the chances are greater than 90% that it will not be culturable by traditional methods (R. Fulthorpe, pers. comm.).

The discovery of previously unknown species has been made possible by the development of superior methods to purify, amplify, clone and sequence DNA from environmental samples (Giovannoni, Britschgi, Moyer & Field, 1990; DeLong, Wu, Prézelin & Jovine, 1994). For various structural characteristics, rDNA has become a key target of such studies. DNA can be extracted from both fresh material, or from dry or frozen samples, and from samples preserved in alcohol. It is therefore

recommended that any further studies on microbial populations of whale skin include such techniques.

4.5 Conclusion

This is the first study that describes microbial associations on skin taken from presumably healthy free-swimming mysticetes on both their breeding and feeding grounds, as well as from stranded animals. The absence of any diatoms from the *Bennettella* genus on the skin taken from Antarctic and South African animals may reflect the difficulty of sampling such patchy organisms by biopsy, or it may imply that right whales exhibit high cellular proliferation rates, not unknown in cetaceans, which prevent diatomaceous films from forming. The predominance of "microfloral" aggregations on late season cows and calves seems to suggest that these micro-organisms are acquired in coastal waters and not in the Antarctic. The presence of fungal mycelia and spores, yeast colonies and bacteria add the southern right whale to the list of cetaceans (and mammals) on which these micro-organisms have been found, both in coastal and Antarctic waters. The invasive *Candida*-like infestation of the skin of a freshly stranded neonate is the first such record reported in this species and may, as has been thought in other cetaceans, be directly related to the death of this animal. The positive identification of the various species in the microbial association would enhance our understanding of the marine ecosystem by giving us the potential to determine whether the aetiologies of these, and future, infestations are perhaps due to steadily increasing levels of pollution i.e anthropogenic (and possibly pathogenic) in nature or not. Such studies might provide us with biomarkers of any related changes within the marine environment. The importance of appropriate collection, handling and preparation of samples for microbial analyses is hereby highlighted.

CHAPTER 5

FATTY ACID COMPOSITION OF THE BLUBBER IN SOUTHERN RIGHT WHALES, *EUBALAENA AUSTRALIS*.

5.1 Introduction

Whale blubber is a fatty layer of subcutaneous tissue that completely envelops the external surface of the whale. Blubber is normally considered to perform important physiological functions, such as forming an insulating layer to conserve body heat (Matthews, 1958; Bryden, 1964; 1972), as a fat (energy) depot, and probably has other functions such as reducing the resistance to the flow of water during swimming (Ackman, Eaton & Jangaard, 1965). The blubber layer underlies a relatively thin dermal covering, with which it is intimately connected, and overlies the musculature, from which it is separated only by a thin loose layer of blood-permeated connective tissue that is flexible and permits independent movement of the muscle and blubber (Lockyer, McConnell & Waters, 1984) (Plate 69).

The thickness of the blubber layer varies along and across the body (Slijper, 1948) and it was suggested that the differences in the thickness of blubber play an important part in the formation of the general body-outlines, i.e. in the streamlining of the body. In large whales, the blubber represents about 15-43% of total body mass (Lockyer, 1976). Most lipid storage in cetaceans occurs within the blubber layer (Ackman *et al.*, 1975a, b; Lockyer *et al.*, 1984; Ackman & Lamothe, 1989; Aguilar & Borrell, 1990; Møller, Born, Dietz, Ruzzante, Huag & Øien, 2000).

The biochemical roles of lipids as metabolic energy reserves and structural components of biomembranes are well understood and information concerning the physiological status of organisms studied can be obtained (Sargent, Parkes, Mueller-Harvey & Henderson, 1987). Micro-organisms form the basis of the marine food web and although much remains to be discovered about the lipids of these organisms, current knowledge is sufficient to enable lipids to be used as 'biomarkers" in marine

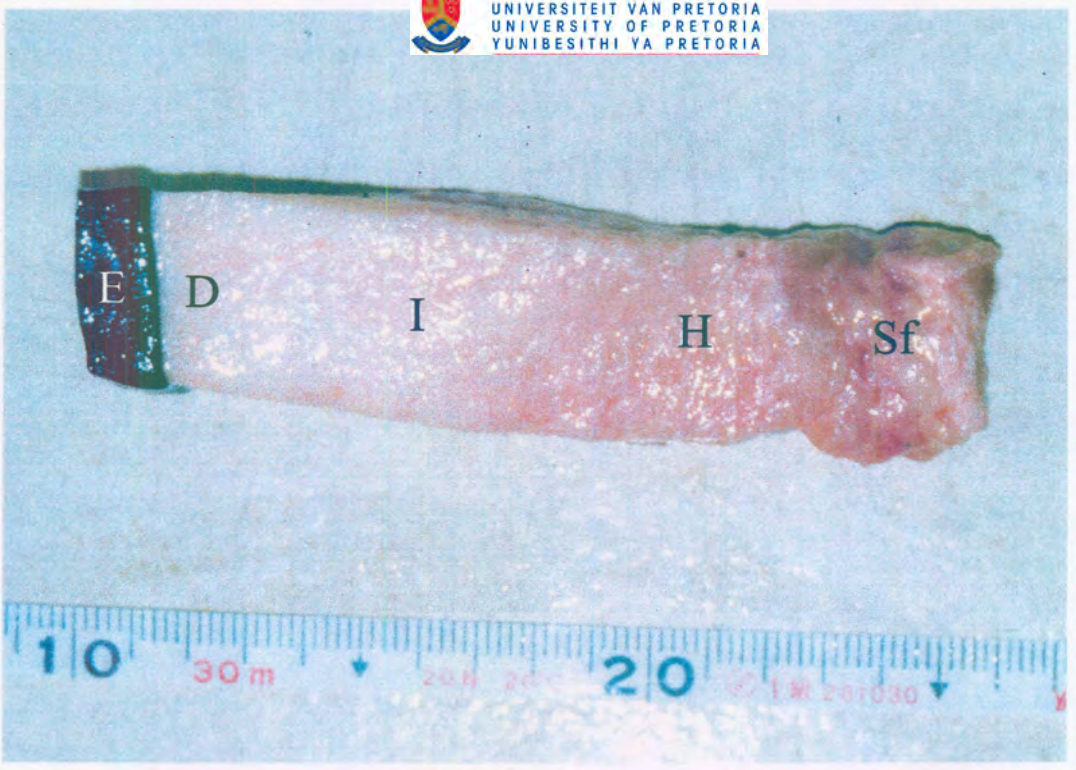

Plate 69: The integument of a neonatal southern right whale. The separation of the layers for lipid analysis is possible on gross inspection of the integument, dermal layer (D), intermediary fibrous/fatty layer (I), fatty hypodermal layer (H), superficial fascia (Sf), epidermis (E).

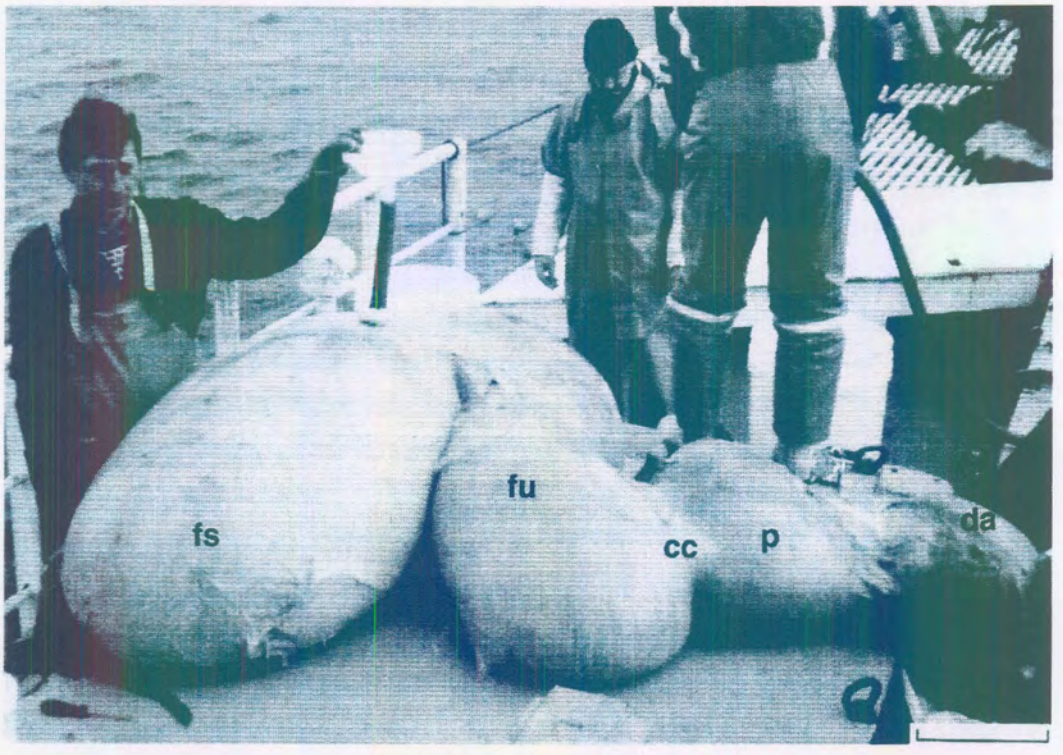

Plate 70: A maximally expanded minke whale (*Balaenoptera acutorostrata*) stomach and proximal duodenum, showing the forestomach (fs), the fundic chamber (fu), the pyloric chamber (p) and the duodenal ampulla (da). The connecting channel (cc) is situated between the fundic and the pyloric chamber. Scale bar = 30 cm (from Olsen *et al.*, 1994).

ecosystems (Sargent *et al.*, 1987). Biomarkers are defined as chemical components of organisms, which can be analysed directly from the environment and ideally can be interpreted both quantitatively and qualitatively in terms of *in situ* biomass (Sargent *et al.*, 1987). Lipids are particularly useful biomarkers since they are relatively easily extracted, identified and quantified as compared with other major biochemical constituents such as protein and carbohydrate (Sargent *et al.*, 1987).

On macroscopic examination, the histological structure and composition of whale blubber is not homogeneous across its thickness over the body (Ackman, Hingley, Eaton, Logan & Odense, 1975a; Lockyer, McConnell & Waters, 1985). In large whale species so far investigated, considerable variation in blubber structure and lipid content exists between the innermost deep and the superficial outermost strata (Heyerdahl, 1932 in Aguilar & Borrell 1990; Klem, 1935; Pedersen, 1950; Ackman *et al.* 1965; 1975a; 1975b; Lockyer *et al.* 1984; 1985; Aguilar & Borrell, 1990; Lambertsen *et al.*, 1993). The blubber can be divided into layers on the basis of physical appearance, fat content, iodine value (a measure of the proportion of unsaturated fats), and fatty acid distribution (Ackman *et al.* 1965, 1975a, b; Lockyer *et al.* 1984). The outermost blubber layer next to the skin is thought to be the most stable biochemically, serving chiefly for insulation and thermoregulation, although it, too, is known to vary in fat content and iodine value across the body in individual whales (Ackman *et al.*, 1975a). Analysing the triacylglycerol (TAG) fraction of lipids, various authors have been able to determine that metabolic energy storage is carried out in the inner layers, which respond much more rapidly to changes in metabolic state such as pregnancy, fattening up, and starvation (Ackman *et al.*, 1975a, b; Lockyer *et al.*, 1984; Aguilar & Borrell, 1990). Especially in periods of fattening, the deep blubber will reflect differences in diet between individuals (i.e. thereby representing a relatively short time scale) (Ackman & Lamothe, 1989; Møller, Born, Dietz, Ruzzante, Huag & Øien, 2000). The superficial blubber, on the other hand, represents a longer time scale of habitat exploitation, fingerprinted not only by the diet but also by endogenously produced fatty acids which optimise the insulation properties of the blubber closest to the cold environment (Ackman & Lamothe, 1989; Møller, Born, Dietz, Ruzzante, Huag & Øien, 2000).

Lipid metabolism is very similar in all mammals, with variations in metabolic processes arising primarily from the consumption of different diets. Borobia, Gearing, Simard, Gearing & Béland (1995) conducted a study on the humpbacks and fin whales of the Gulf of St Lawrence. The results of this study showed that these two species modify dietary fats to the same extent, in the formation of their blubber fatty acids, and that these acids do accurately reflect the food supply of the whales. These researchers therefore suggested that globally, differences in the fatty acid composition of whale blubber are set by variations in dietary intake more than by species-specific metabolic differences. This implies that depot fats of species of cetaceans and their prey items from the same geographical area can be compared in an attempt to discriminate between dietary and other factors affecting fatty acid composition (Ackman *et al.*, 1971; Sargent *et al.*, 1987).

Besides for dietary factors, another exogenous factor thought to affect the fatty acid composition of cetaceans is the process of microbial fermentation (Morii, 1972; 1979; Morii & Kanazu, 1972; Herwig *et al.*, 1984; Herwig & Staley, 1986; Mathiesen, Aagnes & Sormo, 1990; Olsen, Aagnes & Mathiesen, 1994a; Olsen, Nordøy, Blix & Mathiesen, 1994b; Olsen & Mathiesen, 1996). Baleen and toothed whales have multi-chambered stomach systems (Carte & Macalister, 1868; Jungklaus, 1898; Schulte, 1916; Hosokawa & Kamiya, 1971; Herwig, Staley, Nerini & Braham, 1984; Tarpley, Sis, Albert, Dalton & George, 1987) (Plate 70), which resemble that of ruminants (Hungate, 1966). Although the functional organisation of the baleen whale stomach is different from that of ruminants, evidence for forestomach microbial fermentation (as in ruminants) has been observed in the forestomach of some small odontocetes (Morii, 1972; 1979; Morii & Kanazu, 1972) as well as some mysticetes, e.g. bowhead whales (*Balaena mysticetus*), grey whales (*Eschrichtius robustus*), fin whales (*Balaenoptera physalus*) and minke whales (*Balaenoptera acutorostrata*) (Herwig *et al.*, 1984; Herwig & Staley, 1986; Mathiesen, Aagnes & Sormo, 1990; Olsen, Aagnes & Mathiesen, 1994a; Olsen, Nordøy, Blix & Mathiesen, 1994b; Olsen & Mathiesen, 1996).

Tsuyuki & Naruse (1963) and Tsuyuki & Itoh (1970) described the fatty acid composition of North Pacific right whale blubber and although both North Atlantic and southern right whales were also exploited commercially (Best, 1994; Tormosov *et al.*, 1998; IWC, 1998) and processed mainly for their oil, there are no published accounts of fatty acid analyses of these species.

This study provides the first description of the fatty acid composition of southern right whale blubber. Deep-core samples (generally including the outer, mid and portions of the inner layers) were acquired from free-swimming calves and cows in order to investigate the fatty acid exchange between these two groups. Details on the distribution of fatty acids in the inner, mid and outer blubber layers, as well as in various positions along the body are also provided for stranded individuals. The use of lipids as biomarkers for prey identification in this species is discussed, and the implication for the existence of microbial fermentation, based on the fatty acid composition, is indicated.

5.2 Materials and Methods

5.2.1 Study area

Samples of integument (skin and blubber) from living southern right whales were collected during the August and October field seasons of 1998 and 1999, as well as during early November 2000. The study area included Walker Bay (Gansbaai), Struisbaai, De Hoop Marine Reserve and St. Sebastian Bay, all on the south coast of Southern Africa (Plate 1). Samples were taken from stranded animals in the above areas as well as in the Cape Peninsula, Dwarskersbos and Elands Bay, along the west coast of Southern Africa (Plate 1).

5.2.2 Sample collection

A total of 143 blubber samples (comprising 26 biopsies from adults, 36 biopsies from calves, 36 positional and 45 separated-layer samples from stranded animals (Section 5.2.2.2)) were processed for fatty acid determination (Tables 10 and 11).

Table 10: Details of southern right whales biopsied along the South African coastline for lipid analysis.

Sample #	Date	Location	Age*	Sample #	Date	Location	Age
2	28/08/98	Witsand	Cow	20	31/10/98	Gansbaai	Calf
10	29/10/98	Witsand	Cow	21	31/10/91	Gansbaai	Calf
13	30/10/98	Gansbaai	Cow	22	28/08/99	Witsand	Calf
25	28/08/99	Witsand	Cow	24	28/08/99	Witsand	Calf
27	28/08/99	Witsand	Cow	26	28/08/99	Witsand	Calf
29	28/08/99	Witsand	Cow	28	28/08/99	Witsand	Calf
31	28/08/99	Witsand	Cow	30	28/08/99	Witsand	Calf
34	28/08/99	Witsand	Cow	33	28/08/99	Witsand	Calf
37	29/08/99	Witsand	Cow	36	29/08/99	Witsand	Calf
39	29/08/99	Witsand	Cow	40	29/08/99	Witsand	Calf
41	29/08/99	Witsand	Cow	43	05/09/99	Witsand	Calf
46	05/09/99	Witsand	Cow	44	05/09/99	Witsand	Calf
48	05/09/99	Witsand	Cow	45	05/09/99	Witsand	Calf
51	05/09/99	Witsand	Cow	47	05/09/99	Witsand	Calf
53	05/09/99	Witsand	Cow	49	05/09/99	Witsand	Calf
55	05/09/99	Witsand	Cow	52	05/09/99	Witsand	Calf
60	05/09/99	Witsand	Cow	54	05/09/99	Witsand	Calf
70	01/11/99	Struisbaai	Cow	56	05/09/99	Witsand	Calf
74	01/11/00	Witsand	Cow	63	28/10/99	Struisbaai	Calf
78	02/11/00	Witsand	Cow	66	01/11/99	Struisbaai	Calf
79	02/11/00	Witsand	Cow	68	01/11/99	Struisbaai	Calf
81	08/11/00	Gansbaai	Cow	71	03/11/99	Struisbaai	Calf
82	08/11/00	Gansbaai	Cow	72	03/11/99	Struisbaai	Calf
85	08/11/00	Gansbaai	Cow	73	01/11/00	Witsand	Calf
86	08/11/00	Gansbaai	Cow	75	01/11/00	Witsand	Calf
89	10/11/00	Gansbaai	Cow	76	01/11/00	Witsand	Calf
1	28/08/98	Witsand	Calf	80	02/11/00	Witsand	Calf
3	28/08/98	Witsand	Calf	83	08/11/00	Gansbaai	Calf
9	29/10/98	Witsand	Calf	84	08/11/00	Gansbaai	Calf
14	30/10/98	Gansbaai	Calf	87	08/11/00	Gansbaai	Calf
15	30/10/98	Gansbaai	Calf	90	10/11/00	Gansbaai	Calf

* Based on the assumption that all adults accompanying calves were their lactating mothers

Table 11: Details of southern right whales that stranded along the South African coastline and that were sampled for lipid analysis.

