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

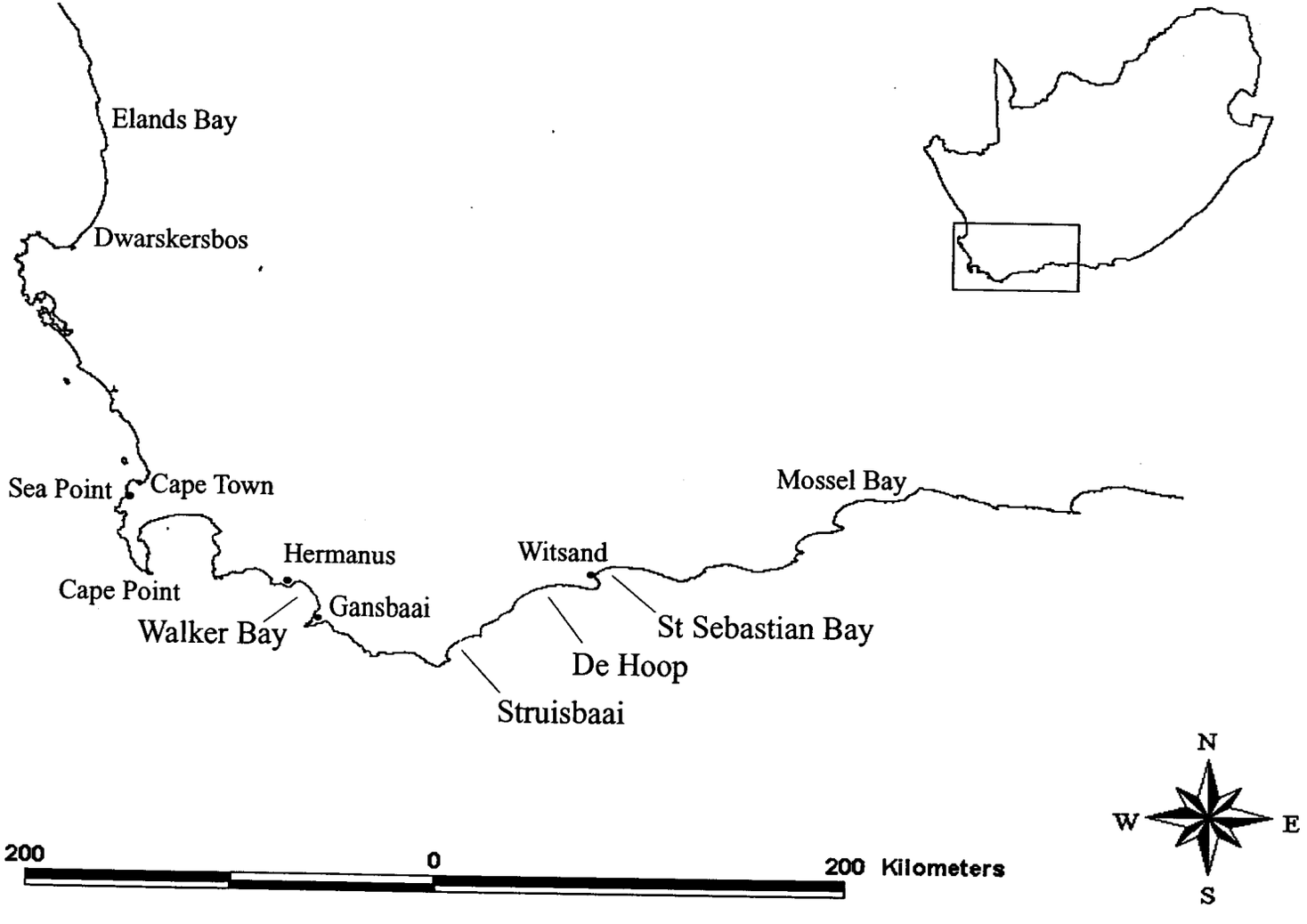

Plate 1: Map of sampling areas along the South African coastline.

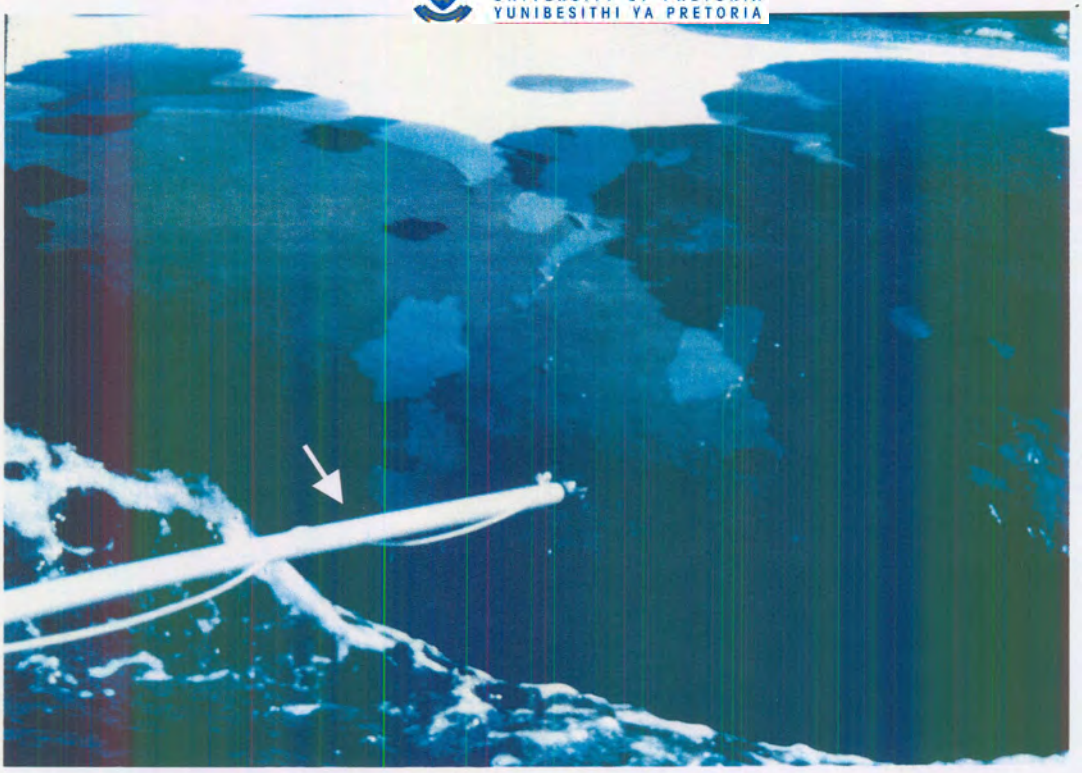

Plate 2: Biopsy pole (arrow) was inserted perpendicularly into the whale and immediately retracted. Note dorsally-located white and grey patches.

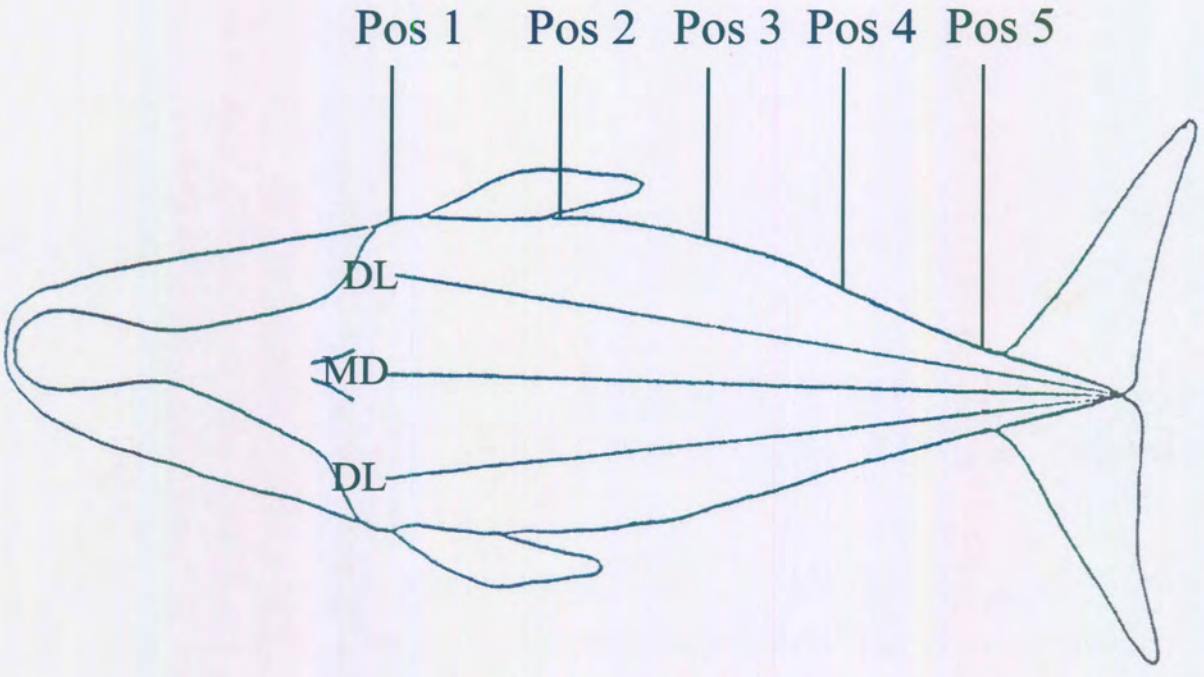

Plate 3: Sampling positions (Pos 1-5) along the bodies of stranded southern right whales. Dorso-lateral (DL) and mid-dorsal (MD) planes (lateral and mid-ventral planes are not indicated, but were sampled when possible).