Sample #	Position	Date	Location	Age	Gender	Length (m)
84/27	mid-dorsal	09/08/84	Gansbaai	Juvenile	?	9.25
86/32	mid-dorsal	09/02/86	De Hoop	Neonate	Male	4.85
89/30*	mid-dorsal	12/06/89	Gansbaai	Adult	Male	14.7
90/29	mid-dorsal	16/08/90	Hermanus	Neonate	Female	4.8
91/18	mid-dorsal	13/09/91	De Hoop	Neonate	Male	6.65
94/12	mid-dorsal	22/09/94	Witsand	Juvenile	Female	11.23
98/09	Mid-dorsal, pos 1	20/08/98	Witsand	Neonate	Female	3.9
98/09	Mid-dorsal, pos 2	20/08/98	Witsand	Neonate	Female	3.9
98/09	Mid-dorsal, pos 3	20/08/98	Witsand	Neonate	Female	3.9
98/09 *	Mid-dorsal, pos 4	20/08/98	Witsand	Neonate	Female	3.9
98/09	Mid-dorsal, pos 5	20/08/98	Witsand	Neonate	Female	3.9
99/05	Mid-dorsal, pos 1	16/09/99	Hermanus	Neonate	Male	4.84
99/05	Mid-dorsal, pos 2	16/09/99	Hermanus	Neonate	Male	4.84
99/05	Mid-dorsal, pos 3	16/09/99	Hermanus	Neonate	Male	4.84
99/05 *	Mid-dorsal, pos 4	16/09/99	Hermanus	Neonate	Male	4.84
99/05	Mid-dorsal, pos 5	16/09/99	Hermanus	Neonate	Male	4.84
99/05	Mid-ventral, pos 1	16/09/99	Hermanus	Neonate	Male	4.84
99/05	Mid-ventral, pos 2	16/09/99	Hermanus	Neonate	Male	4.84
99/05	Mid-ventral, pos 4	16/09/99	Hermanus	Neonate	Male	4.84
99/05	Mid-ventral, pos 5	16/09/99	Hermanus	Neonate	Male	4.84
99/05	Mid-lateral, pos 2	16/09/99	Hermanus	Neonate	Male	4.84
99/05	Mid-lateral, pos 3	16/09/99	Hermanus	Neonate	Male	4.84
99/05	Mid-lateral, pos 4	16/09/99	Hermanus	Neonate	Male	4.84
99/05	Mid-lateral, pos 5	16/09/99	Hermanus	Neonate	Male	4.84
00/09*	Mid-dorsal, pos 3	24/07/00	Witsand	Neonate	Male	5.91
00/11*	Mid-lateral, pos 1	06/09/00	Sea Point	Juvenile	Female	9.85
00/11*	Mid-lateral, pos 2	06/09/00	Sea Point	Juvenile	Female	9.85
00/11*	Mid-lateral, pos 3	06/09/00	Sea Point	Juvenile	Female	9.85
00/11*	Mid-lateral, pos 4	06/09/00	Sea Point	Juvenile	Female	9.85
00/11*	Mid-lateral, pos 5	06/09/00	Sea Point	Juvenile	Female	9.85
00/12 *	Mid-dorsal, pos 4	18/09/00	Dwarskersbos	Neonate	Male	4.43
00/14 *	Dorso-lateral, pos 1	13/10/00	Cape Point	Subadult	Male	15.7
00/14*	Dorso-lateral, pos 2	13/10/00	Cape Point	Subadult	Male	15.7
00/14*	Dorso-lateral, pos 3	13/10/00	Cape Point	Subadult	Male	15.7
00/14*	Dorso-lateral, pos 4	13/10/00	Cape Point	Subadult	Male	15.7
00/14*	Dorso-lateral, pos 5	13/10/00	Cape Point	Subadult	Male	15.7

* Layer analysis was done these samples

Biopsied blubber samples were not subdivided into inner, mid and outer layers as material was limited, instead all layers were analysed together (Section 5.2.2.1). Samples from stranded animals were halved, one half was further subdivided into inner, middle and outer layers, each analysed separately and the other half was analysed with all layers combined. Histological analysis (Chapter 2, Page 47) revealed that deep-core rather than complete integumentary samples were obtained from free-ranging southern right whales. Although the length of the adult biopsy needle (19.2-20.5 cm) fell within mean mid-lateral (Tormosov *et al.*, 1998) and mid-dorsal (C. Miller, unpubl. data) blubber thickness measurements from southern right whales, the orientation of the biopsy needle relative to the whale at the time of sampling was a varying factor that determined the depths of the samples retrieved.

5.2.2.1 Biopsies

Each animal was approached perpendicular to its long axis and sampled by inserting the biopsy head (on the end of a 9 m aluminium pole) into the dorso-lateral surface of the whale and immediately retracting it (Plate 2). Once a successful biopsy attempt was made, the sample was removed from the biopsy head, placed in foil and into a labelled plastic bag and then put into a cooler box with "blue ice". The biopsy heads were cleaned in 99% chloroform between samples, and the barbs reset or, if necessary, replaced. Back on land, the samples were measured, noting epidermal and blubber thicknesses. The pigmented epidermis was cut away from blubber samples (the cut was made on the blubber side of the intersection between the epidermis and dermis) using a sterile scalpel and the blubber samples were placed in tin foil and then into labelled plastic bags and stored at –20 °C until analysed. The duration of time between sample collection and freezing was approximately 3-9 hours.

5.2.2.2 Stranded animals

Only carcasses that were in visibly good ("fresh") condition were sampled. Five neonates (98/09, 99/05, 00/09, 00/10, 00/12) were sampled within 24 hours of their estimated time of death, all neonates prior to this time were sampled within 48 hours of their estimated time of death. Total body length as well as blubber thickness

measurements were taken (Table 1) and full core samples were placed in foil and frozen at -20 °C within a few hours of collection. Skin and blubber thickness measurements were taken from five positions along the mid-dorsal, lateral and mid-ventral surfaces from animals that stranded from 1998 onwards (Plate 3). Full core samples were taken from the same positions. In most instances, the positioning of the animal prohibited the collection of samples from both the mid-dorsal and mid-ventral surfaces and in other instances the location of the animal made it impossible to take measurements and collect samples from all positions along the various surfaces. Larger samples were subdivided along the vertical plane, one half was used in its entirety for fatty acid analysis and the second half was further divided into 3 macroscopic sections a, b, c and each layer was individually analysed for fatty acid composition. Layer a consisted of the fibrous papillar dermis, layer b represented the less fibrous reticular dermis and the lower, fatty hypodermal layers were contained in layer c.

5.2.3 Lipid analysis

Trial analyses were conducted, on two gas chromatographs, fitted with capillary columns, on two separate occasions, at the Animal Research Council, Pretoria and Medical Research Council, Cape Town. This was done in order to compare the results from these systems with trial analyses run on the 2 m packed column gas chromatograph. Although only thirteen peaks were identified using the 2 m packed column, nineteen individual fatty acids were identified using capillary (ARC and MRC) and 30 m (0.53 mm internal diameter) packed columns (MRC, used for quantitative analysis). Apart from the fatty acids identified on the 2 m packed column, the other systems detected C18:2, C18:3n6, C18:3n3, C20:2, C20:3, C20:4. However, together with some unidentifiable peaks, each of these fatty acids was generally present in amounts between 0.01 % and 3 % of the total (Figures 6 and 7). As funding was limited and considering the substantial amount of samples to be analysed, qualitative analysis was continued using the available 2 m packed column system described below.

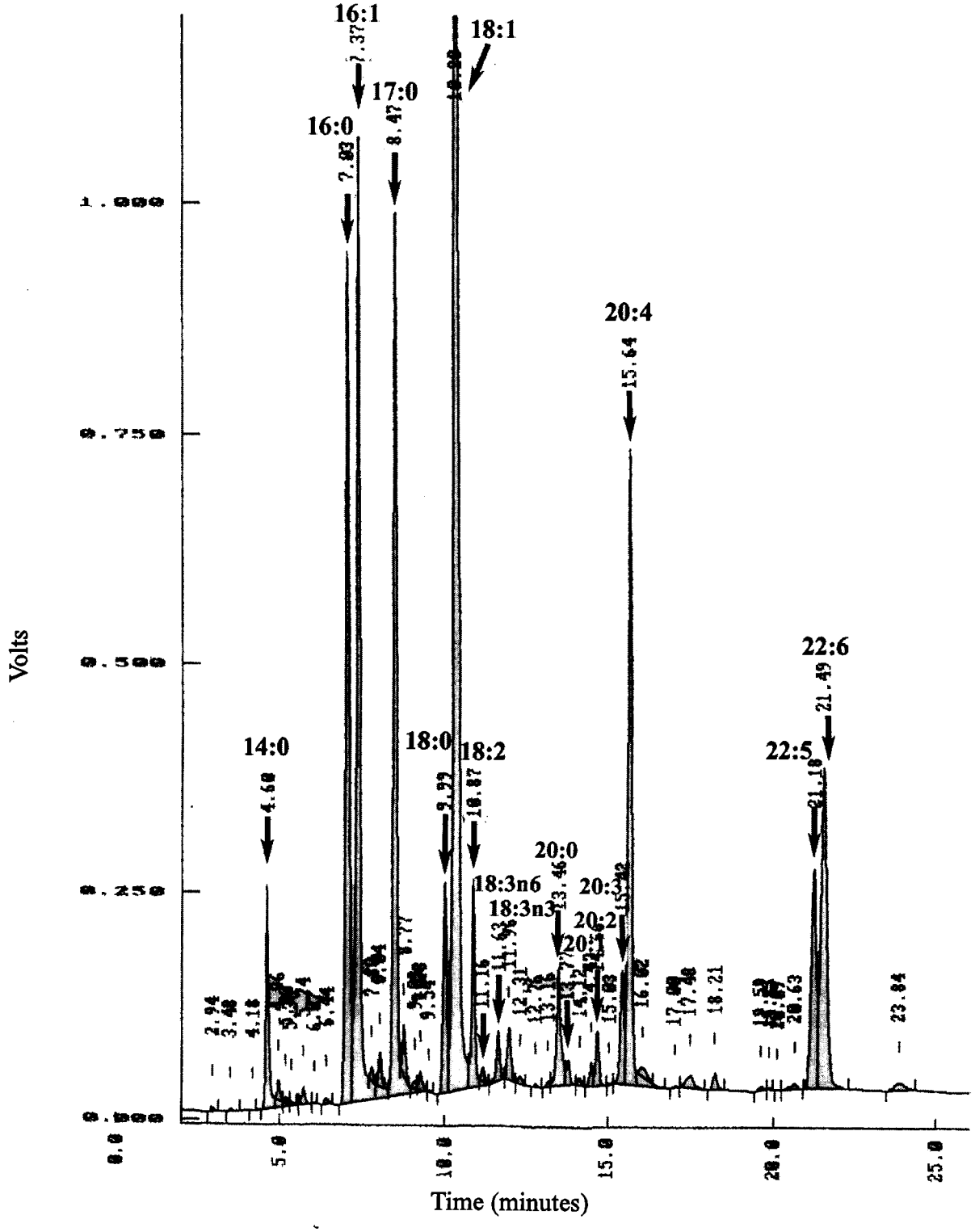

Figure 6 : Chromatograms showing the fatty acid profile from the blubber of a southern right whale calf (# 87), indicating the major fatty acids as well as unidentified peaks, detected using gas chromatography (MRC).

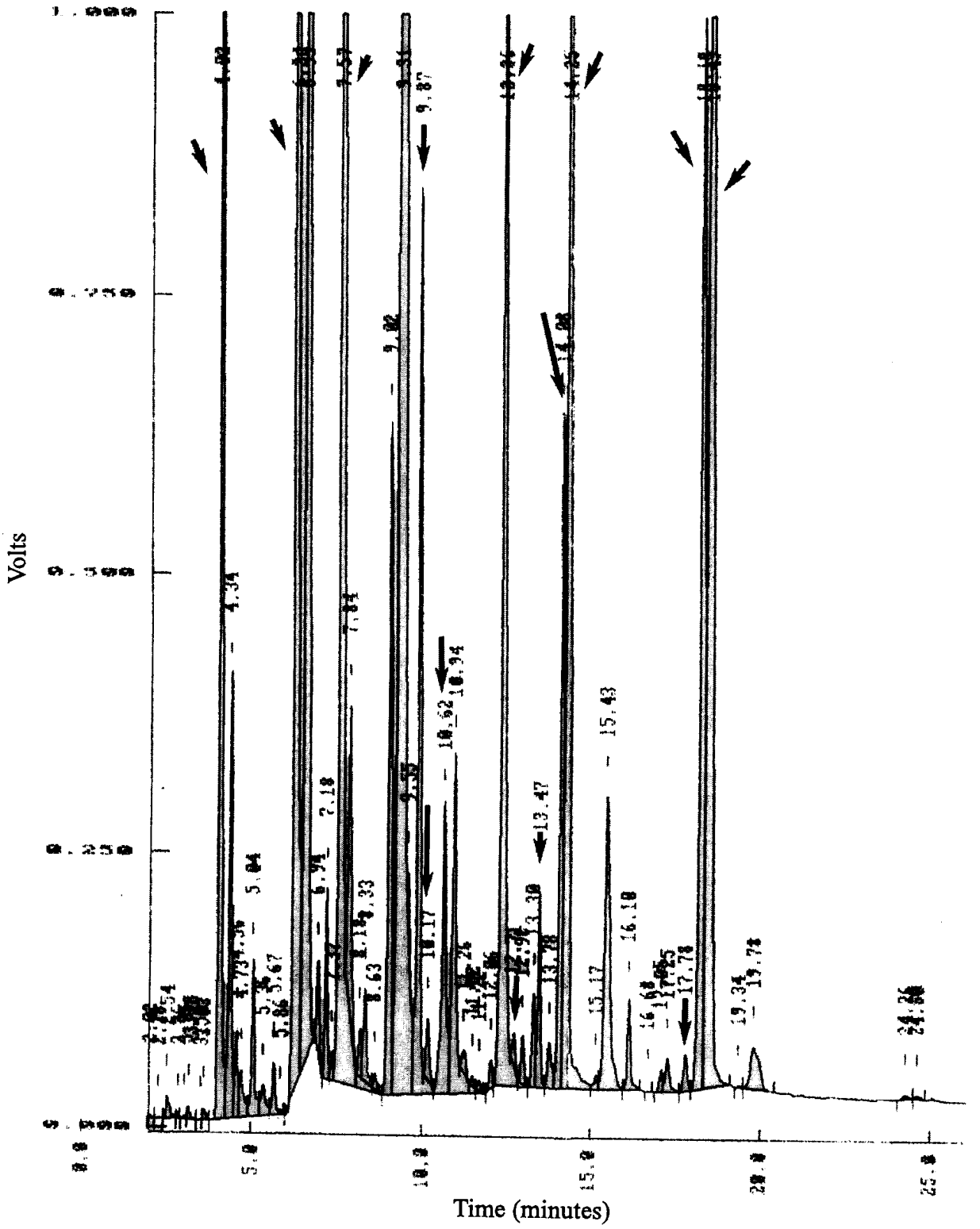

Figure 7: Chromatograms showing the fatty acid profile from the blubber of a southern right whale mother (# 86), indicating the major fatty acids as well as unidentified peaks, detected using gas chromatography (MRC).

5.2.3.1 Preparation of fatty acid methyl esters (FAMEs) for composition analysis

A minimum of 0.5 g of fatty tissue was cut along the long axis of each sample, (consisting of dermal and hypodermal tissue) and placed in a glass vial with a tight-fitting poly top lid. Chloroform was added to the sample. The quantity of chloroform added depended on the weight of the samples but was always 10 times the weight, i.e. 5 ml for 0.5 g tissue. Using a glass rod, the sample was gently crushed and thoroughly blended with the chloroform. This mixture was stored at 2-4 °C and shaken occasionally. The mixture was left to extract for a minimum of 24 hours and a maximum of 72 hours in a refrigerator. Samples left to extract for longer generally resulted in better-separated gas chromatograph peaks.

Lipids were extracted with chloroform: methanol (2:1; v/v) (Folch, Lees & Sloane-Stanley, 1957; Ways & Hanahan, 1964; Webb, De Smet, Van Nevel, Martens & Demeyer, 1998; De Smet, Webb, Claeys, Uytterhaegen & Demeyer, 2000). 0.5 ml of the sample extract was added to 1 ml 2M sodium hydroxide in methanol solution (8 g NaOH in 100 ml methanol) (AOAC, 1975) and 5 ml chloroform, in a heat-resistant centrifuge tube. The samples were tightly sealed, mixed thoroughly and placed in a waterbath at 60 °C for 30 minutes. Thereafter the samples were removed from the waterbath, lids were loosened slightly to release the pressure and allowed to cool. Once cool, the samples were centrifuged at 5 000 rpm for 15 minutes. The clear supernatant was pipetted into a clean plastic vial and either immediately injected into the gas chromatograph or stored in a freezer until required. This extract was used within 48 hours in order to prevent any autoxidation of the fatty acids. Fatty acids were measured by gas chromatography (Webb, Casey & Van Niekerk, 1994; Webb & Casey, 1995) and expressed as proportions of long-chain fatty acids (w/w %) present in the sample.

5.2.3.2 Settings and specifications of the gas chromatograph (G.C.) column for composition analysis

The gas chromatograph used was a Varian model 100, equipped with flame ionisation detection, using a 2 meter glass column (ID: 3 mm, packed with 10 % SP 2330 on Chromosorb W/HP 100/120). Gas flow rates were: H_2 - 300 KPa; O_2 - 300 Kpa; Carrier gas: N_2 - 300 KPa (25 ml/min) at 18 psi (cold). GC Attenuation was set at 64, temperature rise at 5 °C/min. Starting temperature was 150 °C and final temperature was 210 °C. Integrator settings were: chart speed: 0.5 cm/min, injector temperature: 230 °C, detector temperature: 240 °C. Method was completed in 25 minutes.

An aliquot (1 µl) of the esterified sample was injected into the gas chromatograph using an analytical syringe (Scientific Glass Engineering). The syringe was cleaned in between samples using 2-octanol. The column was conditioned after every batch of samples was injected by setting the oven temperature at 225 °C overnight. The carrier gas flow was maintained at all times at 15 ml/min. Identification of the sample fatty acids was then made by comparison of the relative retention times of the fatty acid methyl ester (FAME) peaks from the samples with those of the standard mixture (Table 12). The peaks of fatty acids with chains longer than C22:1 were identified by comparing chromatograms with those obtained using the ARC and MRC chromatographs (Table 12).

5.2.3.3 Standard for composition analysis

A mixed standard solution containing methyl esters of the fatty acids to be detected (C13:0 – C22:1) was prepared by combining 3 Sigma standards ("stock solution" Nu-Chek-Prep. Inc., Elysim, Minnesota, USA) (Table 12). This standard (1 µl) was injected into the gas chromatograph in order to determine and check the retention times of the different fatty acids. The standard solution was injected into the gas chromatograph every day, before any samples were injected.

Table 12: List of fatty acids in standard mixture used for qualitative analysis (University of Pretoria).

Fatty acid	% in standard mixture	Retention time
C13:0	~ 7.00	6:85
C14:0	~ 7.00	7:35
C15:0	~ 7.00	8:70
C16:0	~ 13.30	10:00
C16:1	~ 8.23	10:80
C17:0	~ 7.00	11:25
C18:0	~ 7.00	12:50
C18:1	~ 14.90	13:20
C20:0	~ 7.00	15:30
C18:3	~ 7.00	15:90
C20:1	~ 8.23	17:00
C22:1	~ 8.60	19:45
C22:5	Compared to ARC and MRC retention times	21:18
C22:6		22:50

5.2.3.4 Preparation of fatty acid methyl esters (FAMEs) for quantitative analysis

Due to the small amount of available tissue, only 13 samples from late season animals (5 calves, 8 adults) were left for quantitative analysis, after the extraction process for fatty acid composition was carried out. This was undertaken at the Medical Research Council, Cape Town, using accepted Association of Official Analytical Chemists (1984) methodology and procedures.

5.2.3.5 Settings and specifications of the gas chromatograph column for quantitative analysis

The gas chromatograph used was a Varian model 3300, equipped with flame ionization detection, using a 30 m fused silica megabore DB-225 columns (ID 0.53 mm) (J&W Scientific, Folsom, CA). Gas flow rates were: H_2 at 25 ml/min; O_2 at 250 ml/min; Carrier gas: Hydrogen at 5-8 ml/min. Temperature programming was linear

at 4 °C/min, initial temperature 160 °C, final temperature 220 °C, injector temperature 240 °C and detector temperature 250 °C. A mixed standard solution containing methyl esters of the fatty acids to be detected was prepared (Nu-Chek-Prep, Inc. Elysian, Minnesota, U.S.A) (Table 13) and injected into the gas chromatograph every day in order to correlate retention times. The sample solutions were spiked with a known concentration of a C17:0 standard in order to determine the ratio of peak area to weight.

5.2.4 Data Analysis

Data were recorded as a listing of the various proportions (peak areas) of the total area covered by individual long-chain fatty acids in each sample (w/w %), with gravimetric data being expressed as g/100 g. Fatty acids were classified into saturated (SFA, no double bonds), monounsaturated (MUFA, 1 double bond) and polyunsaturated (PUFA, more than one double bond.

5.2.5 Statistical Analysis

Fatty acid (FA) proportions are reported herein as the percentages by wet weight of the total blubber sample. In general, non-parametric tests were used for analysis of percentage data. Statistical comparisons of FA between age groups and across seasons, were analysed using the Student's t-test for parametric data and Mann-Whitney test for non-parametric data. The Kruskall-Wallis One Way ANOVA on ranks test was used for comparisons of proportions across seasons, and, together with One-Way ANOVA, to compare the fatty acid groups of positional samples. Significant differences are quoted at the $P < 0.05$ level. Analyses were performed using SigmaStat (Version 2.0, Jandel Scientific) for Windows.

5.2.6 Proximate analysis on faecal sample (AOAC, 1984)

A sample of faeces from a southern right whale was collected in 1992 by R. B. Abernethy in Stompneus Bay along the west coast of South Africa. Feeding

Table 13: List of fatty acids in standard mixture used for quantitative analysis (MRC).