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

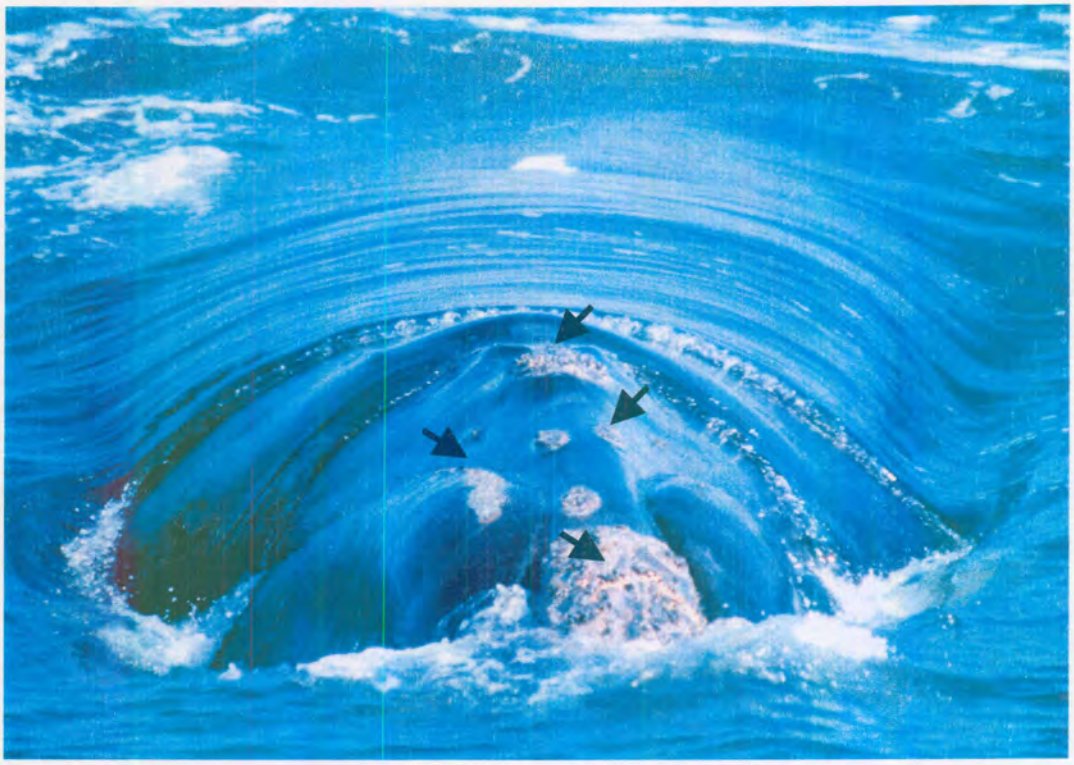

Plate 4: Raised areas of skin (arrows) on the rostrum and mandible of an adult southern right whale. Note white patch on the mid-dorsal surface used for individual identification.

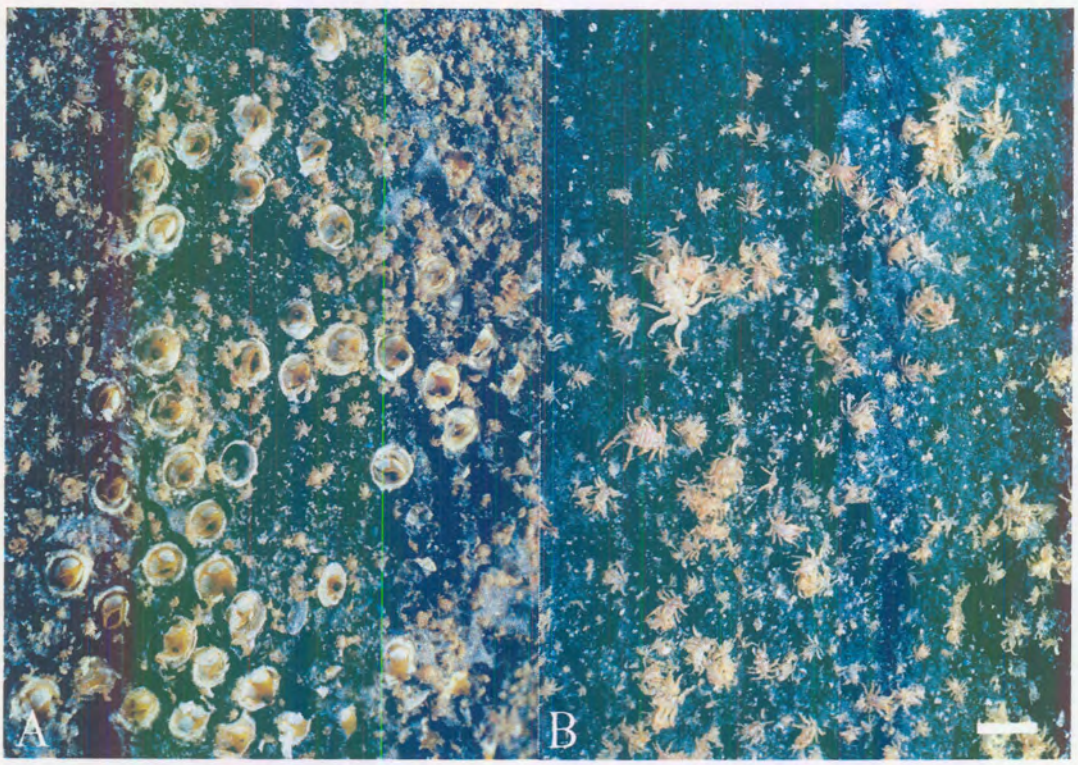

Plate 5: Barnacles (a) and cyamids (b) on the bonnet callosity of a southern right whale. Scale bar = 1.5 cm.

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.

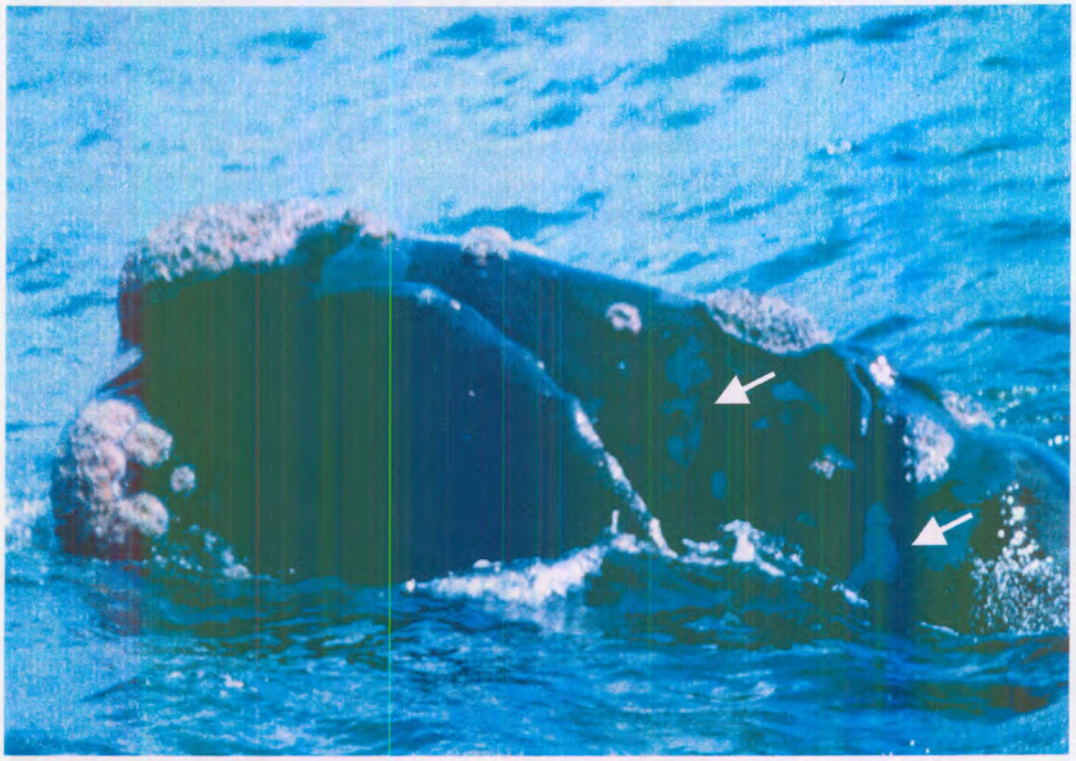

Plate 7: Grey patches (arrows) caused by sloughing of the superficial stratum corneum on the head of an adult southern right whale.

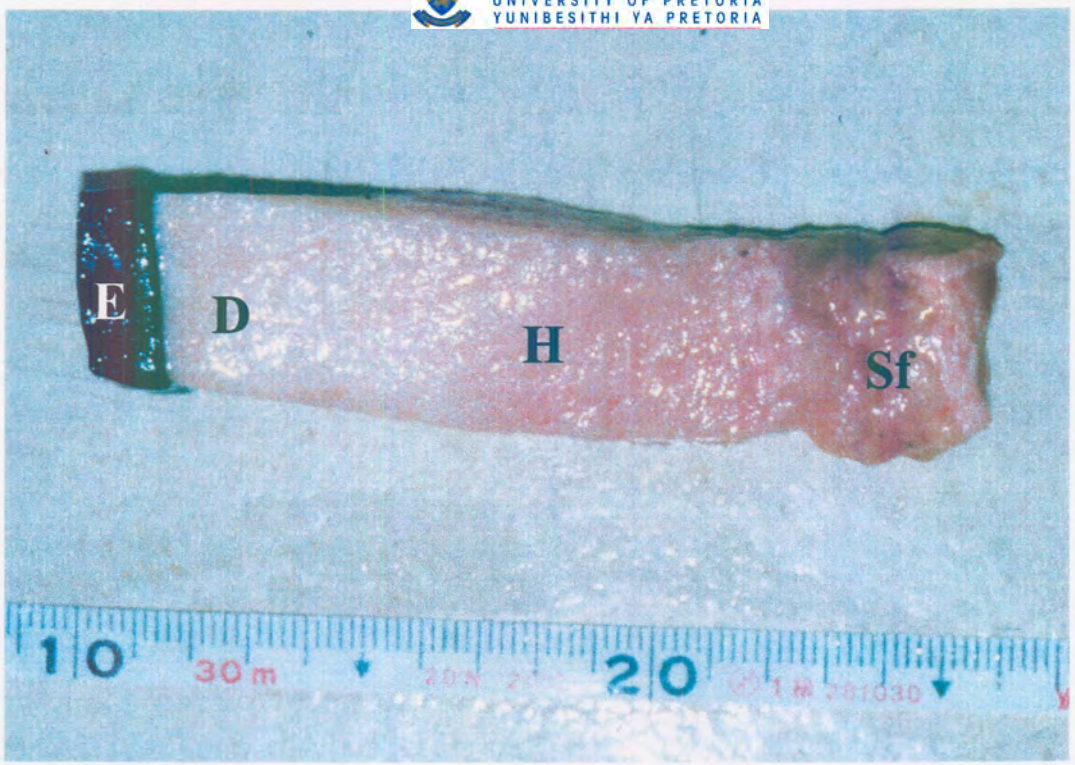

Plate 8: A core sample taken through the integument of a neonatal southern right whale. The epidermal layers (E) are heavily pigmented and noticeably thick. Dermal layer (D), hypodermal layer (H), superficial fascia (Sf) are shown.

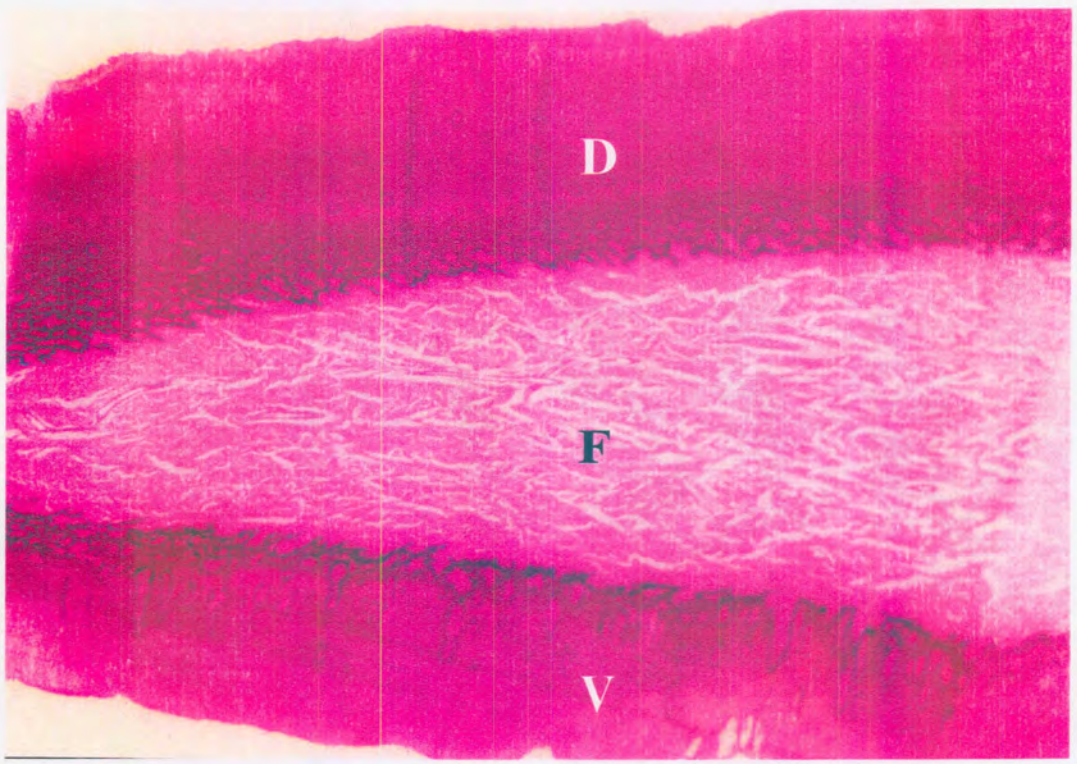

Plate 9: Longitudinal section through the fluke tip of a southern right whale calf. Note the thicker dorsal epidermis (D) compared to the ventral epidermis (V) and extensive collagen fibres (F), coloured light pink, ramifying through core of fluke. (H/E, Mag 8X).

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).

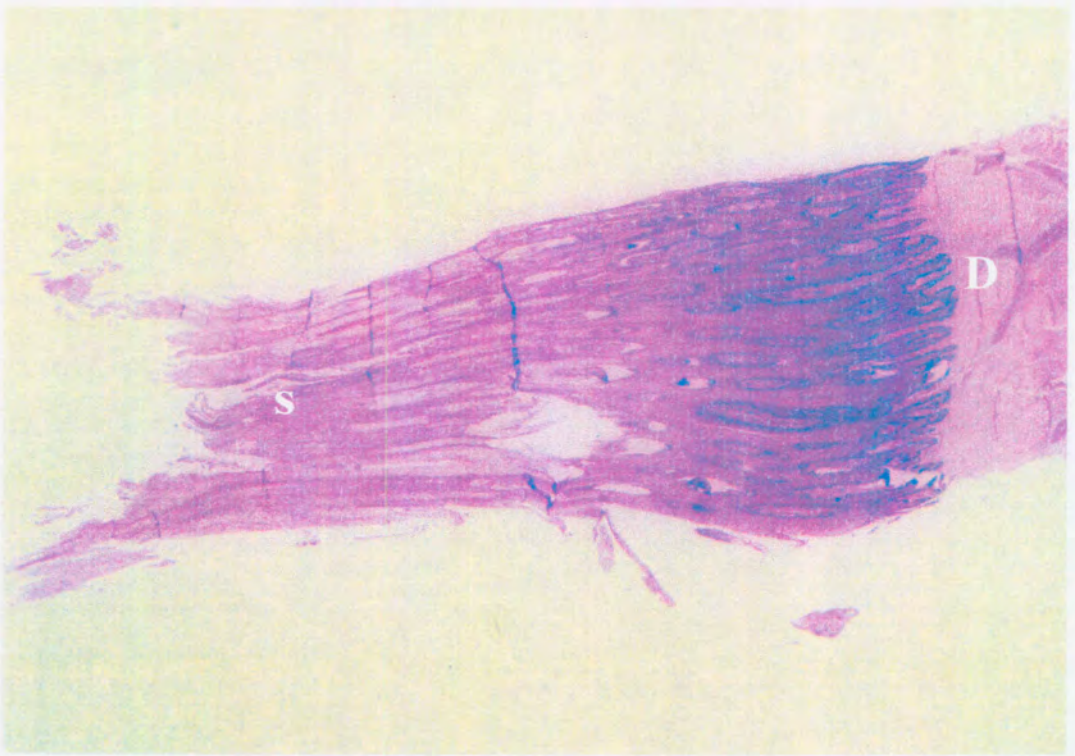

Plate 11: Longitudinal section through the epidermis of a southern right whale calf (99/05) indicates the presence of flattened stratum spinosum cells (s) and the absence of the stratum corneum. Dermal layer (D). (H/E, Mag 10X).

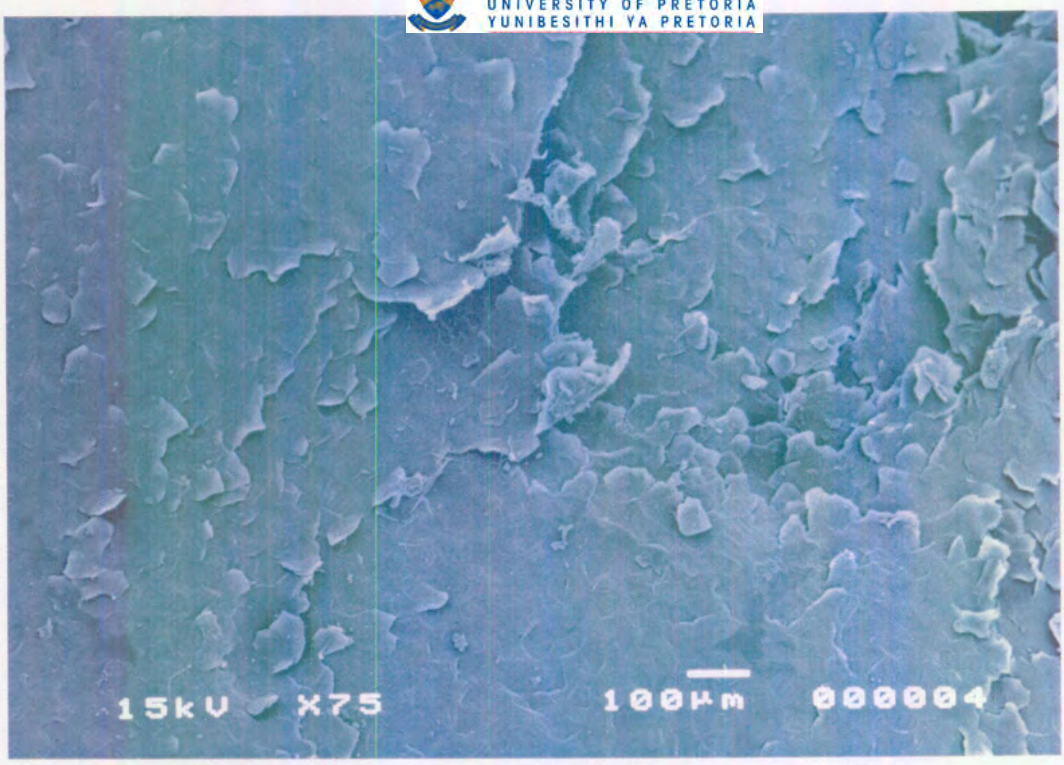

Plate 12: SEM showing the flaking of superficial squamosal keratinocytes of a late season southern right whale calf. (Mag 75X).

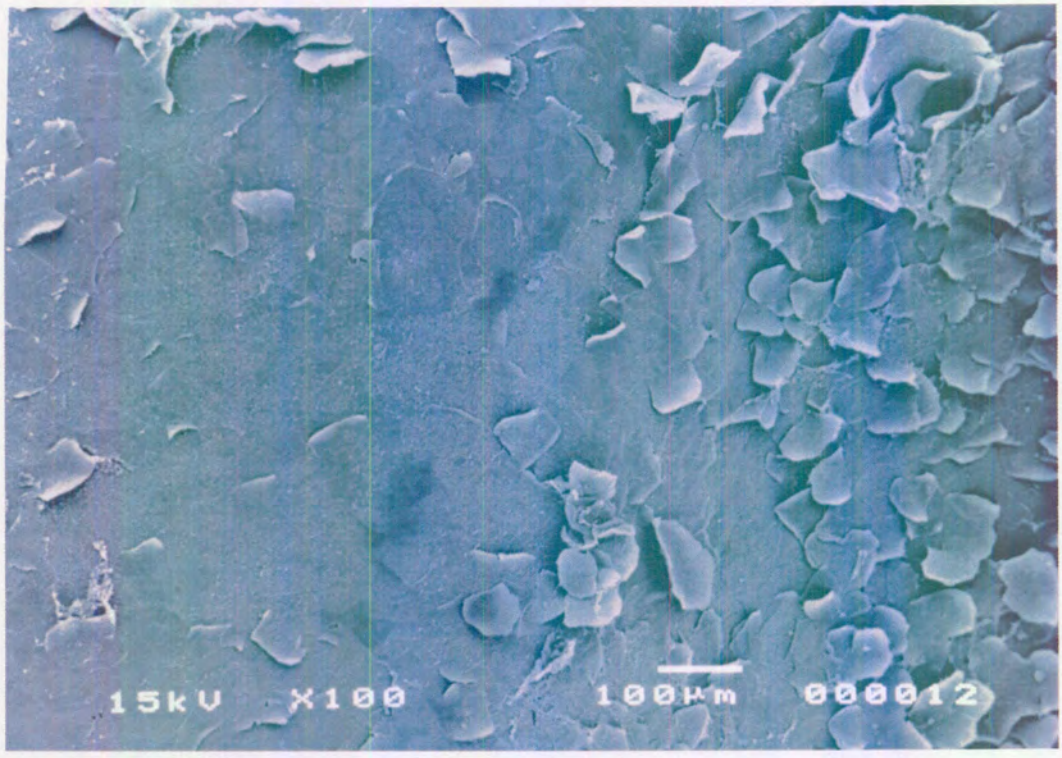

Plate 13: SEM showing the flaking of superficial squamosal keratinocytes of an adult southern right whale sampled in Antarctic waters. (Mag 100X).