Fatty acid	**% in standard mixture**	**Retention time (minutes)**
C14:0	3.85	5:10
C16:0	3.85	7:69
C16:1	3.85	8:05
C17:0	3.85	9:20
C18:0	3.85	10:78
C18:1	3.85	11:08
C18:1	3.85	11:18
C18:2	3.85	11:67
C18:3n6	3.85	11:98
C18:3n3	3.85	12:45
C20:0	3.85	14:02
C20:1	3.85	14:33
C20:2	3.85	14:86
C20:3	3.85	15:29
C20:3	3.85	15:81
C22:0	3.85	17:65
C22:1	3.85	18:10
C20:4	3.85	15:46
C20:5	3.85	16:36
C22:2	3.85	19:07
C22:3	3.85	20:41
C22:4	3.85	20:01
C22:5	3.85	21:48
C22:6	3.85	21:75
C24:0	3.85	23:21
C24:1	3.85	23:99

behaviour was recorded at the same location. The sample was frozen soon after collection.

In order to do crude fat analysis on the faecal sample, the frozen sample was dried for 72 hours at 230 °C. The sample was then ground and placed in a glass bottle in a dessicator until required.

5.2.6.1 Determination of crude fat (ether extract)

Approximately 3 g of dried, ground faecal sample was placed into a Soxhlet extraction flask with approximately 175 ml of petroleum-ether (60 °C - 80 °C boiling point) and heated in a Soxhlet apparatus for 8 hours. After the ether had completely evaporated the flask was placed overnight in an oven at 70 °C. The flask was then placed in a dessicator to cool down and then weighed. The difference between the weight of the flask before and after the extraction was the weight of the crude fat.

5.2.6.2. Determination of crude protein (Macro Kjeldahl method)

An aliquot (1 - 2 g) of wet faeces was weighed out in a Kjeldahl flask (750 ml). Three glass beads were placed in the flask and then 10 g Na_2SO_4 and 0.4 g Selenium was added. After 25 ml concentrated H_2SO_4 (98%) was slowly added to the solution, the flask was gently swirled to make sure that nothing stuck to the sides of the flask. The flask was placed in an upright position on the Kjeldahl rack and the hot plates and extractor fan were switched on. The solution was boiled rapidly until clear in colour and then left for an additional half an hour. Boracic acid (35 ml) and indicator were put into a 500 ml Erlenmeyer flask and placed under the tubes of the distillation apparatus.

The following was added to the Kjeldahl flask:

a) 350 ml distilled water

b) 2 Zn balls (approximately 3 mm in circumference)

c) ± 100 ml 45 % NaOH (to ensure alkalinity).

Once the above were added, the cooling water of the apparatus was switched on and the contents of the Kjeldahl flask boiled until there was 200 ml of solution in the Erlenmeyer flask.

The titration value was corrected with a blank reading and an automatic buret was used to determine the titration value.

$$\% N = \frac{F \times (\text{Titration value} - \text{Blank})}{\text{Sample weight,}}$$

Where F = 0.1432
Blank = 0.01

% crude protein = % N x 6.25

5.2.6.3 Determination of dry matter

A dry, clean crucible was placed in an oven for 1 hour at 100 °C. The crucible was then removed and placed in a dessicator for at least half an hour. The crucible was weighed and a sample of approximately 1 g wet faeces was placed in the crucible and the exact weight recorded. The dry crucible with sample was placed in a dessicator for 18-24 hours at 100 °C (until weight remained constant) and then left to cool in a dessicator for at least half an hour and re-weighed. Dry mass was obtained by subtracting the crucible weight from the weight of the crucible with sample after being in the oven. Percentage dry matter was obtained by dividing the dry mass by the initial sample weight and multiplying by 100.

5.2.6.4 Ash Determination

The crucible with sample (from dry matter determination) was placed in a cold ashing oven, set at 600 °C, for 4 hours. The crucible was left in the oven and allowed to cool down for at least 2 hours and then placed in a dessicator and allowed to cool for a further half-hour. The crucible with sample was weighed and the crucible weight was subtracted to obtain weight of ash. Percentage of ash in the sample was obtained by dividing the weight of the ash by the weight of the air-dried sample and multiplying by 100.

5.3 Results

5.3.1 Fatty acid composition of southern right whale blubber

Thirteen major fatty acids were identified in all southern right whale blubber samples (Figure 8). C13:0 was the shortest fatty acid chain and C22:6 was the longest fatty acid chain detected. The monounsaturated fatty acids (MUFA) seemed to predominate, with C18:1 making up almost 30% of the total fatty acid composition in all individuals, while the long-chained polyunsaturated fatty acids (PUFA), C22:1, C22:5 and C22:6, seemed to contribute the least to the total fatty acid proportions.

Methodological restraints, however, probably resulted in the non-separation of some short and long-chained fatty acids. Comparing the results of the total fatty acid content (Section 5.3.3) with the results for the combined composition analysis indicated that the unidentified peaks detected made up 6.53 $\pm$ 0.237 percent (mean $\pm$ S.E.) of the total fatty acid content of calf blubber (n=7) and 6.20 $\pm$ 1.301 percent (mean $\pm$ S.E.) of adult blubber. The difference between these proportions was not significant (p=0.628).

5.3.1.1 Effect of position on body

The proportions of saturated fatty acids (SFA), MUFA and PUFA found in positions 1 to 5 from samples collected along the mid-dorsal, mid-ventral and lateral planes of stranded neonatal southern right whales were compared between planes and between positions. No significant differences were detected between any of the planes or positions ($p > 0.05$), however the general patterns of these results are described below.

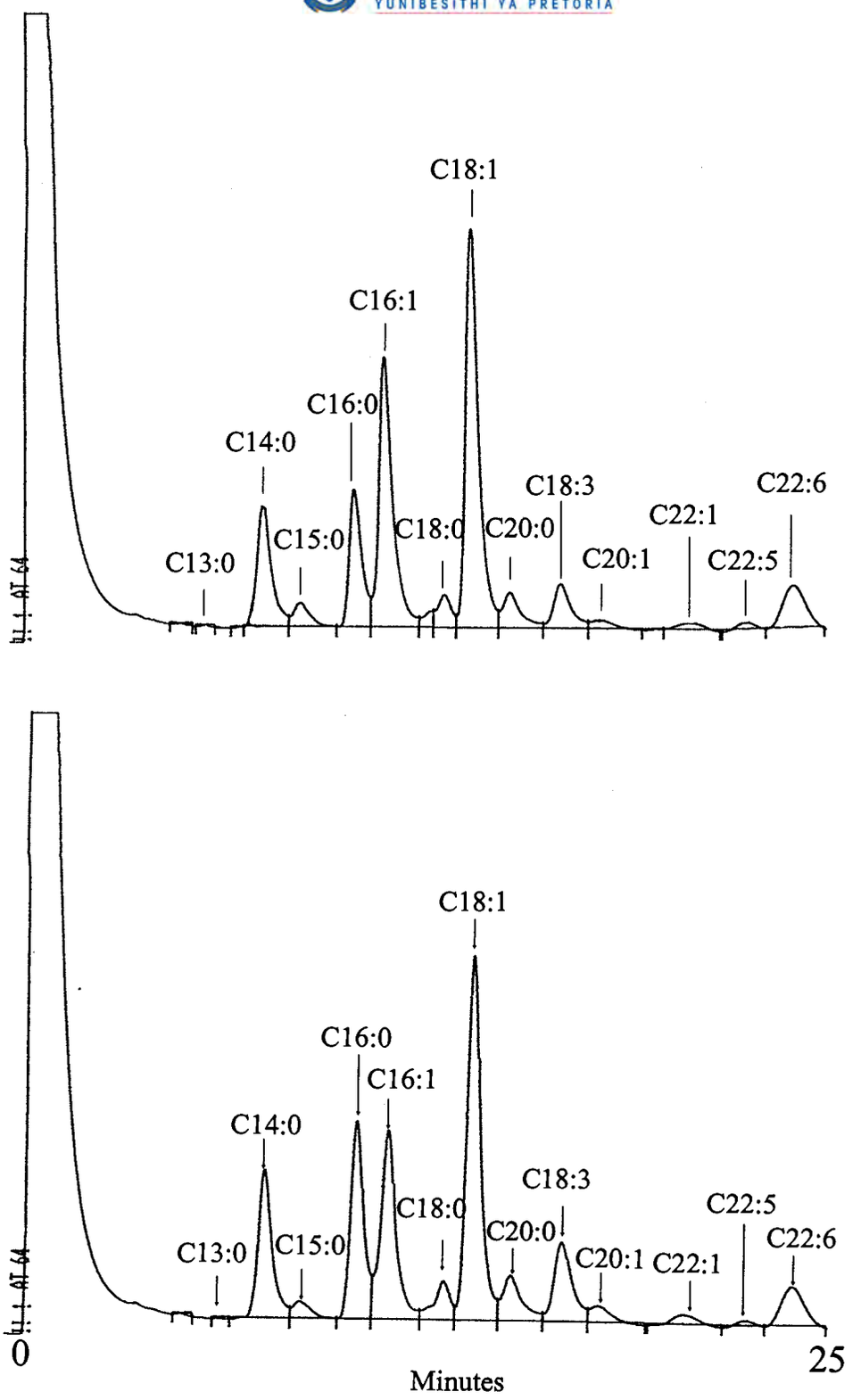

Figure 8: Chromatograms showing the fatty acid profile from the blubber of a southern right whale calf (# 30, top) and mother (# 29, bottom) pair indicating the 13 major fatty acids detected using gas chromatography.

Neonates (n = 4):

A similar fatty acid composition was observed at all positions (1 to 5) along the mid-dorsal plane (Figure 9). C22:6 was absent from samples of positions 2 and 3, while C20:1 was absent from samples of position 1. Small proportions of C17:0 in position 3 and C15:0 in position 1 were noted. The proportion of SFAs increased from position 1 to 4 (mainly due to high amount of C14:0) and then decreased around the peduncle area (position 5) (Figure 10). The distribution of MUFA was fairly uniform with the highest proportions recorded in the peduncle area (Figure 10). C18:1 was the highest of the MUFA in all samples. Compared to the SFA and MUFA, there were far less PUFA found in the mid-dorsal samples of neonates. A very high proportion of C18:3 in a sample from one neonate (26.81%) accounted for the very high proportions of PUFA in position 1, with position 5 having the second highest proportion of PUFA (Figure 10). C17:1, C22:1 and C22:5 were absent in all positions along the mid-dorsal plane (Figure 9).

Along the midventral plane, identified fatty acids were generally present in all positions (1,2,4,5 - Figure 11) except for C15:0 in positions 2 and 5, and the absence of C22:6 in position 2. C17:0, C17:1, C22:1 and C22:5 were absent in all positions. SFA were highest in position 1 due to high amounts of C14:0. SFA tended to decrease towards the middle of the body and then increased in the peduncle area, unlike the MUFA which were higher in the middle regions (Figure 12). Position 2 had the highest amount of MUFA due to high values of C18:1 in this position. C18:1 was the predominant MUFA in all positions. A non-significant increase in the amount of PUFA was noted in a cranio-caudal direction, with C18:3 levels being highest in position 5 (Figure 11).

C15:0, C22:1 and C22:5 were only detected in position 4 and C22:6 was present only in position 5 of the lateral samples from positions 2 to 5 (Figure 13). Marked amounts of SFA were recorded in position 2 due to very high C14:0 values (48.4%). SFA tended to decrease towards the middle and lower back but increased in the peduncle region (Figure 14). MUFA again showed the opposite, being high in the mid-lower regions of the body (Figure 14). Position 3 had the highest amount of MUFA

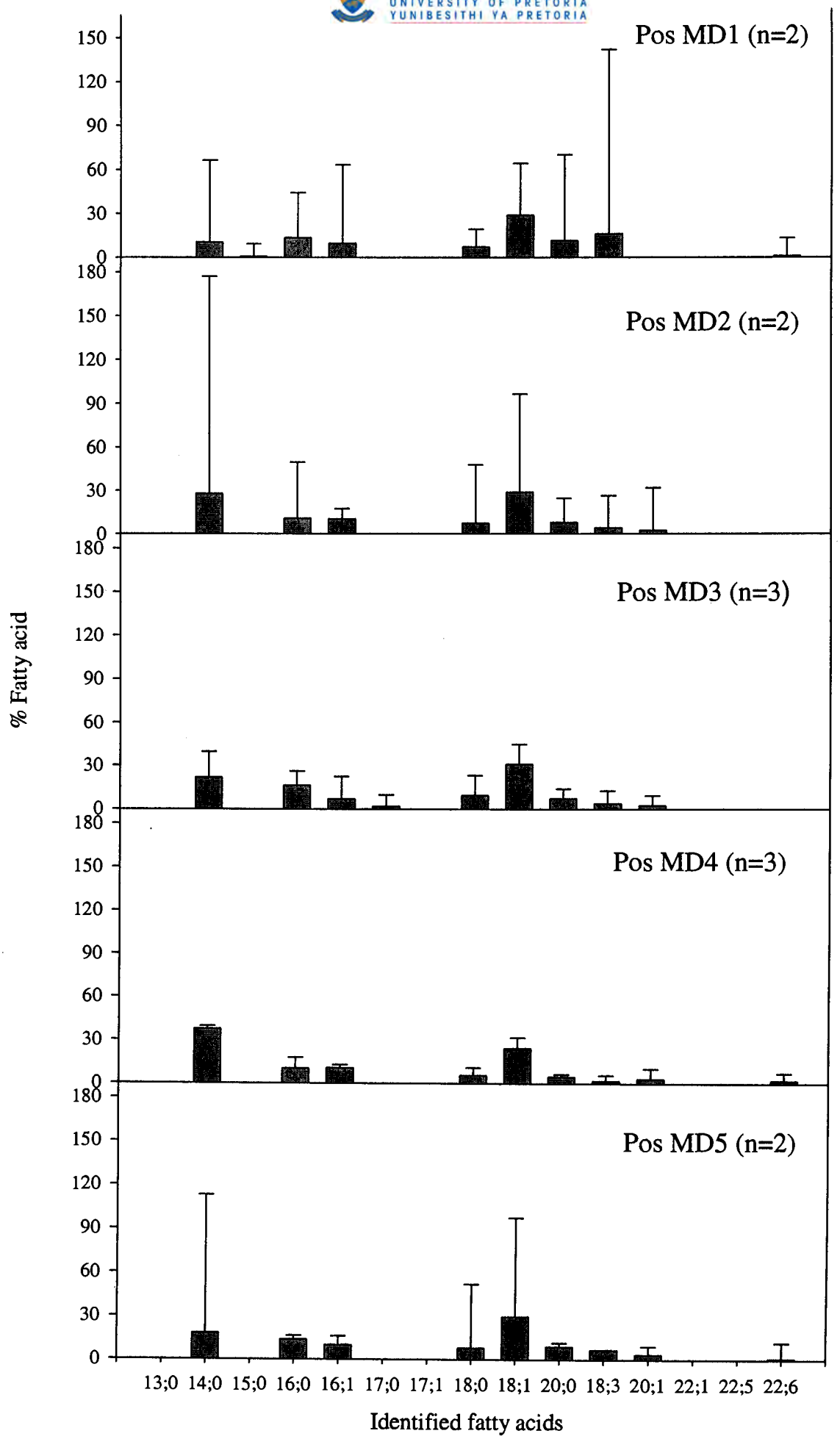

Figure 9: Proportions of fatty acids in positions 1-5 on the mid-dorsal plane along the bodies of neonatal southern right whales (mean ± S.E.).

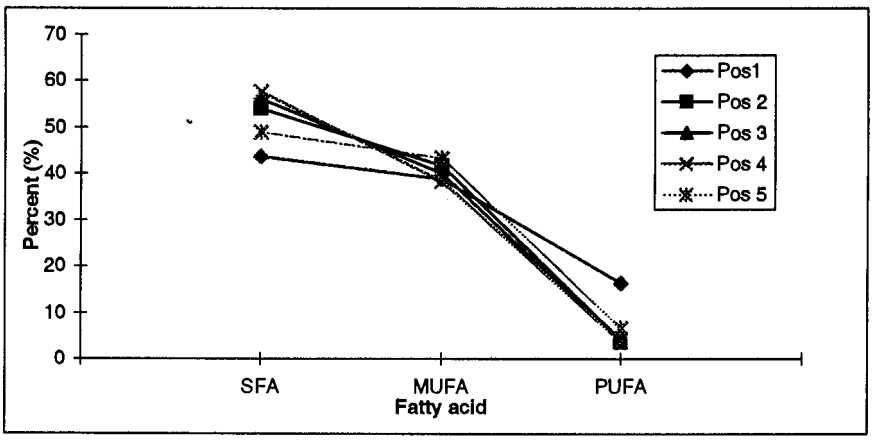

Figure 10: Proportions of fatty acids on the mid-dorsal plane along the body of a male neonatal southern right whale.

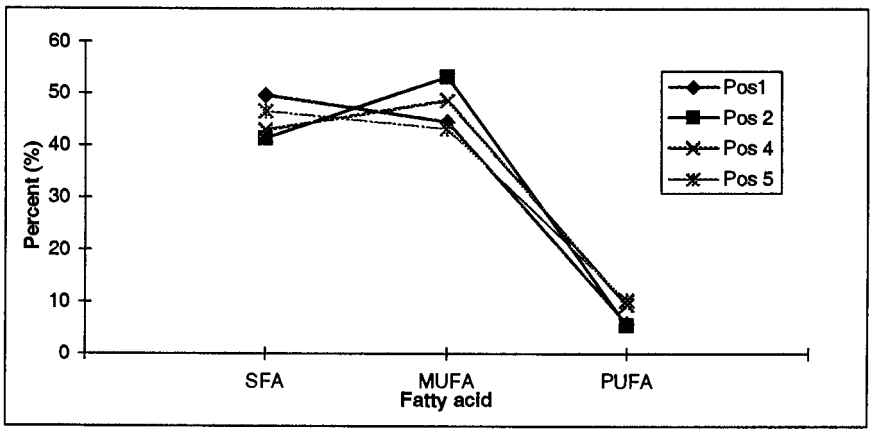

Figure 12: Proportions of fatty acids on the mid-ventral plane along the body of a male neonatal southern right whale.

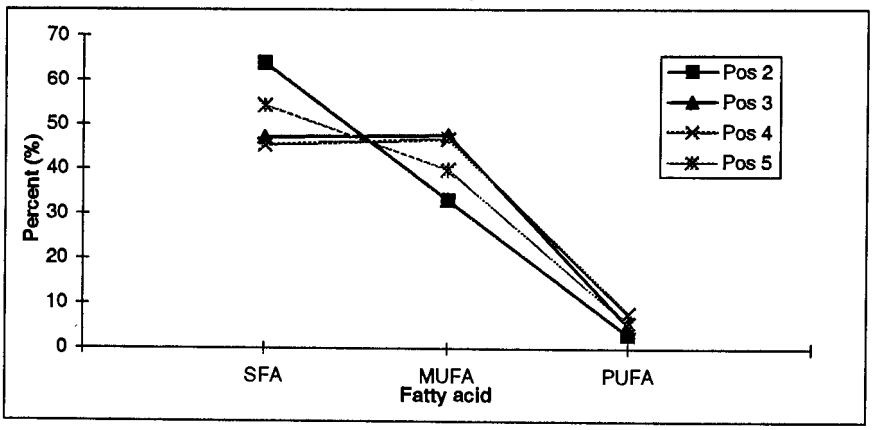

Figure 14: Proportions of fatty acids on the lateral plane along the body of a male neonatal southern right whale.