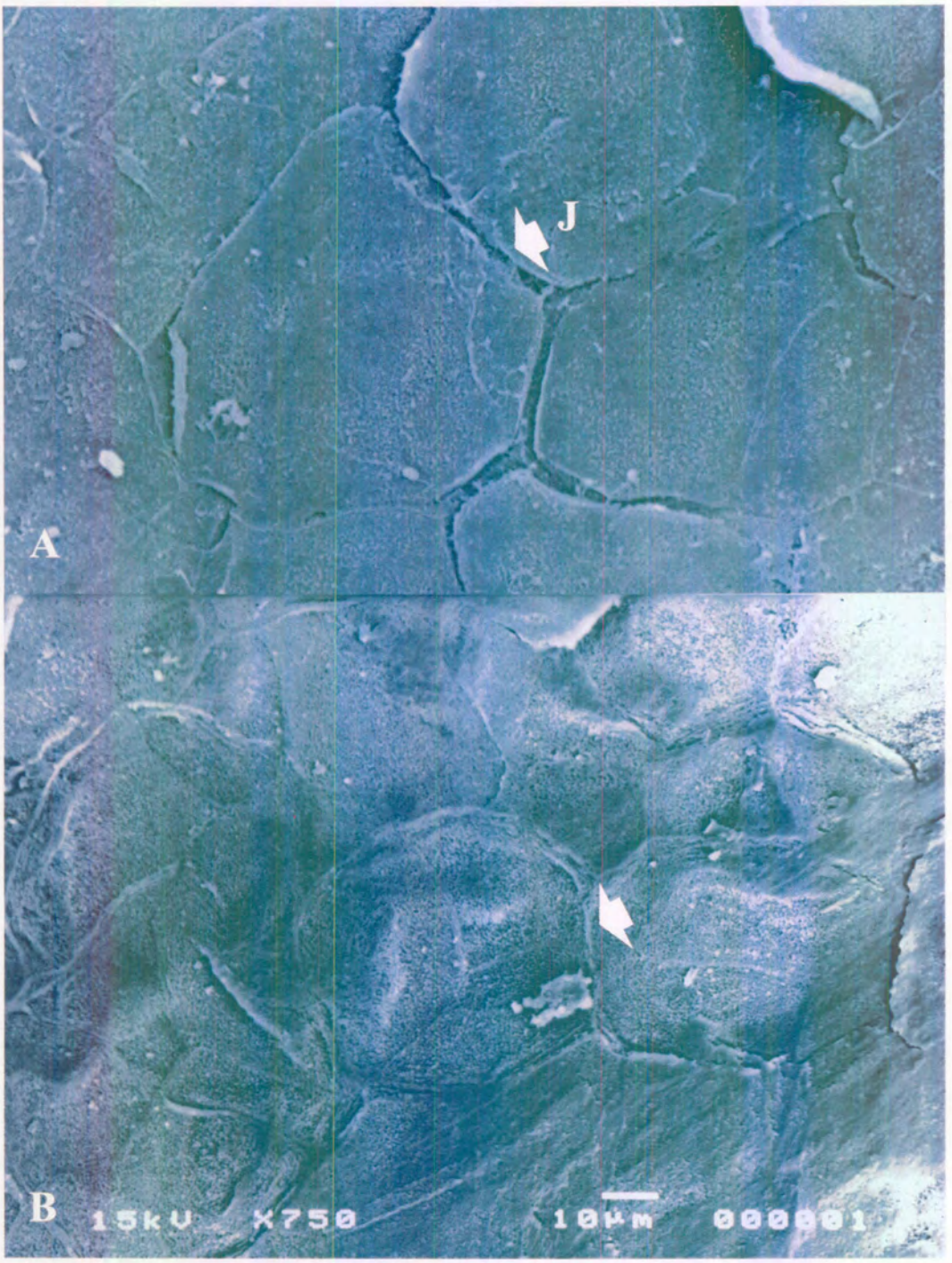

Plate 14: SEM of superficial keratinocytes with distinct pentagonally-shaped cell junctions (j) forming deep surface ridges in a honeycomb-like pattern (arrows). A, individual epidermal cells in the process of sloughing; B, cell boundaries formed by epidermal cells that have already sloughed. (Mag 750X).

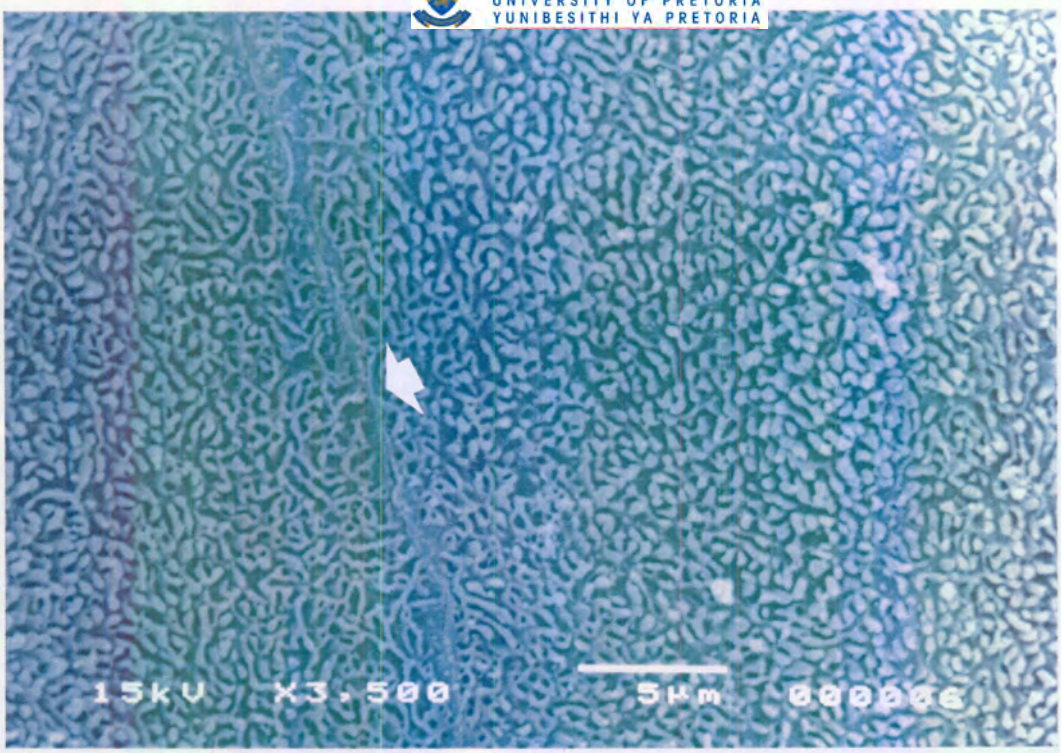

Plate 15: SEM showing the uniformly pitted appearance of the superficial epidermis exposing the disconnected and freed intercellular boundaries (arrow), after sloughing has occurred. (Mag 3 500X).

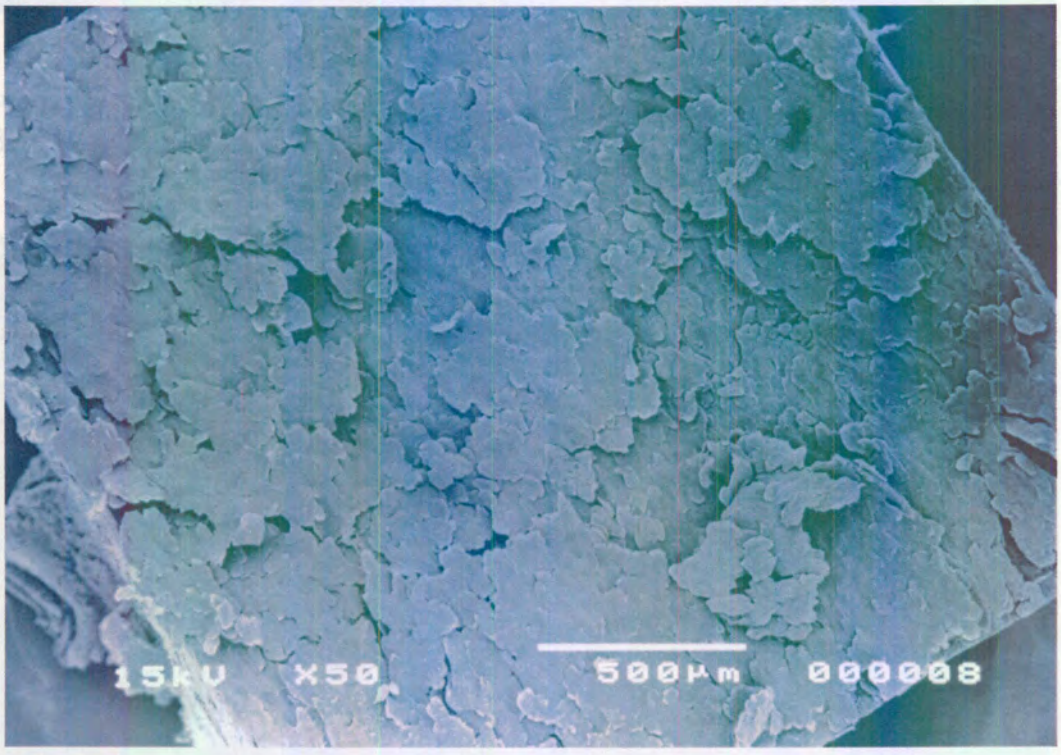

Plate 16: SEM exposing the multi-layered superficial epidermal moult of a subadult southern right whale. (Mag 50X).

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

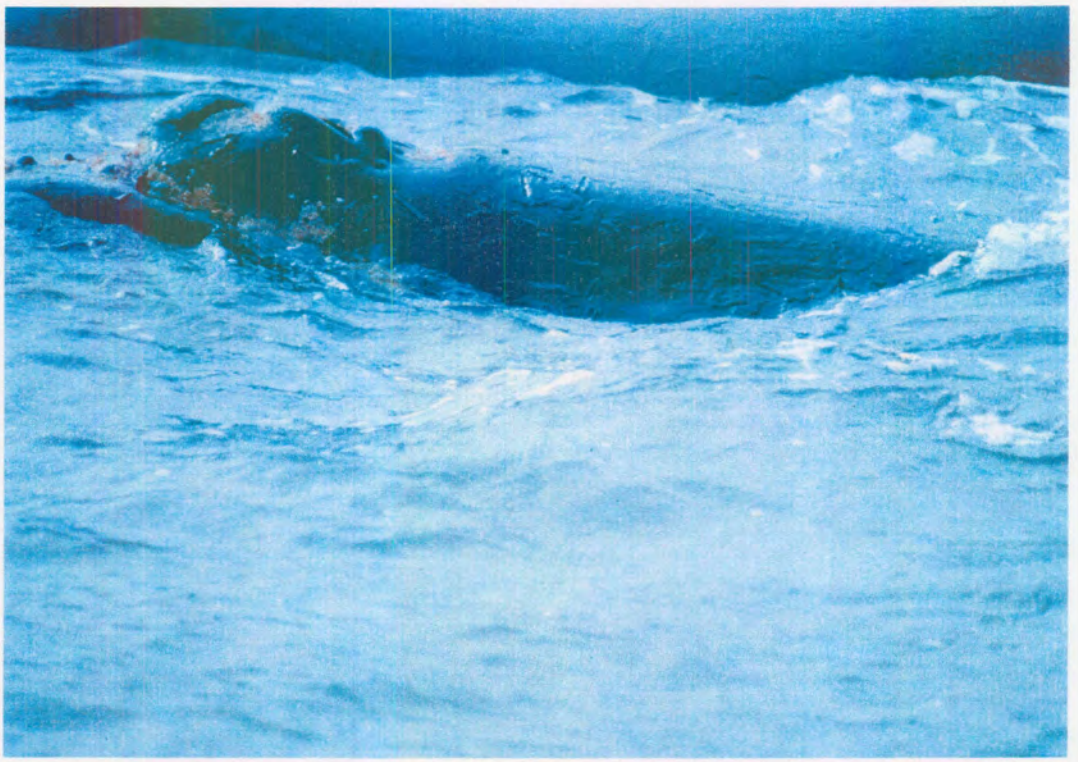

Plate 18: Southern right whale cow and "rough-skinned" calf. Note the light grey colour and rough, broken appearance of the calf's skin.

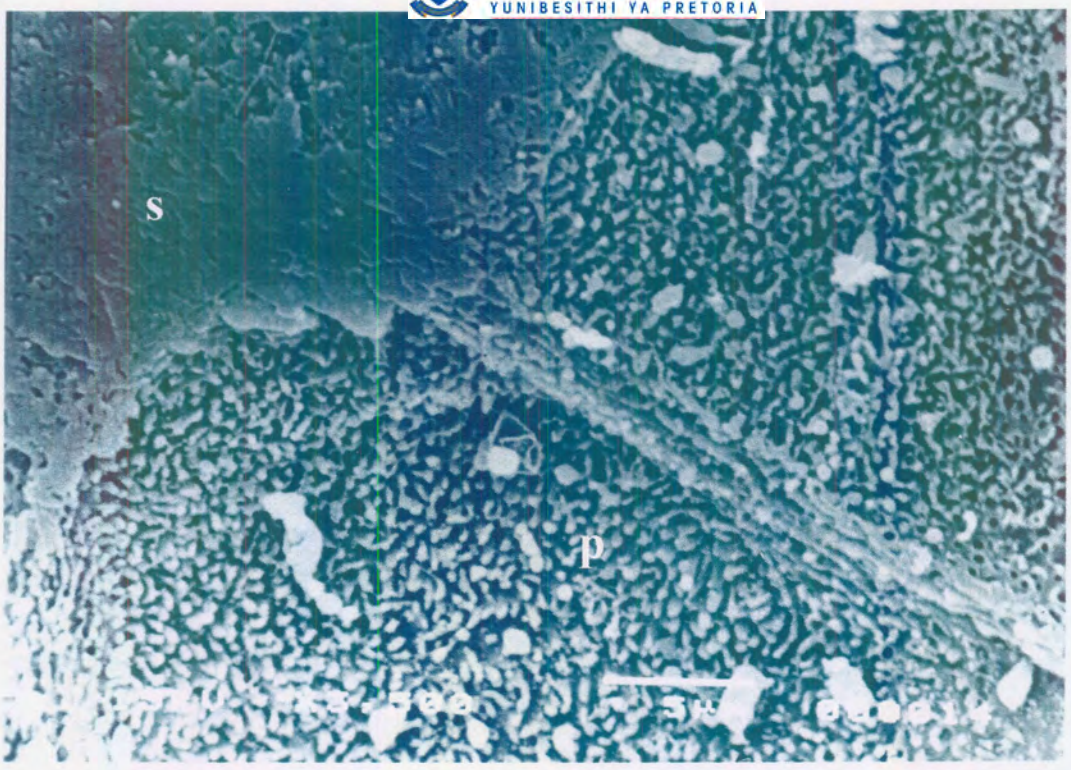

Plate 19: SEM of the skin of an early season, smooth-skinned calf. Note the patches of smooth skin (s) with no honeycomb patterns visible which give way to the exposed, pitted surface of recently sloughed areas (p). (Mag 3 500X).

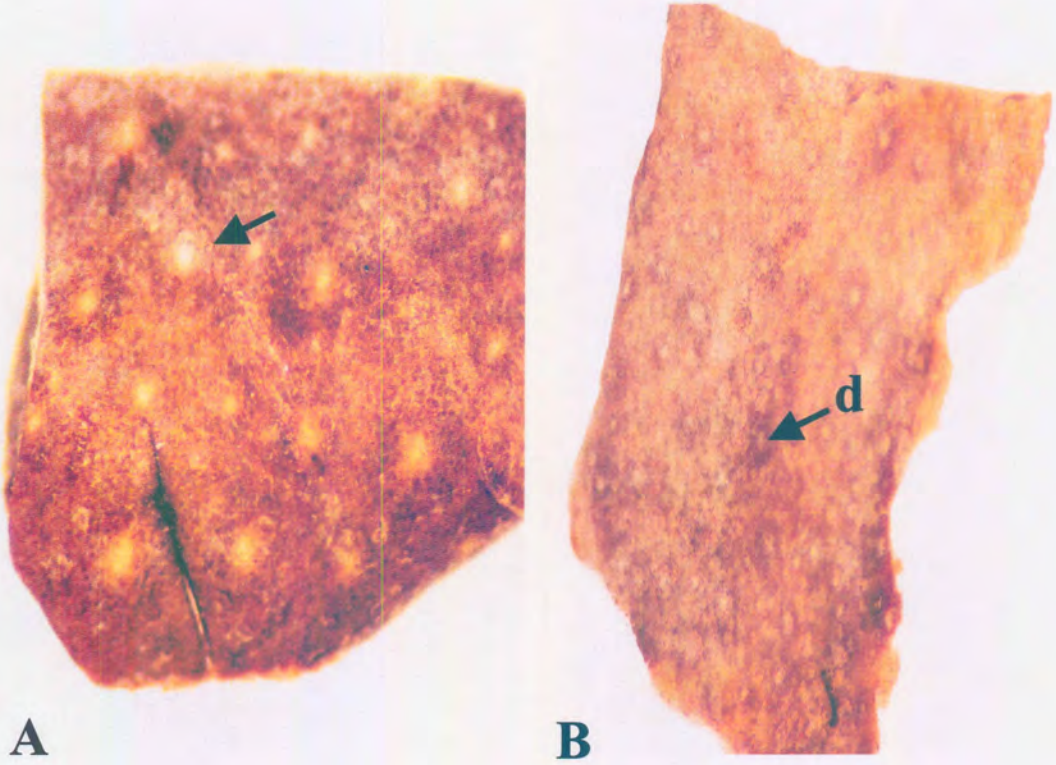

Plate 20: "Dots" on the surface of the skin of a southern right whale sampled in the Antarctic (a) and a brindle-coloured southern right whale calf (b) showing the distinct dark ring (d) around the"dots".

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

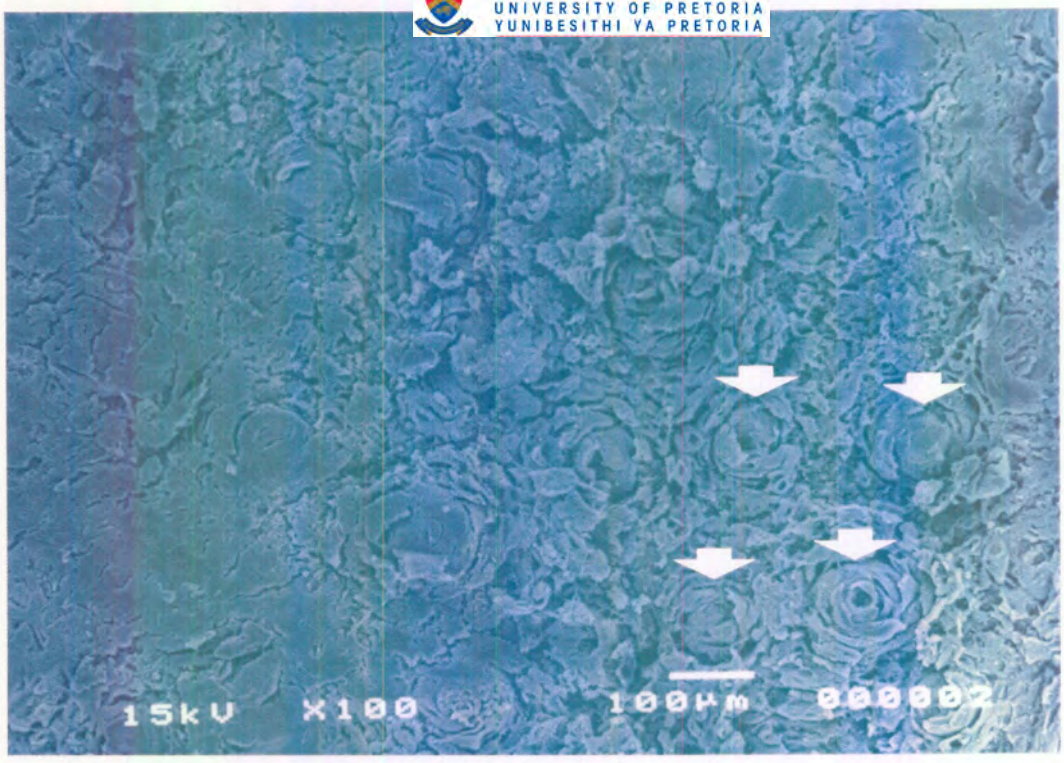

Plate 21: SEM showing the irregular nature of the superficial epidermis of a "rough-skinned" southern right whale calf. Note the exposed keratinocyte rosettes around and superficial to the dermal papillae (arrows). (Mag 100x).