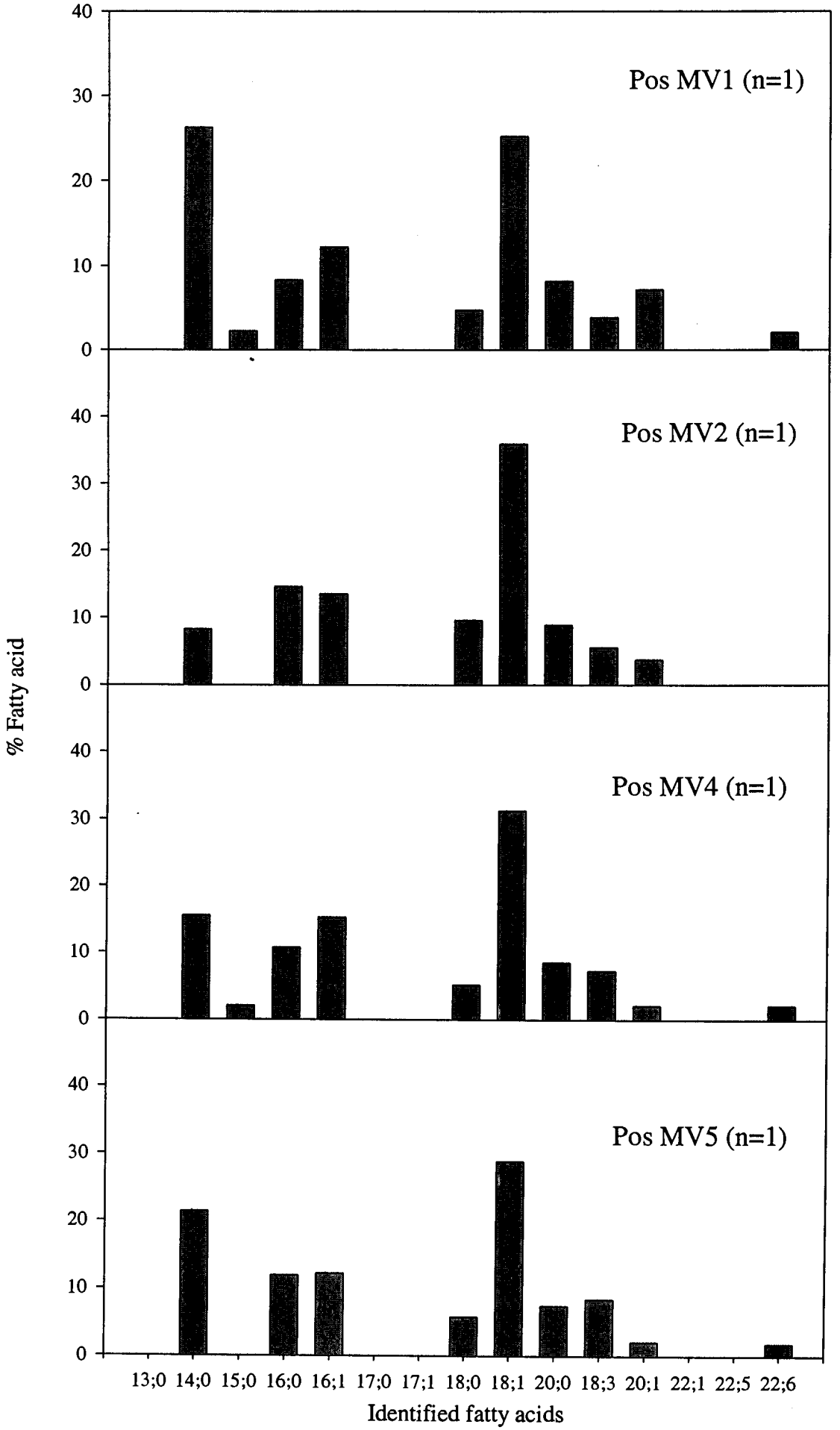

Figure 11: Proportions of fatty acids in positions 1,2,4 and 5 along the mid-ventral plane of a neonatal southern right whale.

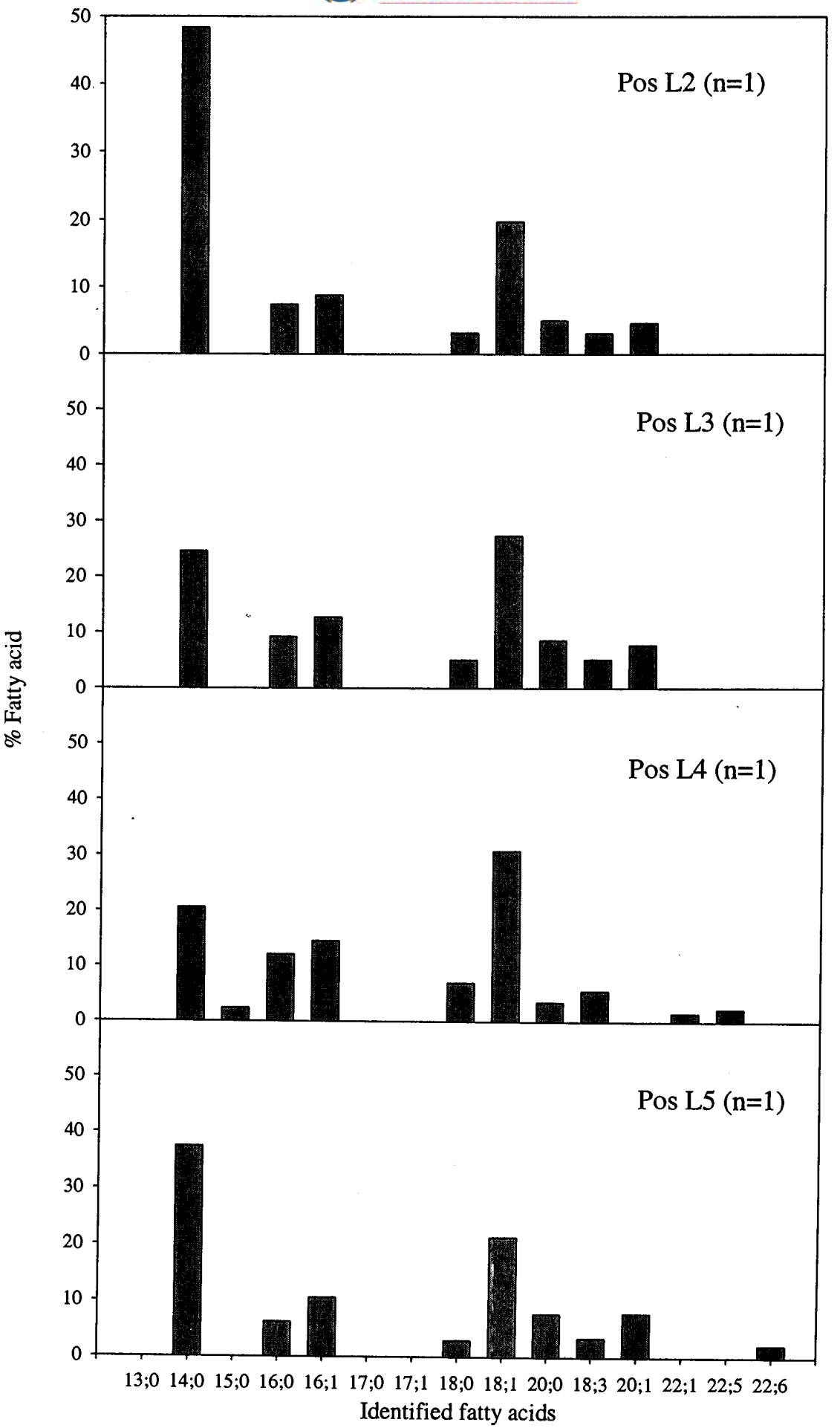

Figure 13: Proportions of fatty acids in positions 2-5 along the lateral plane of a neonatal southern right whale.

due to higher amounts of C20:1, this fatty acid was absent in position 4. PUFA values were lowest in position 2, but highest in the lower back region mainly due to C18:3 values.

Juvenile (n = 1):

No statistical test was possible because of the sample size, but the general trends are discussed below.

Lateral samples from positions 1 to 5 showed C22:5 and C22:6 to be present in all positions (Figure 15). SFA were highest in position 5 which had at least three times the amount of C14:0 than any other position and there was generally more SFA detected in the caudal regions of the animals body (positions 4 and 5) (Figure 16). MUFA seemed evenly distributed along the body, decreasing only in the peduncle region. Higher PUFA values were detected in the middle of the body, with the lowest proportions in the peduncle area (Figure 16). C22:6 values were consistently higher than C22:5 values at all positions along the lateral plane.

Subadult (n = 1):

No statistical test was possible because of the sample size, but the general trends are discussed below.

C22:5 was absent in positions 4 and 5 along the dorso-lateral plane (positions 1 to 5 - Figure 17). SFA seemed to be higher in the mid and lower regions of the animals body, the highest values recorded in position 3 (high amounts of C14:0 (20.8%)) and lowest values recorded in the peduncle area (Figure 18). MUFA levels were highest in the peduncle area as a result of high C16:1 (20.3%) values, however, MUFA were generally higher in the cranial regions, decreasing towards the caudal regions. PUFA

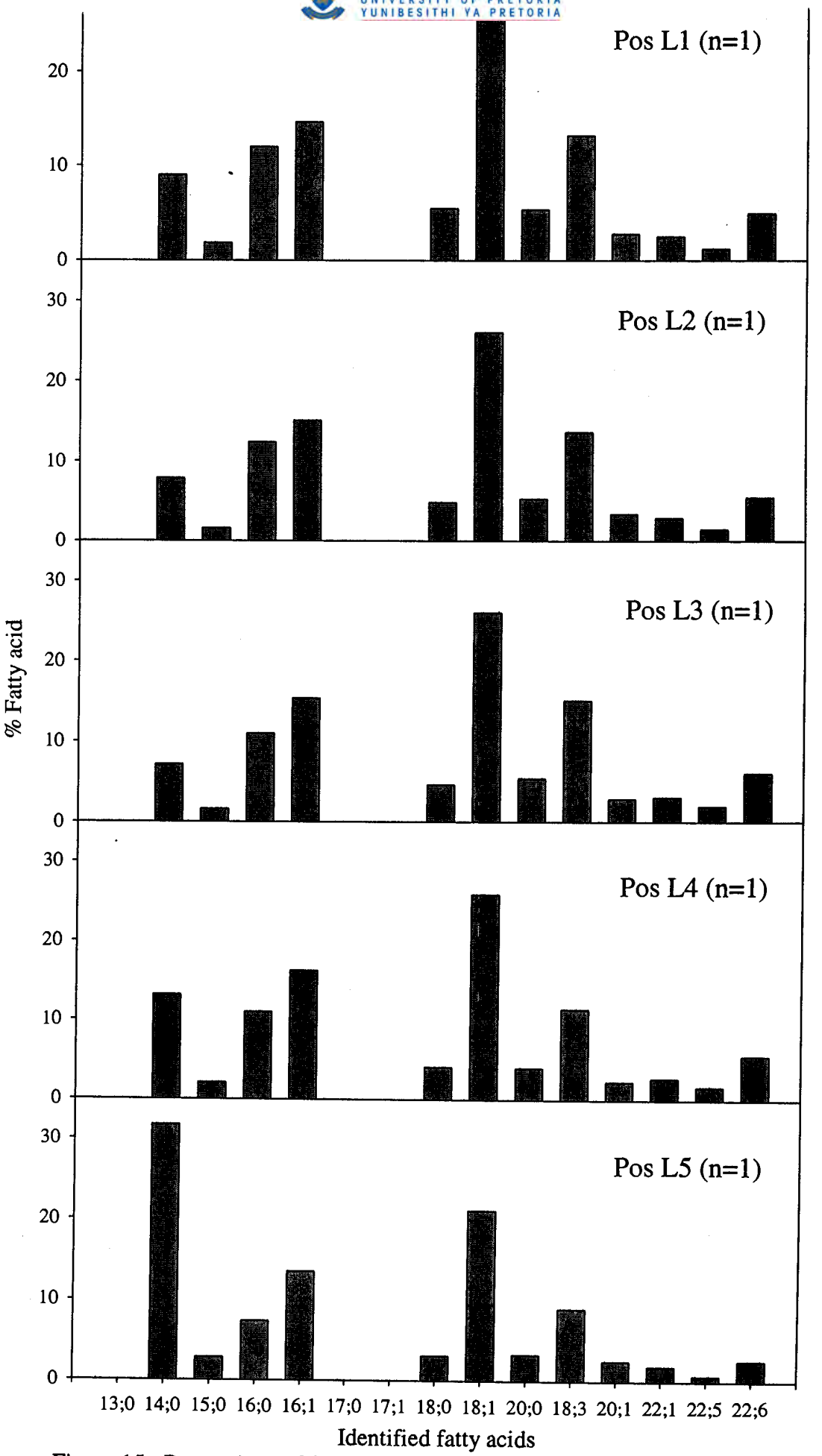

Figure 15: Proportions of fatty acids in positions 1-5 along the lateral plane of a juvenile southern right whale.

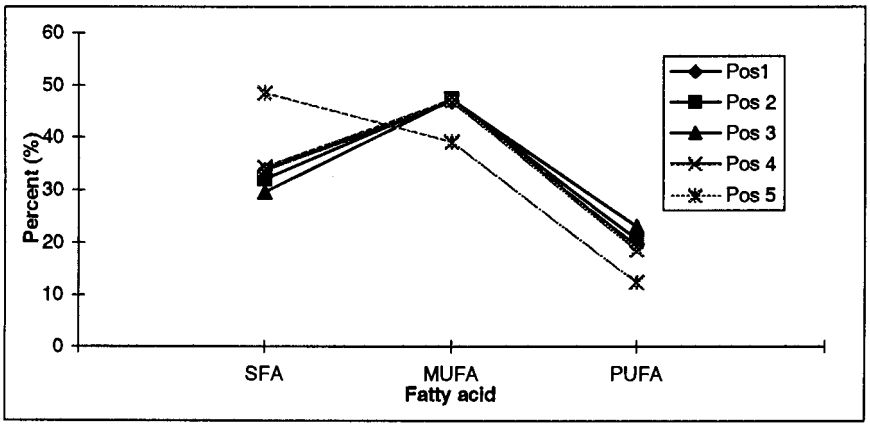

Figure 16: Proportions of fatty acids on the lateral plane along the body of a juvenile male southern right whale.

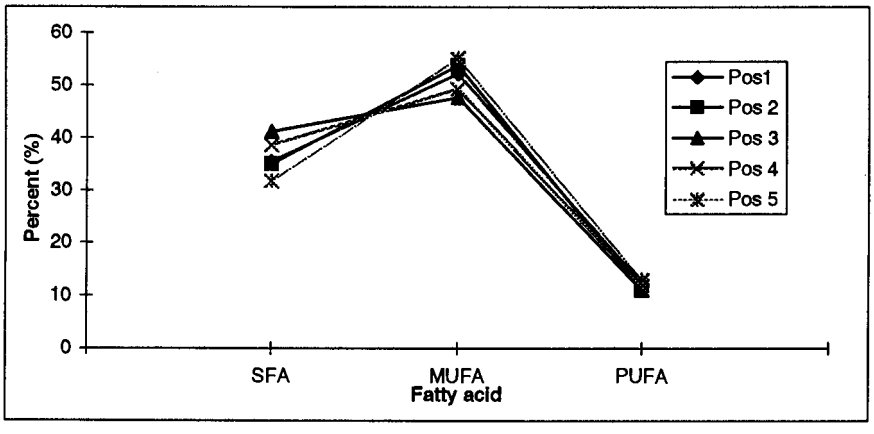

Figure 18: Proportions of fatty acids on the dorso-lateral plane along the body of a subadult male southern right whale.

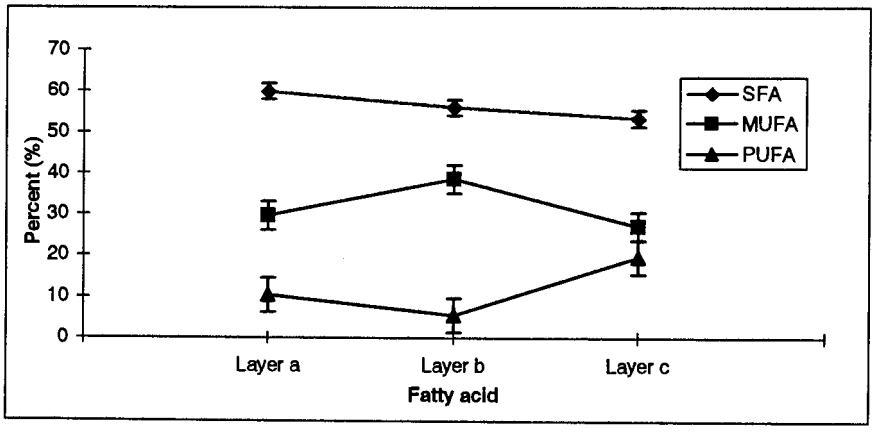

Figure 19: Average proportions of fatty acids in the layers of mid-dorsal position 3 samples from 4 neonatal southern right whales.

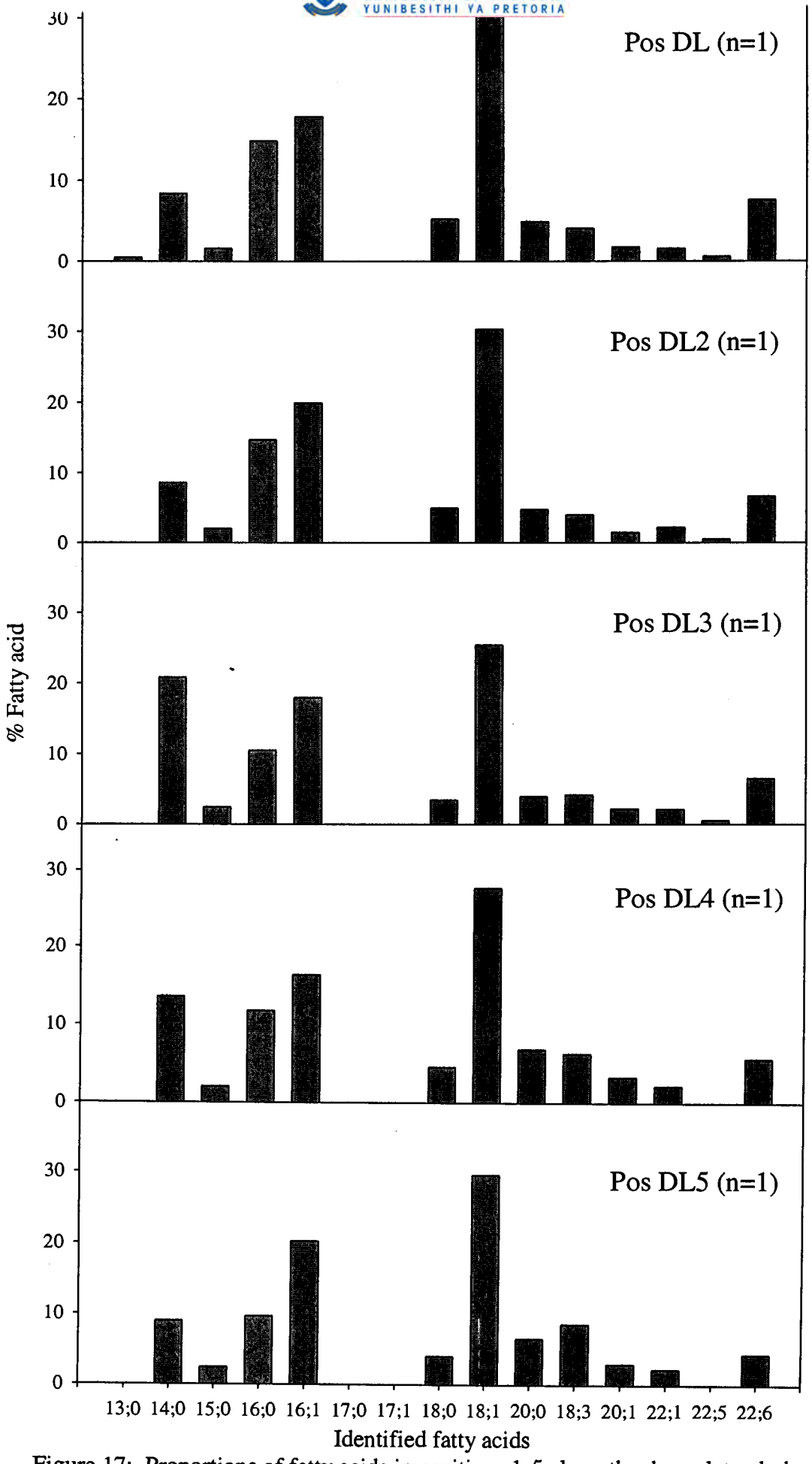

Figure 17: Proportions of fatty acids in positions 1-5 along the dorso-lateral plane of a subadult southern right whale.

values were marginally highest in position 5 and lowest in the lower anterior and middle regions (positions 2 and 3).