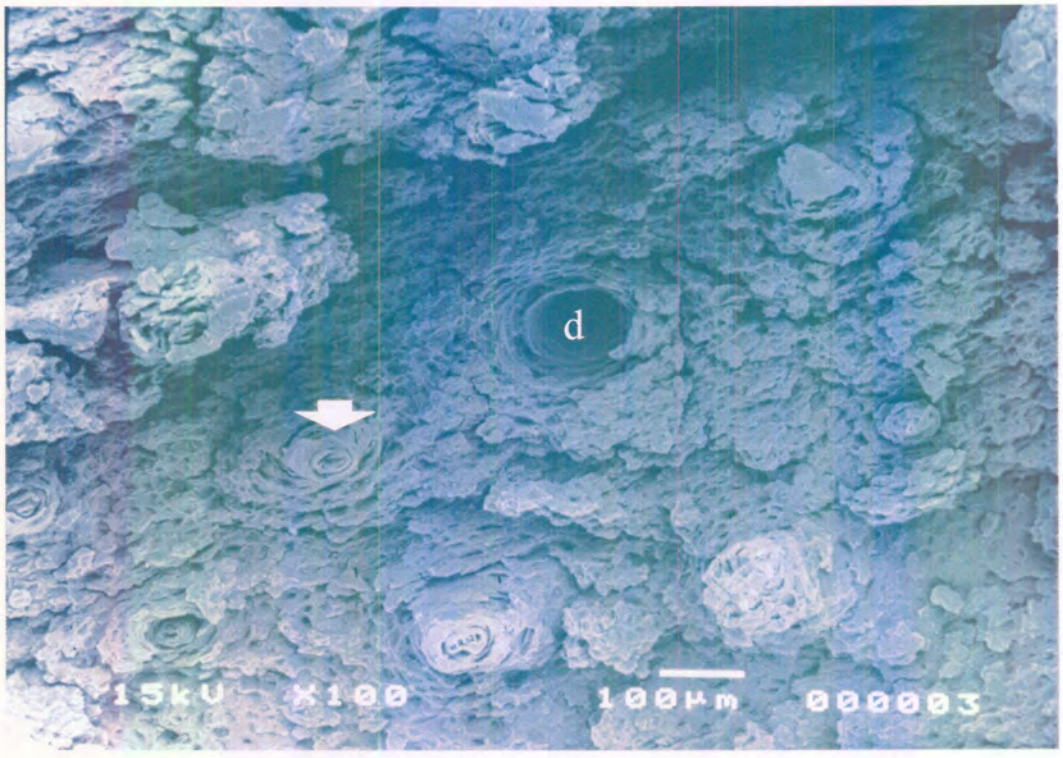

Plate 22: SEM of exposed keratinocyte rosettes around and superficial to the dermal papillae (arrow) of the skin of a stranded adult southern right whale (89/30). Decomposed dermal papilla (d). Absence of stratum corneum, probably due to decomposition. (Mag 100x).

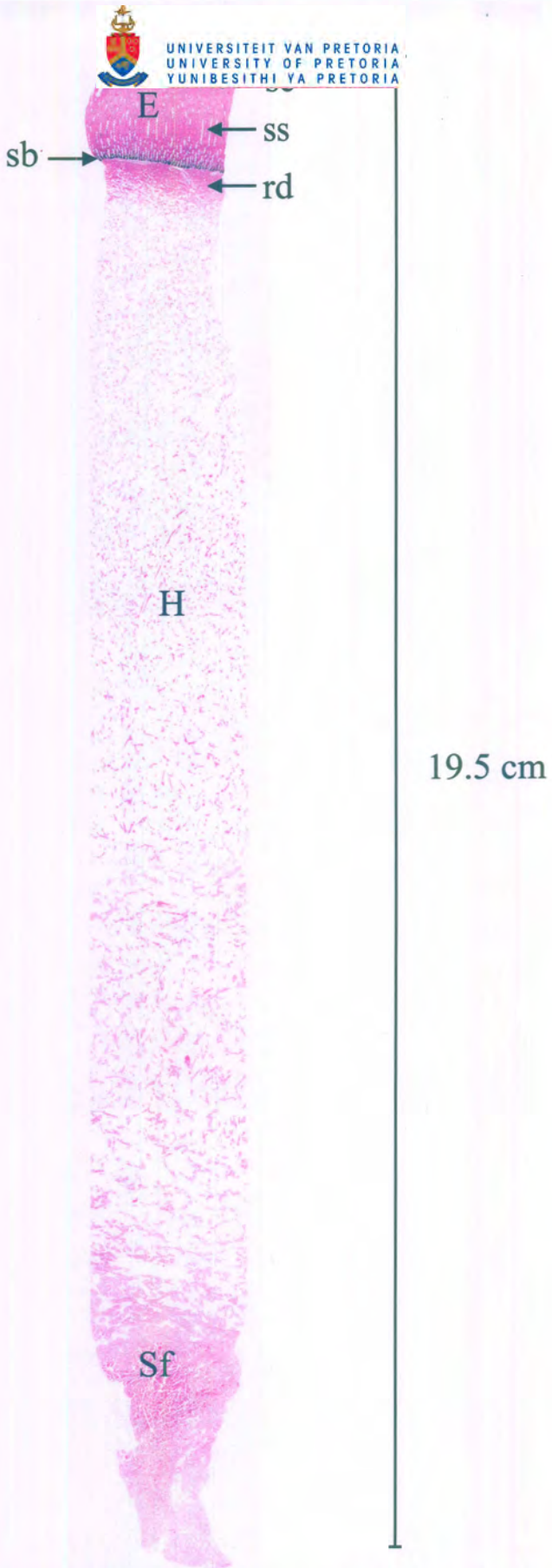

Plate 23a: Longitudinal section through the integument of a juvenile southern right whale (00/11), killed by a boat collision. Epidermis (E), stratum corneum (sc), stratum spinosum (ss), stratum basale (sb), reticular dermis (rd), hypodermis (H) infiltrated with collagen fibres (pink lines) and adipocytes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

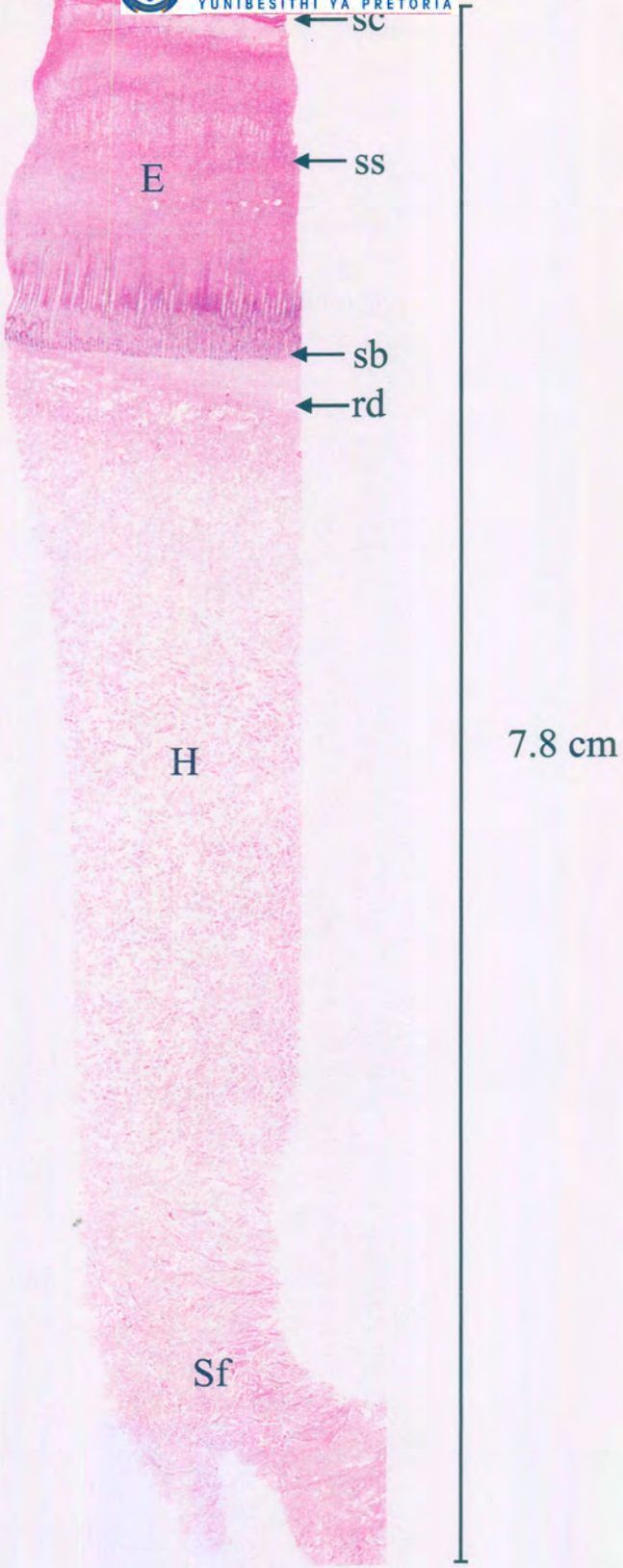

Plate 23b: Longitudinal section through the integument of a neonatal southern right whale (00/09). Epidermis (E), stratum corneum (sc), stratum spinosum (ss), stratum basale (sb), reticular dermis (rd), hypodermis (H) infiltrated with collagen fibres (pink lines) and adipocytes (white spaces), superficial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