5.3.1.2 Effect of layer in blubber

The proportions of SFA, MUFA and PUFA found in blubber layers a to c from samples collected along the mid-dorsal, mid-ventral and lateral planes of stranded neonatal southern right whales, were compared between planes and between positions 1 to 5. No significant differences were detected between any of the planes, positions or layers ($p > 0.05$), however the general patterns of these results are discussed below.

Neonates (n=4):

Mid-dorsal samples from position 3, layers a to c (Figure 19) revealed that SFA decreased from outermost to innermost layer, with high C14:0 values in layers a (36.1 %) and c (31.9 %). MUFA proportions were highest in layer b and lowest in layer c. PUFA were twice as high in layer a and three times higher in layer c, compared to layer b. High PUFA values in layer c was due to a very high proportion of C18:3 (48.37 %) in one neonate. A small proportion of C15:0 was present in layer a and C17:0 was present in layer b only. C22:6 was absent in layer b and C13:0 was absent in layer c. C22:1 and C22:5 were not detected in any of the layers.

Juvenile (n=1):

Lateral samples from position 1 to 5 showed that SFA proportions tended to increase with depth of layer (Figure 20). C14:0, C16:0 and C18:0 were the fatty acids that influenced these results. C13:0 was only detected in layer c of position 1 and in layer a of position 4 C16:1 and C20: were primarily responsible for layer b containing the highest proportions

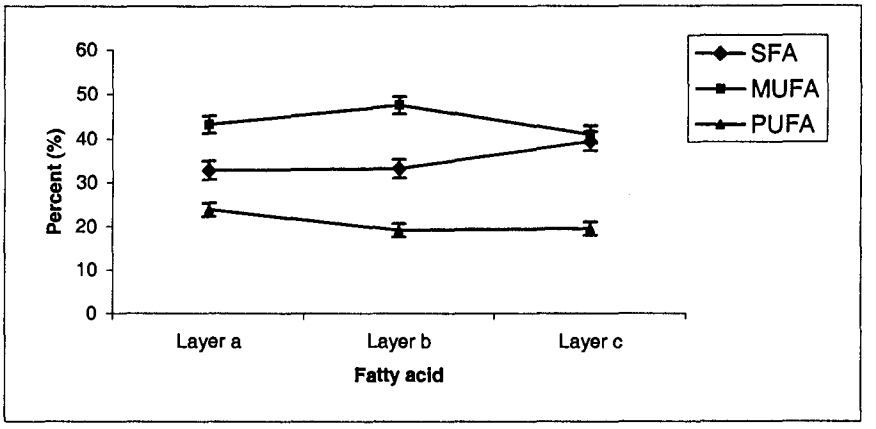

Figure 20: Average proportions of fatty acids in the layers of lateral positions 1-5 from a juvenile southern right whale.

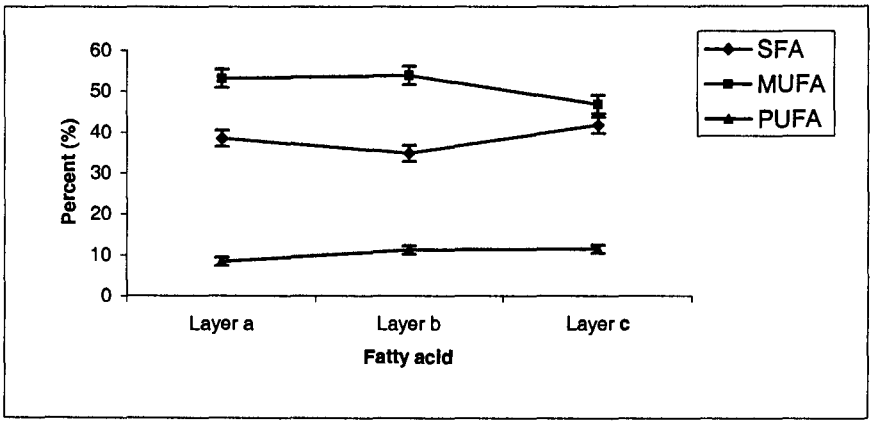

Figure 21: Average proportions of fatty acids in the layers of dorso-lateral positions 1-5 from a subadult southern right whale.

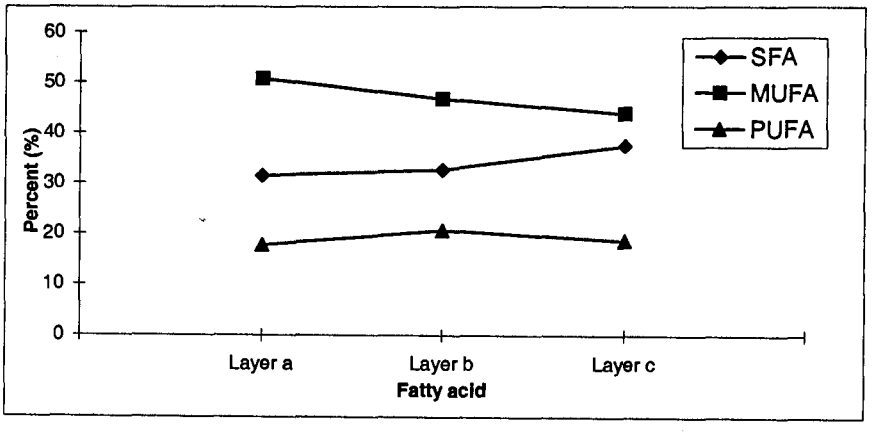

Figure 22: Proportions of fatty acids in the layers of a mid-dorsal position 3 sample from an adult male southern right whale.

of MUFA. The lowest proportions were detected in layer c, probably due to the lack of C20:1 and C22:1 in layer c of position 5. C20:1 was also absent in layer a, from position 3. PUFA were highest in layer a, decreased in layer b and increased slightly in layer c. The high proportions of PUFA in layer a are related to the proportion of C18:3 in layer a and of position 2 (29.6 %) and position 4. No C22:5 was detected in layer c of position 5.

Subadult (n=1):

SFA proportions were highest in layer c and lowest in layer b with high proportions of C16:0 and C14:0 in positions 2 and 4, respectively (Figure 21). C13:0 was only present in layer c. Layer b contained marginally higher MUFA values than those of layer a, with layer c composing the least MUFA. Very high values of C18:1 (~30%) were detected in all layers of position 1 and proportions of C16:1, C18:1 and C22:1 from position 2 further influenced the high MUFA values in layer b. PUFA increased with depth of layer, layer c having the highest proportions of C18:3. C22:5 was not present in layer a, position 1 and in layer b, position 3. C22:6 levels were generally high in this animal (5-8%) compared to the other age groups.

Adult (n=1):

A single mid-dorsal sample taken from an adult male right whale, at position 3 (layers a to c) (Figure 22) revealed that SFA increased with depth of layer due predominantly to C16:0 levels. MUFA decreased with depth of layer, with high proportions of C16:1 and C18:1 in layer a. PUFA were highest in layer b and lowest in layer a.

While results obtained when comparing positional layer data were non-significant, it should be stressed that sample sizes were small and mostly taken from neonates, where one might not expect much stratification to have taken place. As they stand, however, these base-line data seem to indicate that blubber samples obtained using biopsy techniques, and taken anywhere along the mid-dorsal surface of southern right whales, would be representative of the general fatty acid composition of the animals' blubber. This conclusion would be stronger if the samples taken were deep in nature,

as in this study. The following section, in which biopsies taken from early season and late season cows and calves are compared, proceeds on this assumption.

5.3.1.3 Age and seasonal effects on the proportions of fatty acids

The saturated fatty acid, C17:0, was only detected in some neonatal samples and not in samples from any of the other age classes.

C15:0 ($p < 0.0001$), 16:1 ($p = 0.003$), C22:1 ($p = 0.004$), C22:5 ($p = 0.030$) and C22:6 ($p < 0.0001$) were significantly higher in early season calves compared to neonates, while C18:0 ($p = 0.009$) and C20:0 ($p = 0.032$) were significantly higher in neonates compared to early season calves (Table 12). C18:3 was higher in neonates compared to early season calves, however this difference was not significant ($p = 0.973$).

Early season cows showed a similar pattern relative to neonates, with significantly higher C15:0 ($p < 0.0001$), C16:1 ($p = 0.022$), C17:1 ($p = 0.038$), C22:1 ($p < 0.0001$), C22:5 ($p = 0.006$) and C22:6 ($p = 0.022$) values. Similarly, C18:0 ($p = 0.005$) was significantly higher in neonates compared to early season cows (Table 14).

Proportions of C15:0 ($p < 0.0001$), C17:1 ($p = 0.021$), C18:3 ($p = 0.0001$), C22:1 ($p = 0.0001$) and C22:5 ($p = 0.039$) were all significantly higher in early season cows compared to early season calves, while C16:1 ($p = 0.0003$), C18:0 ($p = 0.024$), C18:1 ($p = 0.001$) and C22:6 ($p = 0.002$) were all significantly higher in early season calves compared to early season cows (Table 14).

Seasonal differences between early season and late season calves can be seen in proportions of C14:0 and C15:0 which were significantly higher ($p = 0.013$ and $p = 0.025$, respectively) in early season calves than in late season calves, whereas C18:1 was significantly higher ($p = 0.005$) in late season calves compared to early season calves (Table 14).

Table 14: Mean proportions of fatty acids in seasonal and age groups, Neo = neonates, "Ec" = early season calves, "Ea" = early season adults, "Lc" = late season calves, "La" = late season adults (Mean % ± S.E.).

Fatty acid	Neo (n=6)	Ec (n=18)	Ea (n=15)	Lc (n=18)	La (n=11)
C13:0	0.104 ± 0.104	0.02 ± 0.02	0.00 ± 0.00	0.02 ± 0.018	0.06 ± 0.053
C14:0	19.1 ± 3.763	11.95 ± 0.703^{d}	12.60 ± 0.706	9.77 ± 0.443^{d,e}	11.53 ± 0.805^{e}
C15:0	0.472 ± 0.304^{a,b}	2.10 ± 0.071^{a,c,d}	2.56 ± 0.073^{b,c,f}	1.90 ± 0.052^{d,e}	2.24 ± 0.134^{e,f}
C16:0	13.1 ± 1.859	10.90 ± 0.341	10.80 ± 0.372	11.5 ± 0.273	11.60 ± 0.380
C16:1	7.82 ± 2.903^{a,b}	18.70 ± 0.422^{a,c}	16.30 ± 0.403^{b,c}	19.0 ± 0.311^{e}	16.60 ± 0.670^{e}
C17:0	1.34 ± 0.00	nd	nd	nd	nd
C17:1	nd	0.10 + 0.072^{c}	0.52 + 0.115^{c,f}	0.15 ± 0.082	0.04 ± 0.036^{f}
C18:0	8.04 ± 1.779^{a,b}	4.11 ± 0.154^{a,c}	3.44 ± 0.249^{b,c}	4.34 ± 0.203^{e}	3.68 ± 0.143^{e}
C18:1	27.7 ± 2.836	30.30 ± 0.596^{c,d}	27.30 ± 0.526^{c}	32.7 ± 0.495^{d,e}	28.90 ± 0.578^{e}
C20:0	8.23 ± 2.111^{a}	5.21 ± 0.352^{a}	5.41 ± 0.330	4.80 ± 0.245	4.96 ± 0.253
C18:3	8.92 ± 4.838^{a}	5.05 ± 0.317^{c}	10.94 ± 0.978^{a,c}	5.82 ± 0.672^{e}	10.47 ± 1.183^{e}
C20:1	3.35 ± 1.756	3.46 ± 0.757	2.27 ± 0.237	2.02 ± 0.272^{e}	3.01 ± 0.346^{e}
C22:1	0.110 ± 0.110^{a,b}	0.90 ± 0.102^{a,c}	2.18 ± 0.214^{a,c}	0.93 + 0.093^{e}	1.75 ± 0.213^{e}
C22:5	0.170 ± 0.17^{a,b}	0.79 ± 0.076^{a,c}	1.08 ± 0.114^{b,c}	0.86 ± 0.049	0.85 ± 0.113
C22:6	1.57 ± 1.574^{a,b}	6.35 ± 0.374^{a,c}	4.59 ± 0.341^{b,c}	6.16 ± 0.238^{e}	4.31 ± 0.332^{e}

nd = not detected
[a] significant differences between neonates and early season calves
[b] significant differences between neonates and early season cows
[c] significant differences between early season calves and early season cows
[d] significant differences between early season calves and late season calves
[e] significant differences between late season calves and late season cows
[f] significant differences between early season cows and late season cows

Late season cows showed significantly higher proportions of C14:0 ($p = 0.046$), C15:0 ($p = 0.037$), C18:3 ($p = 0.001$), C20:1 ($p = 0.013$) and C22:1 ($p < 0.0001$) compared to late season calves. However, proportions of C16:1 ($p = 0.002$), C18:0 ($p = 0.027$), C18:1 ($p < 0.0001$) and C22:6 ($p < 0.0001$) were significantly higher in late season calves compared to late season cows (Table 14).

Early season and late season adults showed significant differences in only two fatty acids, namely, C15:0 and C17:1 which were significantly higher ($p = 0.035$ and $p = 0.017$, respectively) in early season adults compared to late season adults (Table 14).

5.3.1.4 Seasonal trends in lipid composition between different age groups

Neonates contained very high proportions of C14:0, which accounted for the much higher SFA in this group compared to all other groups (Figures 23 and 24). However, compared to early and late season calves, neonates had lower proportions of MUFA (low proportions of C16:1 and C22:1) and PUFA (low proportions of C22:5 and C22:6). Calves and adults from both seasonal groups showed the same general trend with MUFA predominating and PUFA making up the least proportion of fatty acids (Figure 23). SFA were slightly higher in early season calves compared to late season calves that had slightly higher proportions of MUFA and PUFA, respectively (Figure 23). The same trend defined fatty acid proportions in early and late season adults, except for PUFA being slightly lower in late season adults (Figure 23).

A general decrease in the proportions of SFA was noted from neonates to late season calves (Figure 23). The differences between neonates and the two seasonal groups of calves were statistically significant, as were the differences between early and late season calves (Table 15). SFA levels remained fairly constant within adult samples and, although the early season cow SFA values were significantly less than that found in the neonates, the difference between early and late season cows was not significant (Table 15). Neonates possessed the lowest amounts of MUFA, which tended to increase from neonates to late season calves (Table 15). Early season calves had significantly higher amounts of MUFA than early season adults, whereas late season calves had significantly higher levels of MUFA than both seasonal groups of cows (Table 15). PUFA increased from neonates to late calves and correspondingly decreased from early to late season adults (Figure 23). Significant differences in this latter group of fatty acids were detected between early season cows and early and late season calves (Table 15).

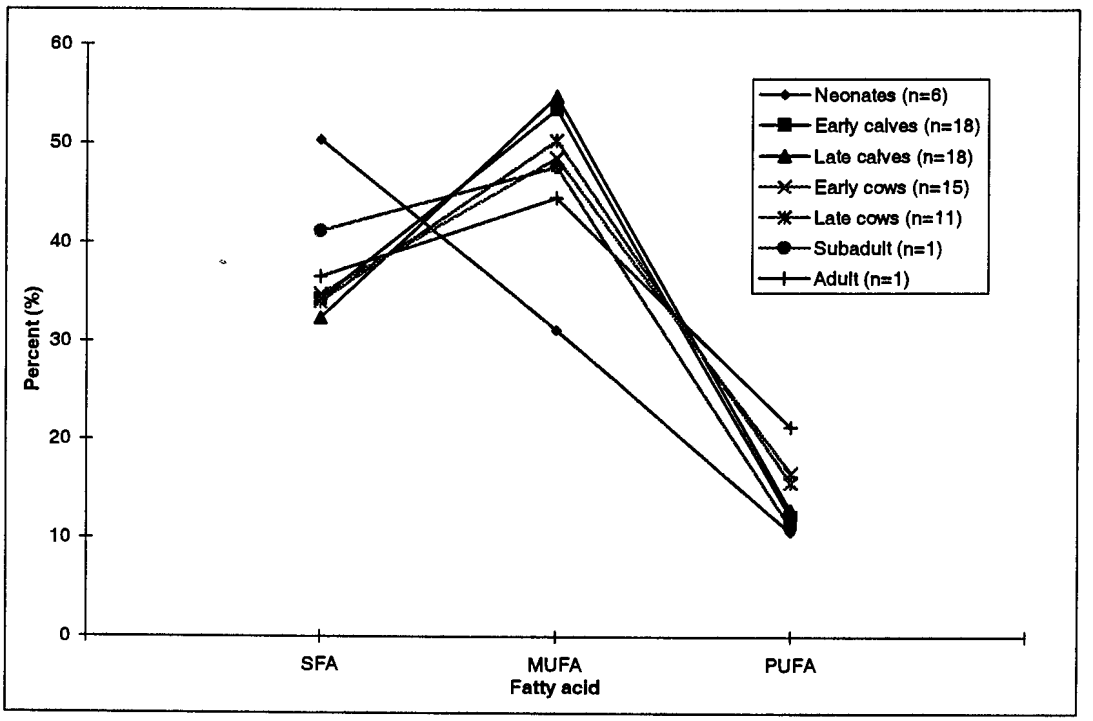

Figure 23: Proportions of saturated (SFA), Monounsaturated (MUFA) and Polyunsaturated (PUFA) fatty acids in various age and seasonal groups.

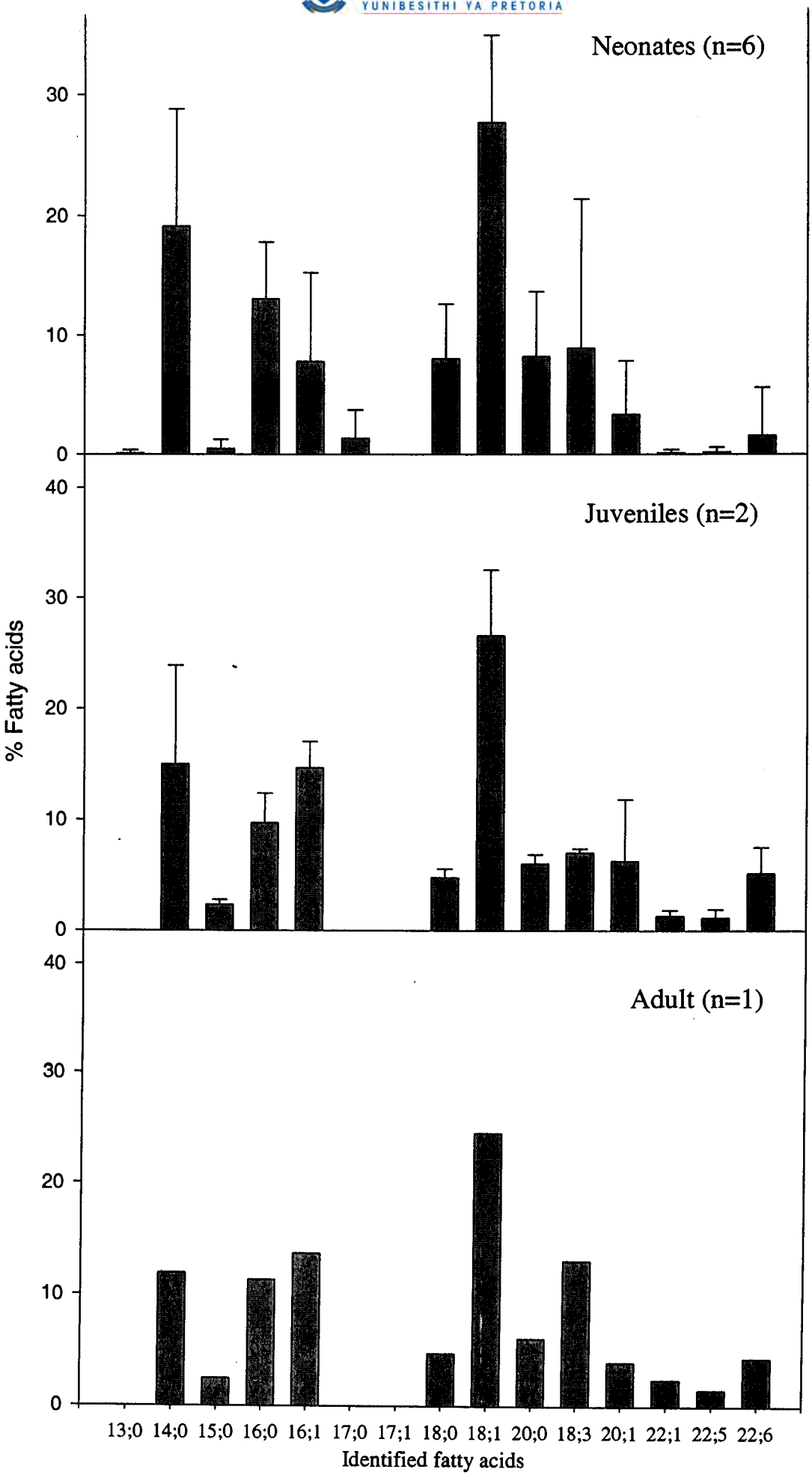

Figure 24: Proportions of fatty acids in various age groups of southern right whales (Mean ± S.E.).