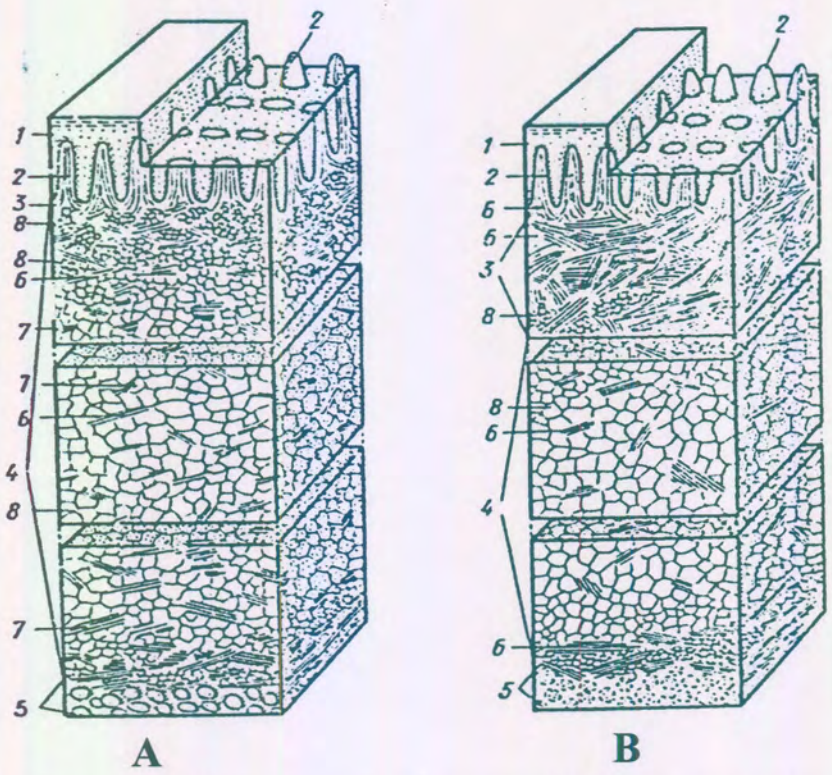

Plate 24: Structure of the integument of rorquals (A) and toothed cetacea (B). Legend: 1 = epidermis, 2 = dermal papillae, 3 = dermis, 4 = hypodermis, 5 = subcutaneous musculature, 6 = bundles of collagen fibres, 7 = bundles of elastin fibres, 8 = adipocytes (from V. Sokolov, 1955).

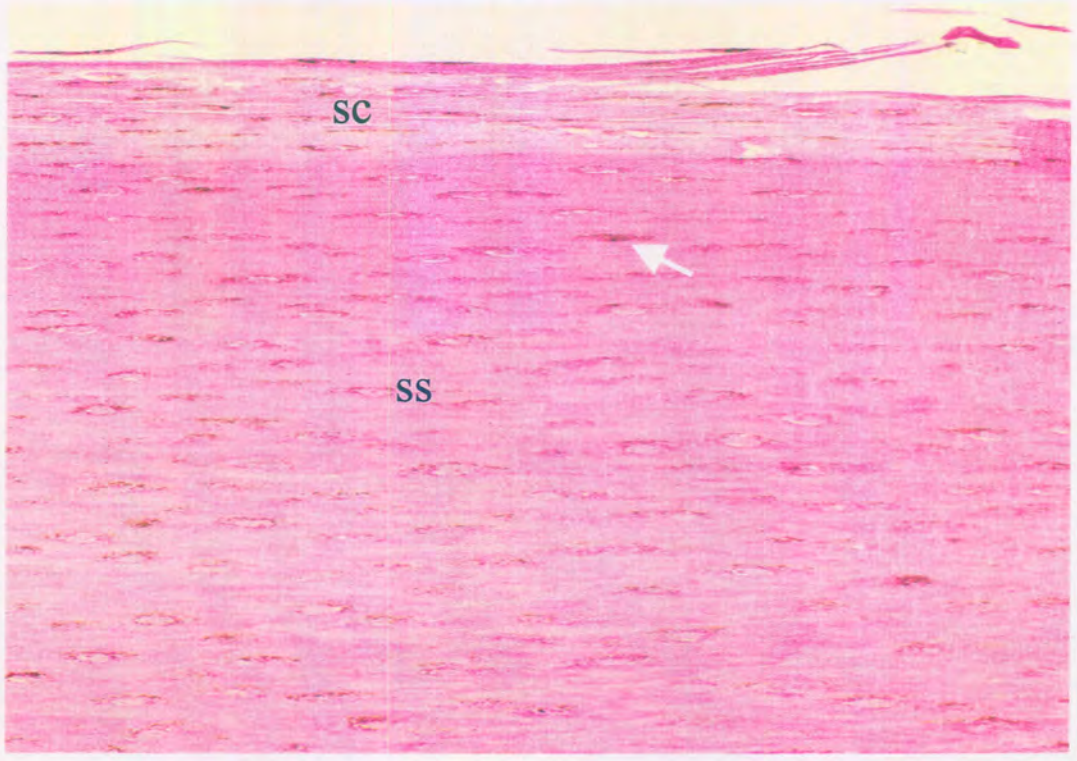

Plate 25: Stratum corneum (sc) and stratum spinosum (ss) layers of the epidermis of an adult southern right whale. Note melanin granules stained black (arrow). (H/E, Mag 200X).

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

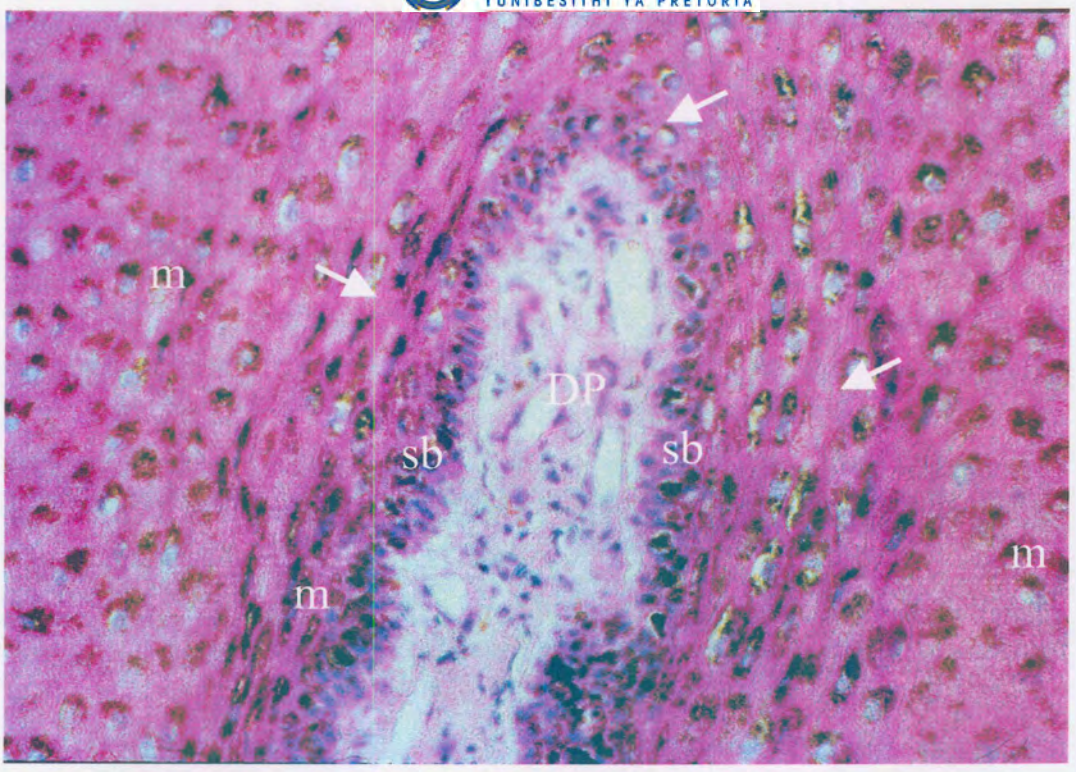

Plate 26: A dermal papilla (DP) protruding into the stratum spinosum of a non-calf southern right whale. Note flattened stratum spinosum cells along the sides and tip of the papilla (arrows), melanin granules (m) stained black and stratum basale. (H/E, Mag 200x).

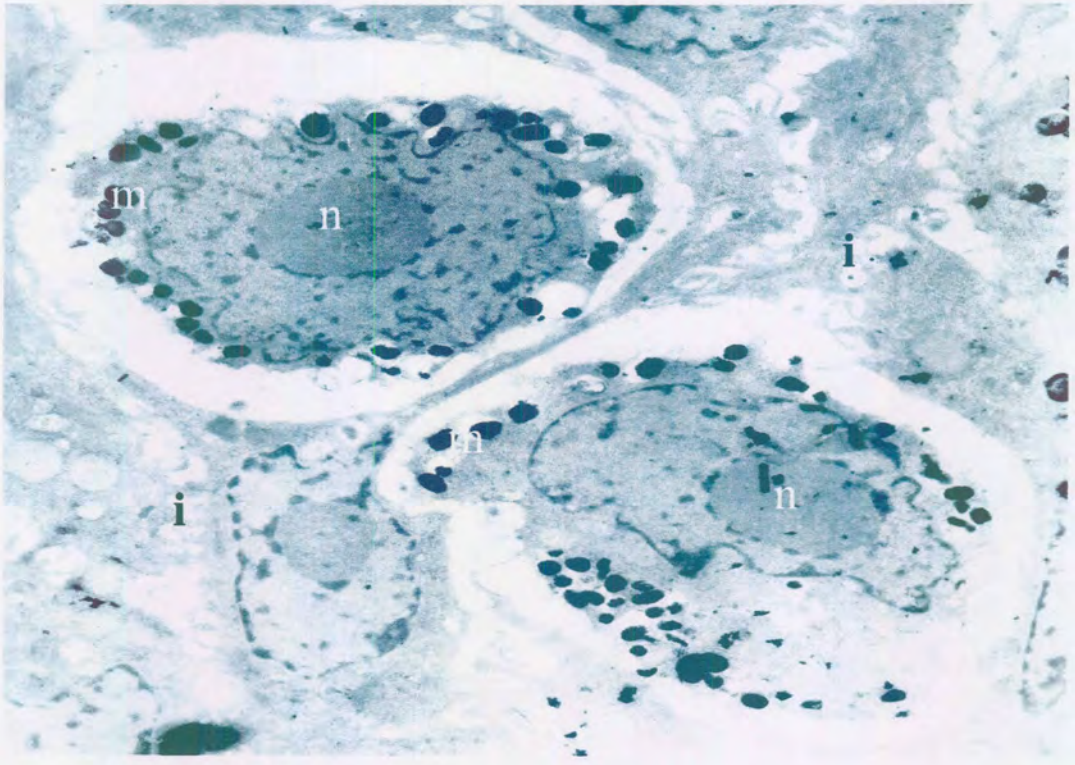

Plate 27: TEM showing the presence of melanin granules (m - black dots) around the nuclei of stratum spinosal cells. Note the thick cell boundaries formed by inter-folding cell membranes (i), nucleolus (n). (Mag 12 000X).