Table 15: Mean proportions of lipid classes in seasonal and age groups (Mean % ± S.E.)

	Neonates MD3 (n=6)	Early calves (n=18)	Late calves (n=18)	Early cows (n=15)	Late cows (n=11)
SFA	50.39 ± 5.05[a,b,c]	34.31 ± 0.698[a,e]	32.37 ± 0.425[b,e,h]	34.81 ± 0.524[c]	34.06 ± 0.779[h]
MUFA	31.13 ± 2.166[a,b,c]	53.51 ± 0.472[a,d,e,f]	54.79 ± 0.399[b,e,h]	48.58 ± 0.662[c,d]	50.31 ± 0.971[f,h]
PUFA	10.66 ± 4.693	12.19 ± 0.511[d]	12.85 ± 0.658[g,h]	16.61 ± 0.801[d,g]	15.63 ± 1.256[h]

[a] significant differences between neonates and early season calves; [b] significant differences between neonates and late season calves; [c] significant differences between neonates and early season cows; [d] significant differences between early season calves and early season cows; [e] significant differences between early season calves and late season calves; [f] significant differences between early season calves and late season cows; [g] significant differences between late season calves and early season cows; [h] significant differences between late season calves and late season cows.

The single sample from a stranded subadult and an adult male showed the same arrangement of fatty acids, i.e. MUFAs, SFAs and PUFAs (Figure 23). The subadult possessed higher proportions of SFAs (due to C14:0 levels) when compared to both the adult male and late season adults. The subadult also had higher proportions of MUFAs (high C20:1 levels) compared to that of the adult male, but lower PUFAs (due to C18:3 levels) than both the adult male and late season adults (Figure 23).

5.3.2 A comparison between the fatty acid composition of North Pacific and southern right whales

There were marked differences in the fatty acid composition of the southern right whales documented here and those presented for a North Pacific right whale by Tsuyuki & Itoh (1970).

The major monounsaturated (monoenoic) fatty acid in the southern right whales was C18:1 as opposed to C20:1 in the North Pacific right whale (Table 16). C20:1 levels are markedly higher in the North Pacific right whale compared to the southern right whales, where C20:1 was only the nineth highest fatty acid. The second highest MUFA detected in southern right whales, C16:1, was also low in the North Pacific right whale sample. Major saturated fatty acids in both southern right whales and the North Pacific right whale were C14:0 and C16:0, although the proportions of these

SFA in the North Pacific right whale were much reduced compared to those found in southern right whales. PUFA were higher in the North Pacific right whale due to the large amount of C20:5 found in this animal, which was not detected at all in southern right whales (Table 16).

Table 16: Fatty acid compositions of blubber from a North Pacific right whale and southern right whales (expressed as average weight percent of total fatty acids).

Fatty acid	Adult male NPRW*	Adult male SRW	Subadult male SRW	SRW cows
C10:0	0.78	Nd	nd	nd
C12:0	0.19	Nd	nd	nd
C13:0	Trace	0	0	0.03 ± 0.023
C14:0	6.6	11.94	20.83	12.17 ± 0.532
C14:1	0.49	Nd	nd	nd
C14:2	0.17	Nd	nd	nd
C15:0	0.21	2.47	2.46	2.43 + 0.076
C16:0	8.85	11.33	10.52	11.11 + 0.275
C16:1	4.05	13.7	17.98	16.42 + 0.36
C16:2	1.16	Nd	nd	nd
C17:0	1.37	0	0	0
C17:1	Nd	0	0	0.31 + 0.082
C18:0	2.93	4.73	3.44	3.54 + 0.155
C18:1	17.79	24.5	25.4	28.00 + 0.413
C18:2	1.96	Nd	nd	nd
C18:3	3.12	13.1	4.17	10.74 + 0.740
C19:0	1.01	Nd	nd	nd
C20:0	2.56	6.1	3.94	5.22 + 0.219
C20:1	21.44	3.96	2.18	2.58 + .209
C20:2	0.82	Nd	nd	Nd
C20:3	0.73	Nd	nd	Nd
C20:4	1.46	Nd	nd	Nd
C20:5	16.65	Nd	nd	Nd
C22:1	1.14	2.39	2.12	2.00 + 0.156
C22:5	1.68	1.45	0.51	0.98 + 0.083
C22:6	2.84	4.33	6.45	4.47 + 0.239

NPRW* North Pacific right whale studied by Tsuyuki & Itoh, 1970
SRW = southern right whale
nd = not detected

Comparing North Pacific right whale results to the two stranded southern right whale males (Table 15), the southern right whales generally seemed to possess higher amounts of all the SFA, MUFA values were comparable between the southern and North Pacific right whale adults, while the North Pacific right whale had higher PUFA values than both the southern right whale males.

Excluding neonatal samples, all southern right whale samples, MUFAs made up the highest proportions of the TAG, followed by SFA and then PUFA, while, in the North Pacific right whale PUFA were higher than SFA.

5.3.3 Quantitative fatty acid analysis

Apart from one comparatively small (0.54 g) sample from a cow (# 74), that did not represent all blubber layers, the average total fatty acid content for late season calves (n = 5) was 41.2 % and for late season adults (n = 7) was 42.9 %, the differences were not significant ($p > 0.05$) (Table 17). Predictably, the short sample obtained from a cow (# 74) contained the lowest total fatty acid value (Table 17). Omitting sample # 74, linear regressions, used to determine whether there was any correlation between length of sample and total fatty acid content for both cows and calves, revealed no correlation between these variables (cows: $p = 0.773$; calves: $p = 0.689$).

Table 17: Total fatty acid values from late season southern right whale cows and calves, biopsied along the South African coast (AOAC, 1984).

Sample #	Date of collection	Age	Sample wt (g)	Sample length (cm)	Total fatty acid (g/100g)	Cow/calf pr
73	01/11/00	Calf	1.2	12.7	44.68	1
76	01/11/00	Calf	1.86	9.7	38.97	
80	02/11/00	Calf	0.45	5.6	50.51	2
87	08/11/00	Calf	1.00	8.4	36.06	3
90	10/11/00	Calf	0.95	8.0	35.73	4
74	01/11/00	Cow	0.54	5.0	24.16	2
78	02/11/00	Cow	2.09	21.2	45.66	1
79	02/11/00	Cow	1.72	20.5	36.11	
81	08/11/00	Cow	1.95	17.3	47.47	
82	08/11/00	Cow	1.92	20.7	41.57	
85	08/11/00	Cow	1.5	20.1	43.21	
86	08/11/00	Cow	1.64	13.0	43.99	3
89	10/11/00	Cow	1.04	12.0	42.21	4

5.3.4 Proximate analysis of faecal sample

The faeces consisted of 81.28 % dry matter and the crude fat extraction of the faecal sample (including a replicate sample) revealed values of 7.0 % and 6.88 %, respectively. Crude protein values were corrected for fat content on a dry matter basis and values of 30.77 % and 24.89 % were obtained for the samples. The faecal sample consisted of 39.9 % ash.

5.4 Discussion

5.4.1 Fatty acid composition of southern right whale blubber

The fatty acid profile of triacylglycerols (TAG) in southern right whale blubber was similar to that of most plants and animals, consisting mainly of 14-22 carbon atoms, in even-numbered straight chains, containing a terminal methyl end and a terminal carboxyl end. The chains were saturated with no double bonds or unsaturated, containing from one to six double bonds (Iverson, 1993). The inability of the 2 m packed column to detect some fatty acids (particularly long chain and polyunsaturated fatty acids) was probably due to coelution (Iverson & Oftedal, 1995). As such, broad peaks may represent several incompletely resolved fatty acids. The proportional pattern of fatty acids, i.e. MUFA>SFA>PUFA, found in all but neonatal southern right whale samples is also found in ruminants and, as in ruminants (Christie, 1981), C18:1 was quantitatively the most abundant fatty acid detected, although the values for southern right whales were slightly less than those recorded in ruminants (*E.C. Webb, pers. comm.).

The general shifts in values of the various lipid classes, between all the age/seasonal groups, were probably due to changes in diet/available nutrients over time (Ackman & Hooper, 1968; Lockyer *et al.,* 1984; Iverson, 1993; Iverson & Oftedal, 1995). Increased saturation of fatty acids is generally caused by fermentation processes typically seen in ruminant animals, or animals in which fermentation takes place within the digestive system (Christie, 1981; Vernon, 1981). Extensive biohydrogenation within the neonatal digestive system as well as the lack of external

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food sources (e.g. reduced suckling) may, therefore, explain the high concentration of SFA present in this age group. A decrease in saturation of fatty acids in ruminants is usually related to the ingestion of the animals' typical diet. C17:0 was only detected in some neonatal samples and since this fatty acid cannot be biosynthesised by mammals (Ackman & Lamothe, 1989), the proportions present in the neonates are probably of pre-natal origin (Iverson, Oftedal, Boness & Sampugna, 1995). The presence of C18:3 and C22:6 in some neonates indicated that these animals did engage in suckling before they died (Iverson & Oftedal, 1995).

The variation in fatty acid composition between neonates and early season calves clearly indicates a shift due to dietary influences (i.e. placental transfer to suckling), resulting in high proportions of mainly C16:1 and C22:6 in early season calves (Bowen et al., 1992; Iverson & Oftedal, 1995; Iverson *et al.*, 1995). Marine mammals characteristically convey large amounts of C16:1 and long-chain unsaturated acids to their milk fat (Glass, Troolin & Jeness, 1967). Also of interest is the amount of C15:0 detected in early season calves. This SFA is acquired exogenously by the young whale via milk (Iverson & Oftedal, 1995), microbial fermentation (Vernon, 1981) or from direct consumption of prey (Ackman & Lamothe, 1989). To date, the fatty acid composition of milk samples from southern right whales have not been analysed, however milk from other baleen whale species studied (Glass *et al.*, 1967; Lauer & Baker, 1969; Kasuya, Kato & Dosako, 1997) indicates low proportions of C15:0 (0, 0.93 and 0.3%, for bowhead, fin and minke whales, respectively). These proportions of C15:0 may therefore imply that either the calves are consuming prey (intentionally – which seems highly unlikely given that these animals are probably less than one month old - or inadvertently), or that microbial processes occurring within the digestive system are responsible (see below).

The variation in C14:0 and C15:0 levels between early and late season calves may also be related to dietary changes. Changes in available fat for mobilisation by the cows, which cause changes in the lipid composition of milk, could possibly account for these fatty acid differences in the calves.

Zooplankton generally contain nominal (1-3%) levels of C20:1 and C22:1 acids in their lipids (Ackman, *et al.*, 1965). The deposition of these acids in high proportions in fin whale blubber (and probably in all baleen whale blubber) supports the view that these acids are important as readily accessible energy reserves in both fish and marine mammals (Ackman & Burgher, 1963; Ackman & Jangaard, 1965). In pinnipeds, these acids may be deposited to a large degree directly from the diet, but in baleen whales a definite conversion of source material (lipid or other) into C20:1 and C22:1 acids is indicated (Ackman *et al.*, 1965). However, these higher molecular weight monounsaturates, eicosenoic (C20:1n9) and especially docosenoic (C22:1n11) acids, have subsequently been found to have a specific source in calanoid copepods (Lee, Nevenzel & Paffenhöfer, 1971; Pascal & Ackman, 1976). Therefore, these fatty acids act as biomarkers within copepod predators, thus, characterising higher level marine food webs. Since the total fat content of zooplankton, although variable, may be frequently less than 5% on a wet weight basis, and contain up to 50% non-saponifiable matter (Fisher, 1962 in Ackman *et al.*, 1965; Yamada, 1964), it is perhaps erroneous to view whale blubber as predominantly representing deposition or conversion of dietary fat. *De novo* synthesis of particular fatty acids from the liver acetate pool seems likely to account for most of the C20:1 and C22:1 acids in whales which do not feed on copepods (Ackman *et al.*, 1965) and may contribute to the levels of these acids in whales that do consume copepods. Exogenous as well endogenous sources of C20:1 and C22:1 may therefore explain the seasonal differences in these fatty acids between late season adults and calves, the latter group not having had as much time for the synthesis or accumulation of these fatty acids.

Fatty acids 16:0, 16:1, 18:1 and 22:6 feature strongly in most marine euphausiids (Lockyer *et al.*, 1984). The high proportions of C16:1, C18:0, C18:1 and C22:6 in both early and late season calves, when compared to their respective cows, indicate a dietary "early and end-of-season" effect (Ackman & Jangaard, 1965; Iverson, 1993). During lactation/fat mobilisation, cows deplete unsaturated fatty acids first (as seen in ruminants) (Vernon, 1981), and since the cows do not replace these fatty acids through feeding, it is understandable that the proportions of these fatty acids would decrease from early to later in the season.

The proportions of SFA found in neonates and in a single sample from a subadult male, when compared to a single adult male and early and late season adults, support previous observations that SFA proportions decrease with age i.e. as ingestion of "typical" prey normalises (as is indicated by the higher proportions of C18:3 in the adult male and early and late season adults) (Bowen *et al.*, 1992; Iverson, 1993; Oftedal *et al.*, 1993).

The value of the samples from stranded southern right whales may be limited due to the small sample size and unknown health status of the animals. Nevertheless, it is of interest to note that the general fatty acid profile of MUFA>SFA>PUFA seen in all the biopsy samples, is similar in the positional samples as well as in middle (b) and deep (c) blubber layers of stranded animals. The outermost layer (a), however, is more variable. It is presumably in this latter layer that most synthesis and deposition takes place. Although not statistically significant, variation in fatty acid deposition along the body was evident between the different age groups studied, which suggest that changes over time in lipid composition take place in a cranio-caudal direction. Individual variation between positions along the body was also noted and although the caudal regions of the body contain higher proportions of SFA in ruminants (Webb *et al.*, 1998; Steenkamp, Webb, De Vos, Van Vuuren, 1999) and in fin whales (Lockyer *et al.*, 1984), this was not obvious along all the planes sampled from southern right whales.

5.4.2 Influence of prey on fatty acid composition of North Pacific and southern right whale blubber

In the Antarctic most baleen whales feed predominantly on euphausiids (almost exclusively *Euphausia superba*) as well as copepods and amphipods (Ackman & Eaton, 1966; Nemoto & Yoo, 1970). A variety of organisms are usually included under the generic name "krill", but in the Southern Oceans the name *Euphausia superba* has been considered almost a synonym for krill (Bottino, 1974).

Previous studies by Matthews (1938) reported krill, *E. superba* in the stomach of a right whale from South Georgia, and Lönnberg (1906) characterised the food of right

whales from the same locality as 'kril (Euphausiids)'. Right whales were also seen feeding off Patagonia on the post-larva of lobster-krill (Matthews, 1932) and copepods (Payne, Brazier, Dorsey, Perkins, Rowntree & Titus, 1983), and Hamner, Stone & Obst (1988) observed right whales feeding on krill off the Antarctic Peninsula. Data provided by Tormosov, Mikhaliev, Best, Zemsky, Sekiguchi and Brownell (1998) include the stomach contents of 249 right whales taken by Soviet whalers in the Antarctic. The majority (72.3%) of these stomachs contained euphausiids ('krill'), with copepods ('Calanus') being the next most important food item (24.9%). Two species of copepods were identified, *Calanus propinquus* and *Pleuromanna robusta* and although the euphausiids eaten were classified as 'krill', it is not known whether some or all of these were *E. superba*. Although well studied, the prey of southern right whales has not been isolated.

Unfortunately fatty acid results were only available for one male North Pacific right whale (Tsuyuki & Itoh, 1970), and analytical methodologies differed, which makes comparison with the southern right whale data problematic. The packed column methodology used for southern right whales might also have resulted in inadequate resolution of long-chain PUFAs. Nevertheless, differences in specific/major fatty acids between southern right whales and the North Pacific right whale are worthy of mention. The proportion of C20:1, for instance, was approximately 10 times that found in southern right whales. This MUFA, as well as docosenoic (C22:1n11) acid, are prominent features of the fatty acid composition found in calanoid copepods (Lee *et al.*, 1971; Pascal & Ackman, 1976). North Pacific right whales are known to consume copepods together with small amounts of euphausiids (Klumov, 1962). The fatty acid composition of the North Pacific right whale sampled (Tsuyuki & Itoh, 1970) seemed to support this observation, as the high levels of C20:1 and the presence of C22:1 imply that the animal had presumably fed on carnivorous copepodid species (Saether & Mohr, 1987) while the notable proportions of C20:5 seem to indicate the consumption of euphausiids. Although variation in lipid composition between planktonic genera and within planktonic species is well known (Ackman & Hooper, 1968; Ackman, Eaton, Sipos, Hooper & Castell, 1970; Morris, 1971; Bottino, 1974, Morris & Culkin, 1976; Kattner, Krause & Trahms, 1981; Saether & Mohr, 1987), the low levels of C20:1 and C22:1 (as well as the relative levels of C16:1 and C18:3 and

the total absence of C20:5) seem to accommodate the assumption that the southern right whales sampled for this study may have been feeding on herbivorous euphausiids (Clarke, 1980) rather than copepods.

Southern right whale cows seemed to have three times the amount of C18:3 compared to that found in the single adult male North Pacific right whale (Tsuyuki & Itoh, 1970). C18:3 is originally derived from plant sources (Ackman, Tocher & McLachlan, 1968) and is recorded in small amounts in *E. superba* (1.2%) (Hansen & Meiklen, 1970; Bottino, 1974), but does not occur in copepods (Lee *et al.*, 1971; Ohman, Bradford & Jillett, 1989). It appears that C18:3 may therefore serve as a good indicator that these balaenids are consuming *E. superba.*

The marked difference in the proportions of C16:1 and C20:5 found in the North Pacific right whale sample and the southern right whale samples could be interpreted as an indication of dietary/prey variation, as mentioned above. High proportions of C20:5 were detected in the North Pacific right whale (16.65%) but was absent in all southern right whale samples. This fatty acid is an important component of most marine euphausiids (11-20%, Nonaka & Koizumi, 1964; Hansen & Meiklen, 1970; Van der Veen, Medwadowski & Olcott, 1971; Clarke, 1984; Lockyer *et al.*, 1984) possibly originating from their phytoplankton prey (Bottino, 1974). Samples of *E. pacifica* (Yamada, 1964) showed an appreciably higher content of C20:5 (25.9%) and C22:6 (14.7%) than the Antarctic, *E. superba* (16.2% and 9.3%, respectively) (Hansen & Meiklen, 1970). In the Northern hemisphere euphausiid, *M. norvegica*, C20:5 makes up 8-9% of the TAGs (Ackman *et al.*, 1970; Lockyer *et al.*, 1984) but this fatty acid only comprises 2-3% of fin whale blubber lipid (Lockyer *et al.*, 1984). C20:5 has also been found in some copepod and amphipod species, e.g. *Calanus helgolandicus* (Lee *et al.*, 1971) and *Parathemisto gaudichaudii* (Bottino, 1978). Species of these genera have been recorded in varying amounts in North Pacific right whale stomachs (Klumov, 1962; Omura *et al.*, 1969). Consumption of different prey species by the different whale species may therefore account for the differences in proportions of C20:5 (Bottino, 1974). The total non-detection of C20:5 in the blubber of apparently euphausiid-consuming southern right whales is, however, surprising (although its non-detection by the packed column systems used cannot be discounted). This may infer

intra-species variation in some fatty acids found in the euphausiids, consumed by these whales.

The fact that C20:1 was the major monoenoic fatty acid in the North Pacific right whale and that these animals possessed less SFA compared to southern right whales seems to imply that there is less biohydrogenation (and therefore less efficient microbial fermentation) or less endogenous synthesis of SFA in the North Pacific right whale (Vernon, 1981). In hypothetical terms, and considering methodological differences, the latter findings may also suggest possible differences in digestive processes between North Pacific and southern right whales, subsequent to differences in prey availability.

C14:0 and C16:0 are both predominant fatty acids found in copepods (Kattner *et al.*, 1981; Ohman *et al.*, 1989) as well as in euphausiids (C16:0 = 22-28%) (Hansen & Meiklen, 1970; Sidhu, Montgomery, Holloway, Johnson & Walker, 1970; Van der Veen *et al.*, 1971; Bottino, 1974). However, the higher proportions (approximately twice the amount in some cases) of almost every SFA in southern right whales compared to the North Pacific right whale further supports the above-mentioned hypothesis that lower proportions of SFA generally occur in the monogastric-like digestive system.

Excluding neonates, MUFA comprised the highest proportions of TAG, followed by SFA and then PUFA (although the proportion of the latter could have been under-represented by the analytical technique used), in the southern right whale samples. This proportional pattern has been noted in some rorqual species (Table 18), even though dietary sources or prey species of these balaenids and balaenopterids differ. The North Pacific right whale values, however, did not follow the same pattern and the proportions of PUFA were higher than SFA. As discussed above, and leaving aside questions of comparability of analysis, it seems that North Pacific right whales are more like monogastric animals and deposit fatty acids without extensive biohydrogenation in a "forestomach". In southern right whales, however, a certain degree of biohydrogenation of ingested unsaturated fatty acids may occur, resulting in a larger proportion of SFA.

Table 18: Fatty acid composition of TAGs in various mysticete species (mean % values)*

Species	n	SFAs		MUFAs		PUFAs		Reference
		%	Important f.a.	%	Important f.a.	%	Important f.a.	
Balaenoptera borealis	?	22.1	16:0>14:0>18:0	57.6	20:1>18:1>22:1	19.2	20:5>22:6>20:4	Sano *et al.*, 1965
Balaenoptera borealis	1	22	14:0>16:0>18:0	57	20:1>18:1>22:1	18	22:6>20:4>20:5	Bottino, 1977
Balaenoptera physalus (Antarctic)	5	25.2	16:0>14:0>18:0	53.7	18:1>16:1>20:1	19.8	20:5>18:2>22:5	Sano, *et al.*, 1965
Balaenoptera physalus (NW Atlantic)	1	17.3	16:0>14:0>18:0	71.2	18:1>20:1>22:1	11.4	22:6>20:5>18:2	Ackman *et al.*, 1965
Balaenoptera physalus (NE Atlantic)	1	18.3	16:0>18:0>14:0	62.7	18:1>16:1>22:1	18	22:6>22:5>20:5	Lockyer *et al.*, 1984
Balaenoptera physalus	19	18	16:0>14:0>18:0	74	18:1>20:1>16:1	7	20:5>22:6>22:5	Borobia *et al.*, 1995
Megaptera novaeangliae	10	16	16:0>14:0>18:0	72	18:1>20:1>16:1	11	20:5>22:6>22:5	Borobia *et al.*, 1995
Eubalaena japonica	1	27.4	14:0>20:0>13:0	51.4	18:1>20:1>16:1	21.2	22:6>20:5>18:3	Tsuyuki & Naruse, 1963
Eubalaena japonica	1	24.5	16:0>14:0>18:0	44.9	20:1>18:1>16:1	29.8	20:5>18:3>22:6	Tsuyuki & Itoh, 1970
Eubalaena australis (cows)	26	34.4	14:0>16:0>20:0	49.4	18:1>16:1>20:1	16.1	18:3>22:6>22:5	Present study

* For additional references, refer to summary by Ackman & Lamothe (1989)

The North Pacific right whale had noticeably higher proportions of PUFAs than either the southern right whales or the rorqual species (Table 16), which may be due to the specific nature of the prey being ingested (Ackman & Eaton, 1966; Ackman, 1980) as well as the fact that this individual was caught on feeding grounds, and was presumably in a positive energy balance accumulating fat (Lockyer, 1981; 1987).

5.4.3 Quantitative lipid values of southern right whale blubber

Variation in lipid content/fatty acid composition of blubber with depth and reproductive status in cetaceans is well documented (Heyerdahl in Slijper, 1948; Feltmann, Slijper & Vervoort, 1948; Ackman & Jangaard, 1965; Ackman & Eaton, 1966; Ackman *et al.*, 1971; Ackman, Hingley, Eaton, Logan & Odense, 1975a; Lockyer, 1986; 1987; Lockyer, McConnell & Waters, 1984; 1985; Aguilar & Borrell, 1990; Lambertsen *et al.*, 1993; Møller *et al.*, 2000; Krahn, Ylitalo, Burrows, Calambokidis, Moore, Gosho, Gearin, Plesha, Brownell, Blokhin, Tilbury, Rowles & Stein, 2001). According to the summary by Lockyer (1976), the average blubber weight for 14 North Pacific right whales, *Eubalaena japonica*, amounted to 20 950 kg. During US whaling operations from 1822 to1910, North Pacific right whales yielded an average of 122 barrels (14 546.14 litres) of oil (Best, 1987), or 14.546 tonnes per whale. Oil therefore constituted an average of 69% of North Pacific right whale blubber on a wet weight basis. Unfortunately no equivalent data are available for southern right whales, but assuming a similar oil content to the North Pacific right whale, the quantitative results obtained from biopsies of late season cows and calves seem low. However, other studies done on the lipid-rich blubber of bowhead whales (*T. O'hara, †G. Ylitalo, pers. comm.) and other whale species (Table 19) indicate marked intra-species variation, and also a dependence on factors such as tissue sampling (i.e. biopsy or necropsy), for example. Total lipid values between 14 and 85% have been obtained in bowhead whale tissue (G. Ylitalo, pers. comm.), which bracket the values obtained here for southern right whales. It is interesting to note that there was no apparent relationship between depth of biopsy sample and total fatty acid content, except for a very superficial sample (5 cm in a cow). This suggests that (for total lipid analysis), the deep-core sampling technique was successful in obtaining representative cores of blubber.

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Table 19: Total lipid values (% w/w, expressed as mean ± S.E.) obtained from various samples from different age and sex classes of some non-stranded mysticetes.

Species	Nature of sample	n	Gender	Total lipid	Reference
Balaenoptera physalus	Dorsal, inner blubber layer	3	Male	52.53 ± 3.7	Ackman *et al.*, 1975a
Balaenoptera physalus	Dorsal, inner blubber layer	3	Female	33.4 ± 14.1	Ackman *et al.*, 1975a
Balaenoptera physalus	Dorsal, outer blubber layer	3	Male	66.23 ± 2.2	Ackman *et al.*, 1975a
Balaenoptera physalus	Dorsal, outer blubber layer	3	Female	63.33 ± 4.2	Ackman *et al.*, 1975a
Balaenoptera borealis	Dorsal, inner blubber layer	2	Male	39.4 ± 9.6	Ackman *et al.*, 1975a
Balaenoptera borealis	Dorsal, outer blubber layer	2	Male	60.3 ± 0.7	Ackman *et al.*, 1975a
Balaenoptera borealis	Dorsal, inner blubber layer	2	Female	39.5 ± 0.5	Ackman *et al.*, 1975a
Balaenoptera borealis	Dorsal, outer blubber layer	2	Female	65.65 + 2.85	Ackman *et al.*, 1975a
Megaptera novaeangliae	Dorsal, inner blubber layer	1	Female	18	Ackman *et al.*, 1975a
Megaptera novaeangliae	Dorsal, outer blubber layer	1	Female	47.3	Ackman *et al.*, 1975a
Balaenoptera borealis	?	1	Male	75.2	Bottino, 1977
Balaenoptera physalus	Ventral blubber	1	Male	41.8	Lockyer *et al.*, 1984
Balaenoptera physalus	Posterior dorsal blubber	1	Male, mature	64.2	Lockyer *et al.*, 1984
Balaenoptera physalus	Posterior dorsal blubber	1	Female, lactating	77.4	Lockyer *et al.*, 1984
Balaenoptera physalus	Posterior dorsal blubber	1	Female, immature	66.9	Lockyer *et al.*, 1984
Balaenoptera physalus	Posterior dorsal blubber	1	Female, pregnant	56.9	Lockyer *et al.*, 1984
Balaenoptera physalus	Posterior dorsal blubber	1	Male, immature	54.2	Lockyer *et al.*, 1984
Balaenoptera physalus	Posterior dorsal blubber	1	Male, immature	66.4	Lockyer *et al.*, 1984
Balaenoptera physalus	Mid-dorsal blubber	20	Mixed ages/genders	56.2 ± 1.1	Lockyer *et al.*, 1985
Balaenoptera borealis	Mid-dorsal blubber	12	Mixed ages and genders	57.9 ± 2.2	Lockyer *et al.*, 1985
Balaenoptera physalus	Posterior dorsal blubber	3	Male, immature (1978)	53.2 ± 7.9	Lockyer, 1986
Balaenoptera physalus	Posterior dorsal blubber	5	Male, immature (1981)	70.6 ± 1.5	Lockyer, 1986
Balaenoptera physalus	Posterior dorsal blubber	4	Male, mature (1978)	52.7 ± 11.6	Lockyer, 1986
Balaenoptera physalus	Posterior dorsal blubber	5	Male, mature (1981)	72.5 ± 2.4	Lockyer, 1986
Balaenoptera physalus	Posterior dorsal blubber	3	Female, immature (1978)	58.5 ± 9.1	Lockyer, 1986
Balaenoptera physalus	Posterior dorsal blubber	6	Female, immature (1981)	71.0 ± 1.2	Lockyer, 1986
Balaenoptera physalus	Posterior dorsal blubber	3	Female, mature (1978)	65.5 ± 0.3	Lockyer, 1986
Balaenoptera physalus	Posterior dorsal blubber	1	Female, mature (1981)	76.8	Lockyer, 1986
Balaenoptera physalus	Posterior dorsal blubber	6	Female, pregnant (1978)	45.0 ± 6.4	Lockyer, 1986
Balaenoptera physalus	Posterior dorsal blubber	3	Female, pregnant (1981)	75.1 ± 3.3	Lockyer, 1986
Balaenoptera physalus	Posterior dorsal blubber	1	Female, lactating (1978)	39.4	Lockyer, 1986
Balaenoptera physalus	Posterior dorsal blubber	1	Female, lactating (1981)	59	Lockyer, 1986
Balaenoptera physalus	Mid-dorsal blubber	31	Males	75.3 cv 10.9	Aguilar & Borrell, 1990
Balaenoptera physalus	Mid-dorsal blubber	27	Females, immature	77.5 cv 7.6	Aguilar & Borrell, 1990
Balaenoptera physalus	Mid-dorsal blubber	8	Females, pregnant	81.4 cv 6.8	Aguilar & Borrell, 1990
Balaenoptera physalus	Mid-dorsal blubber	9	Females, lactating	57.5 cv 15.7	Aguilar & Borrell, 1990
Balaenoptera physalus	Mid-dorsal blubber	7	Females, mature	60.8 cv 27.9	Aguilar & Borrell, 1990
Eschrichtius robustus	?	13	Female, immature	48 ± 6.1	Krahn *et al.*, 2001
Eschrichtius robustus	?	4	Males, immature	48 ± 11	Krahn *et al.*, 2001
Eubalaena australis	Dorso-lateral blubber	7	Females, lactating	42.9± 1.36	Present study

Although the lactating cows were in a negative energy balance (mid-lactation) and the suckling calves were in a positive energy balance, the differences between these two groups were not significant. However, without early season data, it is not possible to determine the relevance of these values, although they do provide the first description of total lipid in southern right whale blubber. These results also seem to indicate the importance of obtaining deep-core blubber samples for quantitative lipid analysis, implying that the collection of superficial blubber layers (using most current projectile biopsy techniques) is not recommended for this type of analysis.

The high collagenous content of balaenid blubber is well known (Yablokov, Bel'kovich & Borisov, 1974; Slijper, 1962; Haldiman & Tarpley, 1993) and may possibly influence these weight-related calculations (Lockyer *et al.*, 1984). Determining collagen content should be included in future studies involving measurement of the total lipid content of balaenid blubber.

5.4.4 Microbial fermentation in southern right whales

Krill has an exoskeleton that consists mainly of chitin, which to some extent may prevent the action of digestive enzymes on other parts of the prey. Degradation of the chitin skeleton will eliminate this barrier and also release the chemical energy bound in the chitin itself. In *E. superba*, for example, the chitin skeleton contributes about 10% to the total energy content of the animal (Clarke, 1980). Both the energy density (Mårtensson, Nordøy & Blix, 1994) and the gross chemical composition (Saether & Mohr, 1987) of *E. superba* and *Thysanoessa* sp. are quite similar. Mathiesen, Aagnes & Sormo (1990) have shown that the forestomach of krill-eating minke whales is rich in chitinase-producing bacteria. Such bacteria are probably responsible for the more efficient digestion of krill by minke whales compared with crabeater seals (Mårtensson *et al.*, 1994). Olsen, Nordøy, Blix & Mathiesen (1994b), suggested that the multi-chambered stomach of minke whales increases passage time and consequently increases the time available for both microbial and enzymatic digestion of such complex structures as the exoskeleton of krill.

Bowhead whales consume pelagic crustaceans, including euphausiids and copepods (Kawamura, 1980). The major food items of gray whales are benthic fauna, mainly amphipods (Zimushko & Lenskaya, 1970), in addition to the occasional consumption of kelp while residing in their wintering areas (Nerini, 1984). Volatile fatty acids and the presence of significant levels of bacteria have been detected in the forestomachs of both these species, supporting the hypothesis that microbial fermentation occurs in the forestomach of bowhead and gray whales (Herwig *et al.*, 1984).

The medium-chained, saturated fatty acid, C15:0, which is usually of dietary origin, was not detected by Ohman *et al.* (1989) in *Neocalanus tonsus*, and is generally present in very low proportions in *E. superba* (Nonaka & Koizumi, 1964; Clarke, 1980). However, the oxidation of C16:0 and C18:0 to C15:0 by rumen protozoa has been described in ruminants (Emmanuel, 1974), so it is a possibility that the C15:0 levels detected in southern right whales may have been acquired from microbial sources.

To date, the fresh biochemical contents of southern right whale stomachs have not been studied and direct evidence for microbial fermentation in this species has therefore not been confirmed. However, taking the above factors into consideration, as well as the extensive biohydrogenation of various fatty acids suggested in previous sections, it seems possible that, like some other cetacean species (Morii, 1972; 1979; Morii & Kanazu, 1972; Herwig *et al.*, 1984; Herwig & Staley, 1986; Mathiesen *et al.*, 1990; Olsen *et al.*, 1994a; 1994b; Olsen & Mathiesen, 1996), microbial fermentation occurs within the digestive system of southern right whales.

The chemical analysis of the southern right whale faecal sample provides the first documented results of lipid (~ 7%) and protein (~ 25%) values (on a dry matter basis) of this kind. Although it is not possible to quantify total energy losses through this route without information on the amount and rate of faecal production, these values for lipid and protein seem quite high, given the energetic constraints under which right whales seem to exist (Kenney, Hyman, Owen, Scott & Winn, 1986; Mayo & Marx, 1990).

5.5 Conclusion

The triacylglycerol composition of southern right whale blubber is similar compared to that found in most plants and animals. The general shifts in values of the various lipid classes, between all the age groups, were probably due to changes in diet/available nutrients over time. Fatty acid composition does not vary significantly between different positions along and around the bodies of stranded southern right whales. There is also no distinct layering effect of fatty acids in the different blubber layers of the stranded animals. The deep-core biopsy sampling of the blubber in the dorso-lateral region undertaken here can therefore be considered to provide data representative of the fatty acid composition. The low levels of C20:1 and C22:1 (as well as the relative levels of C16:1, C18:3 and C20:5) found in such biopsies seem to indicate that the southern right whales sampled for this study were probably feeding on herbivorous euphausiids (Clarke, 1980) rather than copepods. The lower degree of saturation found in North Pacific right whale blubber compared to southern right whales seems to imply that there may be differences in the digestive processes between these congeners (although this conclusion assumes general comparability of analytical procedures). The C15:0 levels detected in the southern right whales may have been acquired from microbial sources and thus suggest that microbial fermentation takes place in the digestive system of this species. Although the total lipid values obtained for 12 southern right whales seemed low, they are similar to results obtained from other studies on bowhead whales. The acquisition of deep-core blubber samples for quantitative lipid analysis is hereby highlighted. A faecal sample seemed to indicate that surprisingly high amounts of lipid and protein were excreted, given the energetic constraints under which right whale exist.

The use of the above-mentioned fatty acid data to describe both dietary influences and inter-species differences must however remain open for revision or confirmation using different, and possibly more sensitive, analytical techniques and fatty acid detection equipment.

CHAPTER 6

A BIOPSY SYSTEM FOR DEEP-CORE SAMPLING OF THE BLUBBER OF SOUTHERN RIGHT WHALES, *EUBALAENA AUSTRALIS*.

6.1 Introduction

It is well known that certain characteristics of blubber tissue may vary with depth in cetaceans, e.g. histological structure (Sokolov, 1955; 1960; Slijper, 1962; Giacometti, 1967; Yablokov, Bel'kovich & Borisov, 1974; Aguilar & Borrell, 1990; Haldiman & Tarpley, 1993) and lipid content/fatty acid composition (Heyerdahl in Slijper, 1948; Feltmann, Slijper & Vervoort, 1948; Ackman & Jangaard, 1965; Ackman & Eaton, 1966; Ackman, Epstein & Eaton, 1971; Ackman, Hingley, Eaton, Logan & Odense, 1975a; Lockyer, 1986; 1987; Lockyer, McConnell & Waters, 1984; 1985; Aguilar & Borrell, 1990; Koopman, 1998; Møller, Born, Dietz, Ruzzante, Huag & Øien, 2000). These variations have implications, for example, for energetic determinations and organochlorine analyses (Aguilar, 1985). To collect representative fatty acid data it is, therefore, important to obtain deep core blubber samples. Previous biopsy systems have successfully obtained epidermal tissue, with small portions of blubber attached, from large whales (Lambertsen, 1987; 1991; Lambertsen & Duffield, 1987). Such samples suffice for genetic analyses, but Woodley, Brown, Kraus & Gaskin (1991) found that larger samples sizes were required to make reliable measurements of organochlorine contamination in northern right whales (*Eubalaena glacialis*). Lambertsen, Baker, Weinrich & Modi (1993) developed a new whale biopsy system, especially designed for multi-disciplinary studies, using darts, delivered with a pneumatic gun, having a punch measuring 12 cm in length. Members of the Balaenidae, however, are known to possess the thickest integument of all cetaceans, exceeding 30 cm in some adults (Sokolov, 1960; Slijper, 1962; Omura, Ohsumi, Nemoto, Nasu & Kasuya, 1969; Yablokov, *et al.*, 1974; Haldiman & Tarpley, 1993), and a new system was therefore required for deep-core sampling of these animals. The generally slow-moving and boat-tolerant behaviour shown by southern right whales allowed the system described below to be hand-held. This mechanism of

delivery was anticipated to have far less impact on the animal and at the same time would enable the collection of high quality tissue samples for histological analysis.

6.2 Materials and Methods

Two aluminium poles (i.d. = 2.9 cm, o.d. = 3.1 cm) were modified to interconnect, forming a 9 m handle biopsy pole. The end of one of the poles was adapted to receive a specially designed stage that in turn attached to the biopsy head. The biopsy head consisted of two parts - a stainless steel "needle" and a brass coupling (Plate 71). The stainless steel needles were made by bending (at 90°) and Argon-welding two pieces of stainless steel plate together to form a rectangular shape. One end of the needle was cut diagonally to form a bevelled surface at the tip (Plate 71). This surface was sharpened on a grindstone. The dimensions of the stainless steel needles, for all the biopsy heads, can be found in Table 20.

Table 20: Outside dimensions of stainless steel needles used in biopsy heads for calves and adults.

Age	Dimensions of stainless steel needles (l/b/ht) (cm)
Calves	11 cm x 0.8 cm x 0.4
Calves	11.7 cm x 0.8 cm x 0.4
Adults (2nd model)	19.2 cm x 1.0 cm x 0.7
Adult "trap-door" barb	20.5 cm x 1.1 cm x 0.7

Approximately 0.6-0.9 cm from the sharpened tip, two slots(~ 0.7-0.9 cm apart) were machined horizontally into the needle in order to house the barb. The barbs were made out of stainless steel spring shim (obtained from the protective slide on a computer diskette). The shim was cut into 1.4-1.8 cm strips (0.7-0.8 cm wide) which were then bent to fit into the slots. One end of the barb was bent approx. 0.4-0.9 cm down the strip, at a 25° angle, forming the front of the barb. The other end of the strip was bent approx. 0.25-0.3 cm from the edge, forming the back of the barb. The front of the barb was slipped into the front slot and the back end of the barb manoeuvred into the back slot (Plate 71E). With a thin, flat metal rod (inserted into the back of the needle), the back end of the barb was pushed forwards and upwards to make it flush with the upper surface of the needle (Plate 71G). This secured the barb. One of the

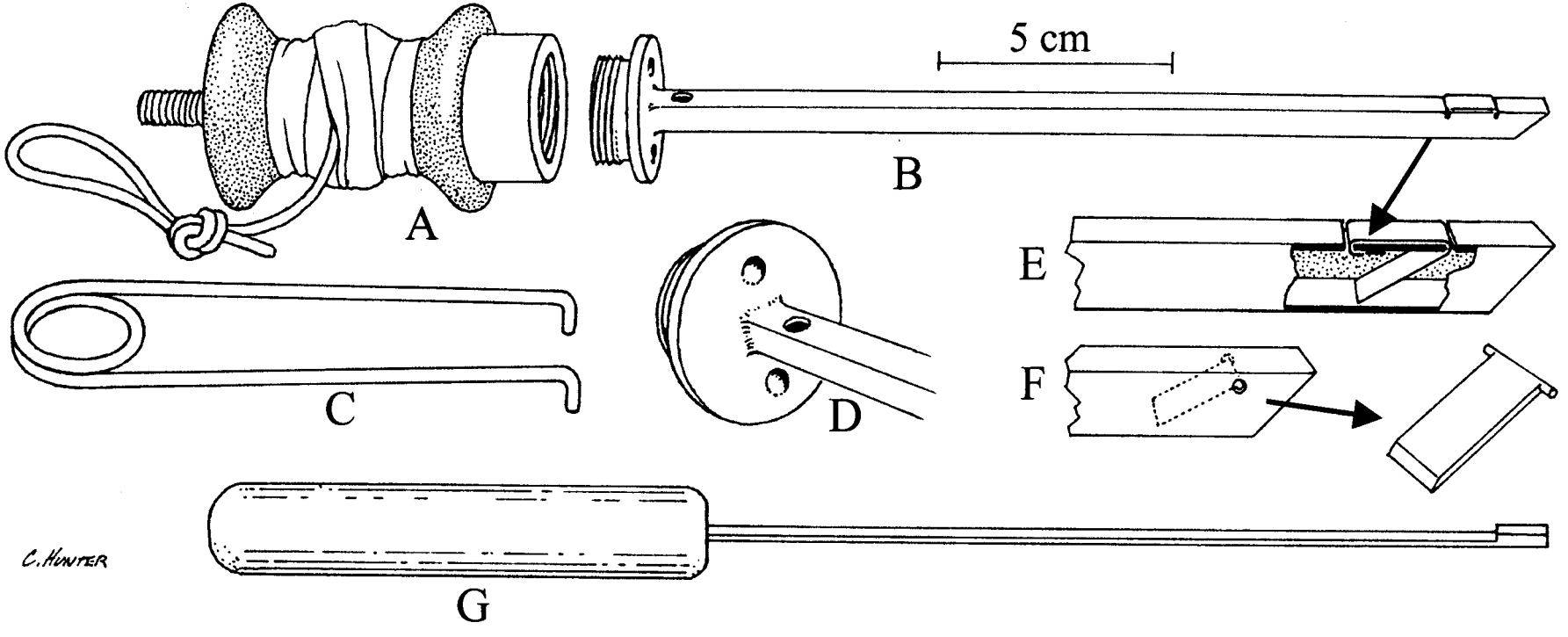

Plate 71: Sketches of the hand-held biopsy system and tools. (A) rubber bush fitted with a brass coupling and nylon cord; (B) brass coupling and stainless steel needle, note the bevelled tip of the needle; (C) spring-steel tool for tightening the biopsy head; (D) brass coupling at the base of the biopsy needle showing indentations for tightening and a hole at the base of the head for air movement; (E) side view of the biopsy head exposing the spring-steel and trap-door barbs (F); (G) tool for setting the spring-steel barb.

larger adult heads was fitted with a "trap-door" barb, hinged at a 45° angle, in line with the lower edge of the tip of the stainless steel needle (Plate 71F).

The intent of both barb systems was to allow the needle tip to cut an undamaged section of skin and blubber and only offer resistance when the needle was withdrawn from the animal. The rectangular cross-section of the needle was designed for greater strength and to allow for a bigger barb than would be possible with a circular cross-section.

The stage consisted of a flexible, hour-glass shaped rubber mount (4 cm in diameter) (in reality a bush from a windsurfer). A brass coupling was manufactured with matching male and female threads (Plate 71A). The female half (o.d. 2.5-3.4 cm, i.d. 1.6-2.5 cm, 1.8-2 cm thick) was attached to one end of the mount, while a rectangle (slightly larger than the dimensions of the stainless steel needles) was removed from the centre of the male half (o.d. 1.8-3.4 cm, i.d. 1.7-2.5 cm, 0.7-1.1 cm thick). The needles were then welded onto/into the male half of the coupling. A small hole (0.3-0.5 cm) was made at the base of each needle (to assist in releasing air during sampling). The intent of the rubber mount was to reduce shearing forces that might bend or break the long needles. In practice, especially with the adult needles, it was found necessary to strengthen the mount by lashing it with insulating tape, to prevent the needle from "ricocheting" on impact (Plate 71A).

Nylon cord (20 m) was tied around the middle of the rubber mount and secured along the aluminium poles with velcro strips. The excess cord was held on deck to act as a tethering system should the mount detach from the poles (which it never did).

The first model of the adult biopsy needle had the same specifications as for the calf needle, except that it was 15 cm in length. In order to lengthen the needle, the other dimensions of the needle had to be increased (specifications listed in Table 20). After the first year of sampling, it was found that the more "square" shape of the adult biopsy needle yielded samples that were more robust. These samples tended to be more complete i.e. this shape provided more support that prevented the deeper fatty tissue layers from tearing away from the rest of the integument. As a result, this

needle was used during the second sampling season on late season calves. However, to reduce the depth of penetration into the calves, 4 stoppers of closed cell foam rubber, the same diameter as the needle collar, were manufactured that could be slipped over the biopsy needle. A small section at the base of the stopper was removed to expose the hole at the base of the needle and thus ensure the movement of air through the needle during sampling. The maximum length of the stoppers was 9 cm, effectively making the biopsy needle 10.2 cm long. This arrangement allowed for more flexibility in sampling, as it obviated the necessity of changing needles when either the cow or calf was more accessible.

To remove the sample, the spring barb was lifted at the front by inserting a sharp object (e.g. long-nose forceps) under the flat section on the outside of the needle. The sample was then blown or pushed out using a thin metal rod. Usually, such barbs were only used once (Plate 72).

6.3 Results

If an attempt was defined as an occasion when the needle was successfully inserted, the success rate for obtaining samples averaged 79.7% for calves and 76.1% for cows (Table 21).

Table 21: Summary of integumentary sampling of southern right whale cows and calves, using hand-held deep-core biopsy system.

Age Group	No. of attempts	No. of samples collected	Mean length of sample+	S.E. of mean
Calves*	79	63	6.25 (n=31) cm	0.53 cm
Cows	46	35	12.3 (n=23) cm	1.37 cm

* Using "calf" and "shortened" adult biopsy heads.
+ Including skin and blubber

The longest samples retrieved from an early season smooth-skinned calf, an early season rough-skinned calf, an early season adult, a late season calf and a late season adult were 11.7, 12.4, 18.6 cm, 13.2 and 21.2 cm, respectively.

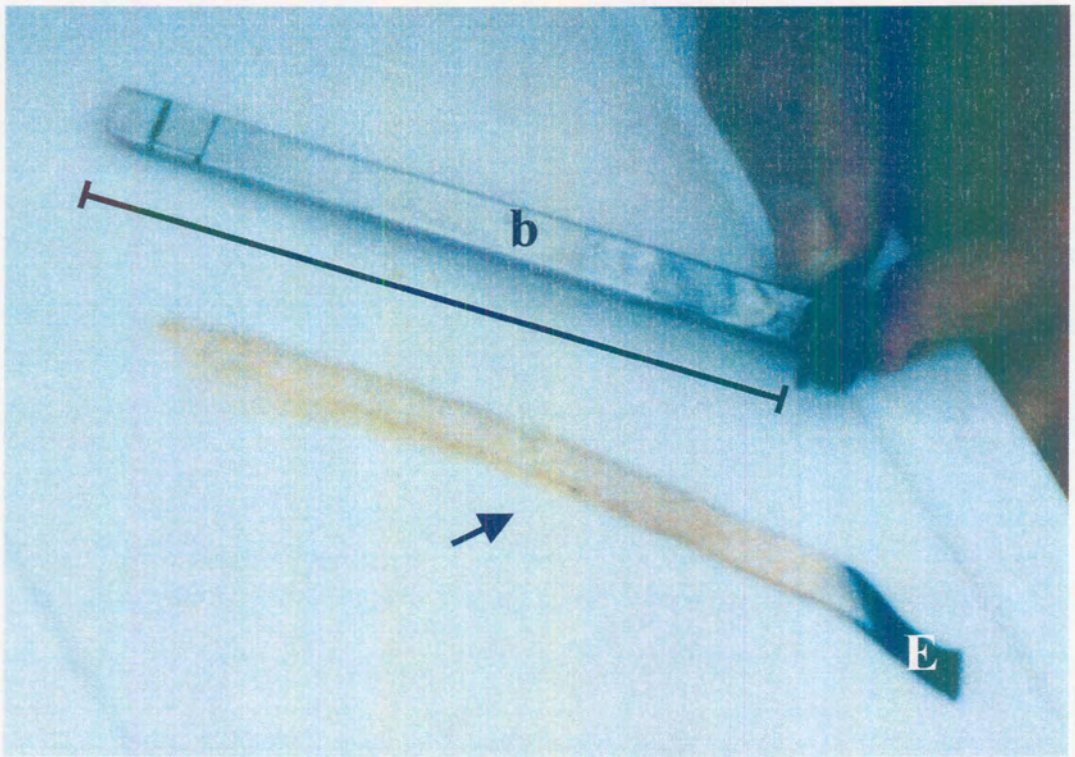

Plate 72: Integument of a southern right whale calf (arrow) and the biopsy head (b) used for sampling (11 cm x 0.8 cm x 0.4 cm). Epidermis (E).

The "trap-door" biopsy needle was only constructed during the last session of late season sampling and used on only one occasion, when it was unfortunately bent. This needle was therefore not fully tested, however the "trap-door" barb design is of obvious benefit in eliminating the need for replacement or re-setting of the barb.

The flexible barb design successfully held the tissue samples and at the same time reduced mechanical damage to the samples that allowed for fine histological study of the pigmented skin and blubber tissue. The shape of the biopsies also provided clean surfaces for histological embedding and sectioning.

Based on the observations of the author, the overall reactions of the whales to this biopsy system were no greater than the reactions of whales to the more superficial sampling using the Paxarms biopsy system, with by far the greatest component of the reaction being to the close approach of the boat. On numerous occasions, when using the Paxarms biopsy system, the author has observed unfavourable reactions by the whales when pneumatic darts missed the target and hit the surface of the water instead. This suggests that the reactions of the whales were possibly exacerbated by the sound/vibrations of pneumatic darts, which is effectively obviated when using this hand-held system. There was only one episode of haemorrhaging seen, following a successful sampling attempt of a neonate. A thin spray of blood came from the biopsy site. The neonate consequently reacted by lifting its head and fluke and then slapping the surface of the water with its fluke before swimming away. Bleeding stopped within minutes and the animal's behaviour appeared normal.

6.4 Discussion

Although the biopsy needles are more difficult and laborious to construct, compared to other biopsy systems, this system is cheap to assemble and can be used from relatively small boats. The head design allows for the collection of samples that can be used for multidisciplinary research on right whales (e.g. histology, toxicology and blubber composition studies), and the acquisition of comparatively deep core samples, which until now have only been available from stranded or harvested animals. Further experimentation with the "trap-door" barb is required. The reactions of the animals to

this biopsy system were no greater than those recorded using projectile biopsy systems, although the biopsy system used in this study requires the vessel to approach the whale closer than projectile systems.

6.5 Conclusion

This hand-held biopsy system is a practical and cheaper alternative to projectile systems, allowing for the collection of deep core samples from southern right whales that can be used for multidisciplinary research.

SUMMARY

Amongst cetaceans, the southern right whale integument is second in skin and blubber thickness only to that of the bowhead whale, *Balaena mysticetus*. Its histological structure has not been previously described. The development of a deep-core biopsy system allowed for the collection of integumentary samples from southern right whales that have been used in the multi-disciplinary research presented in this study.

Histological analysis has revealed the general structure of the integument of southern right whales to be typically mammalian and comparable with that of other cetaceans. The interdigitation of the dermal papillae and epidermal rete (papillomatosis) is particularly marked in this species, as in other balaenids so far studied. This study supports previous findings that the integument of this family, possessing an almost "fat-free" dermal layer, is more like that of odontocetes than that described for balaenopterids. The transition from the dermis to the hypodermis is defined by a gradual increase in the amount of adipocytes in a proximal direction. Unlike bowhead and sei (*Balaenoptera borealis*) whales, southern right whales possess an uninterrupted hypodermal layer that is connected to the muscle layers by a connective tissue layer (superficial fascia).

Scanning electron microscopy (SEM) techniques exposed a typically mammalian pattern of exfoliation of the superficial epidermis. The acquisition of samples from (non-calf) southern right whales in the Antarctic provided the opportunity to determine whether there were seasonal differences in exfoliation between animals in the summer and wintering grounds. Superficial exfoliation was detected on samples from the Antarctic indicating a continuous rather than seasonally-restricted exfoliation in southern right whales. SEM also revealed various microbial aggregations on skin biopsies taken in Antarctic as well as South African waters, including samples that were collected from stranded animals. These microbes included unidentified yeast colonies, various species of fungal mycelia and spores, an unidentified species of cyanobacteria and bacterial cocci. The increased presence of these microbes on cows and calves in October/November when compared to reduced amounts on Antarctic animals, seems to suggest that these microbes are acquired in the coastal waters

around South Africa. An invasive infestation of the skin of a stranded neonate by a *Candida*-like fungus could possibly have contributed to the mortality of this animal. This is the first account of microbial aggregations on and fungal infestations of the skin of southern right whales.

No diatomaceous films were found on any of the stranded whales sampled, and *Bennettella* [*Cocconeis*] *ceticola* (usually associated with films on southern hemisphere cetaceans in cold water) was not present on any of the samples. The unique ultrastructure of southern right whale stratum corneum cells, described previously by other researchers, may allow for high cellular proliferation rates in this species. This suggests that even at higher latititudes, diatom films may not have sufficient time to form on the skin of southern right whales.

Differences in the macroscopic appearance of the skin of neonatal calves were noted during the collection of samples in August. Calves possessed either a characteristically light grey and "rough" skin texture or a dark, smooth skin. Histological analysis of samples from stranded neonates revealed a distinct "double" epidermis, demarcated by a transition line that occurred distal to the tips of the dermal papillae. In noticeably "rough-skinned calves, this line became characterised by intercellular oedema, which caused the cell layers above the line to separate from those below it, essentially forming an ecdysal plane. A similar, although pathological, process known as "spongiosis" has been described in humans, but not before in cetaceans. Although a thick outer epidermal layer ("baby skin") has been noted in bowhead whale calves, this is the first thorough description of this type of moult (ecdysis) in any species of cetacean.

From a combination of aerial and boat-based observations, southern right whale calves complete this ecdysal process approximately one week after birth. This may be an archaic character in balaenids, vestigial evidence of which is found in the more modern balaenopterids.

The extensive and well-developed blubber layer, so characteristic of balaenids, played an important role in fuelling the whaling operations around the South African

coastline. The quantitative and qualitative properties of right whale blubber have remained, until now, relatively uninvestigated. The current study made use of analytical methods that did not exclude the possibility of coelution, but which were supported by trial analyses on more accurate systems. These systems indicated that all the major fatty acids (> 5% of total) were detected, and revealed previously unidentified peaks. Blubber samples were collected from various positions along the bodies of stranded southern right whales in order to determine whether there was any significant variation in the fatty acid composition from one region to the next. Such samples were also sub-divided into three macroscopically distinct layers and analysed for the same purpose. Statistical tests revealed no significant differences between positions around and along the body of a neonate. Differences in fatty acid composition between the layers of individual samples, the layers from various positions and between the layers of samples from animals of different ages (neonates, a juvenile, a subadult and an adult) were also not significant. Noting sample sizes as a limiting factor, these results seemed to indicate that random and relatively superficial (i.e. outer third/half) sampling of southern right whale blubber is acceptable for the purposes of qualitative fatty acid analysis, although clearly deeper core samples would minimize the effects of any possible stratification. The fatty acid composition of this exceptional energy and insulation depot provides possible indications of the prey species consumed by these whales as well as reflecting physiological processes within the digestive system of the southern right whale. For example, the polyunsaturated fatty acid (PUFA), C18:3, is derived originally from plant sources and is recorded in small amounts in *Euphausia superba*, but not at all in copepods, the other prey species consumed by southern right whales. This fatty acid may therefore serve as a good indicator of euphausiid consumption. The low levels of C20:1 and C22:1 (as well as the levels of C16:1, C18:3 and C20:5) seem to allow for speculation that the southern right whales sampled for this study were possibly feeding on herbivorous euphausiids rather than copepods, although confirmation of this requires further analysis of additional samples on capillary column gas chromatographs.

Thirteen major fatty acids were detected in the blubber of the southern right whales sampled for this study. Although this composition was generally consistent in all the seasonal and age classes, significant differences in the proportions of fatty acids were

detected between these groups which were predominantly related to changes in diet/available nutrients over time.

To date, the fresh biochemical contents of southern right whale stomachs have not been studied and evidence for microbial fermentation in this species has therefore not been found. The presence of the medium-chained, saturated fatty acid, C15:0, which has not been detected in southern right whale prey species, but which is usually of dietary origin, suggests that it may have been acquired from microbial sources. This fact, together with the extensive biohydrogenation of various fatty acids evident, may suggest that microbial fermentation occurs within the digestive system of southern right whales.

The general pattern of monounsaturated fatty acids (MUFA) composing the highest proportions of triacylglycerols (TAGs), followed by saturated fatty acids (SFA) and then PUFA, in non-neonatal southern right whales, follows that seen in some rorqual species. Although there are doubts about the comparability of analytical techniques, the values for North Pacific right whales, provided in the literature, do not conform to this pattern and have PUFA occurring in higher proportions than SFA. It was important to note that the slow-swimming North Pacific and southern right whales had higher proportions of SFA (for storage) and lower proportions of metabolically accessible MUFA in their blubber compared to the faster-swimming rorquals described in the literature. Differences in specific fatty acids between the North Pacific and southern right whales are detectable, possibly indicating the consumption of different prey species, however the high MUFA (especially C20:1) and low SFA content of the North Pacific right whale blubber seemed to indicate less biohydrogenation or less endogenous synthesis and possibly less efficient microbial fermentation in this species, compared to southern right whales.

The first description of total fatty acid values from southern right whale blubber tissue is given. The results presented are bracketed by results obtained from bowhead whales. The comparison of results from late season cows and calves revealed no significant differences and a linear regression revealed that the total fatty acid values were not correlated to length of sample in either of the age groups. However the

shortest sample obtained from a cow, consisting predominantly of dermal tissue, possessed the lowest total fatty acid values which indicated that very superficial sampling of blubber tissue for quantitative analysis will probably not provide true representations of the blubber tissue as a whole.

It is well known that certain characteristics of blubber tissue may vary with depth in cetaceans, e.g. histological structure and lipid content/fatty acid composition. These variations have implications for example, for energetic determinations and organo-chlorine analyses. In order to collect representative data, it is therefore important to obtain deep core blubber samples. The generally slow-moving and boat-tolerant behaviour shown by southern right whales allowed for the development of a hand-held biopsy system. This mechanism of delivery is anticipated to have less physical impact on the animal and at the same time enables non-destructive tissue sampling for histological analysis. The system is a practical and cheaper alternative to projectile systems, allowing for the collection of deep core samples from southern right whales that can be used for multidisciplinary research.

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