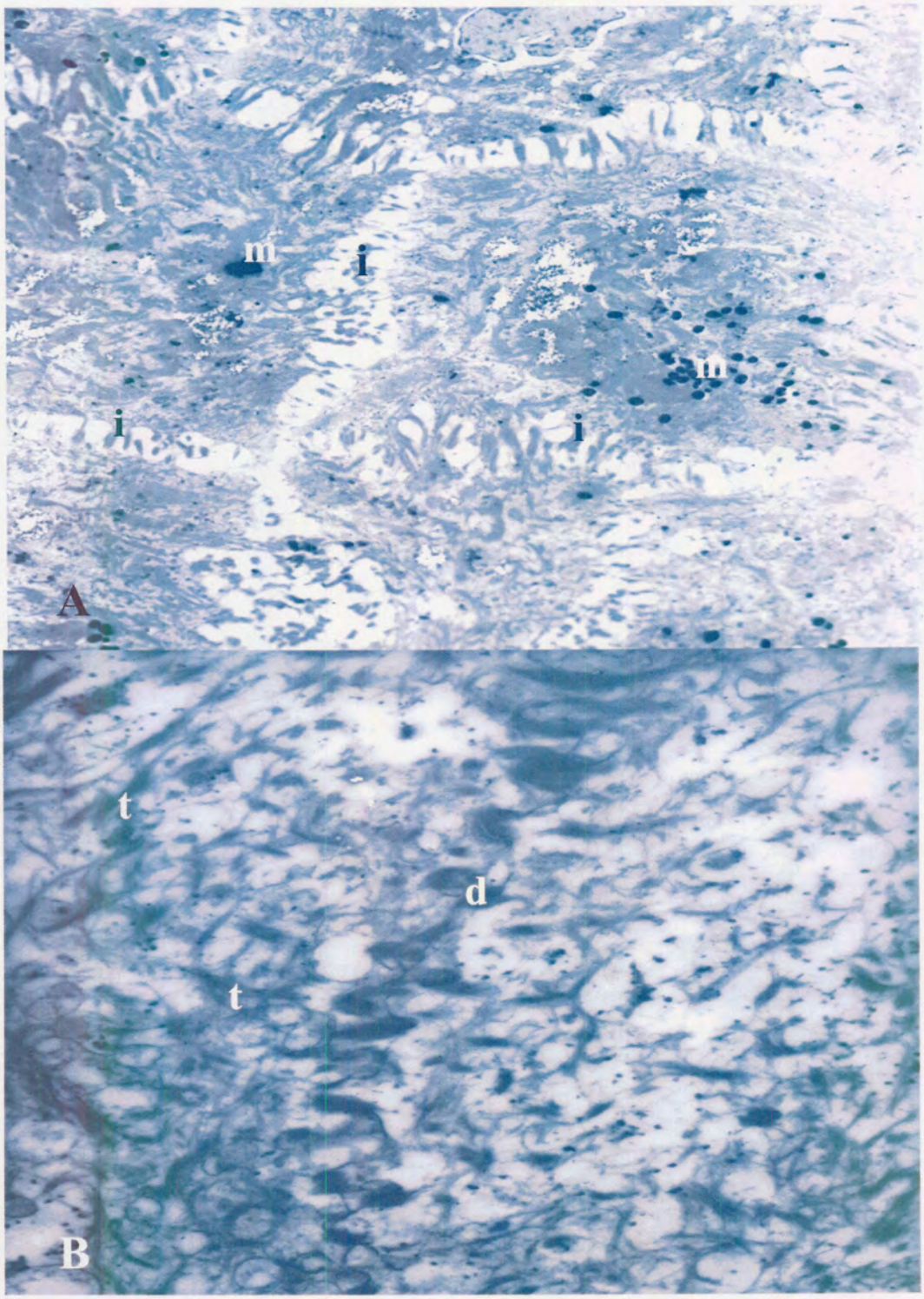

Plate 28: TEM showing the extensive interfolding of stratum spinosum cell membranes (i) connected by desmosomes (d). Tonofilaments (t) are present in parallel bundles within these cells, melanin granules (m) are also present. (A = Mag 4 500X, B = Mag 5 000X).

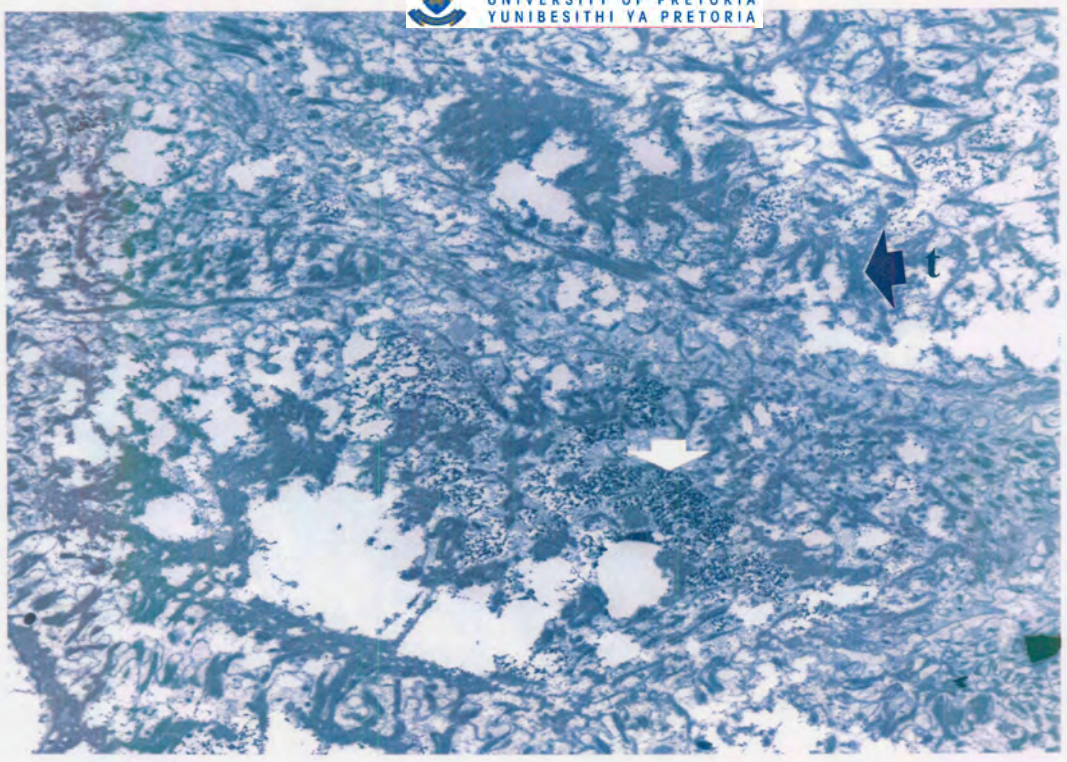

Plate 29: TEM showing large groups of glycogen granules (arrow) in the cytoplasm of spinosal cells. Tonofilaments (t with arrow). (Mag 6 000X).

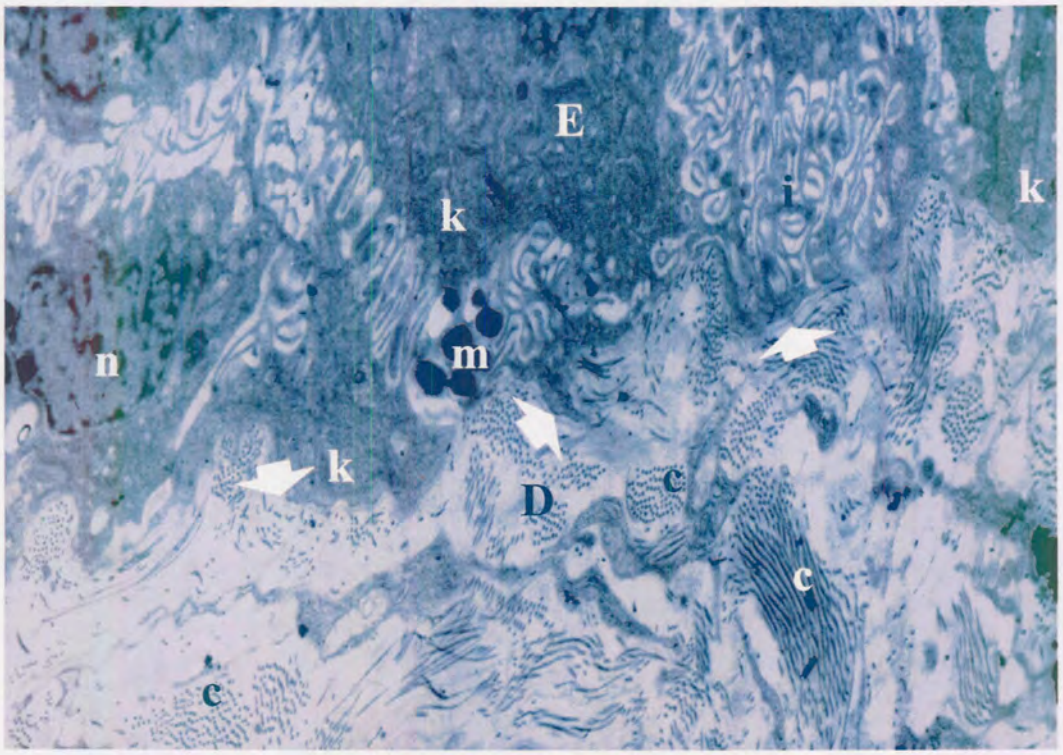

Plate 30: The stratum basale of the epidermis consisted of a layer of variably shaped keratinocytes (k) which interdigitate with the basal lamina (arrows) separating the epidermis (E) and the dermis (D). Melanin granules (m), nucleolus (n), interconnecting cell membranes (i), collagen fibres (c). (Mag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

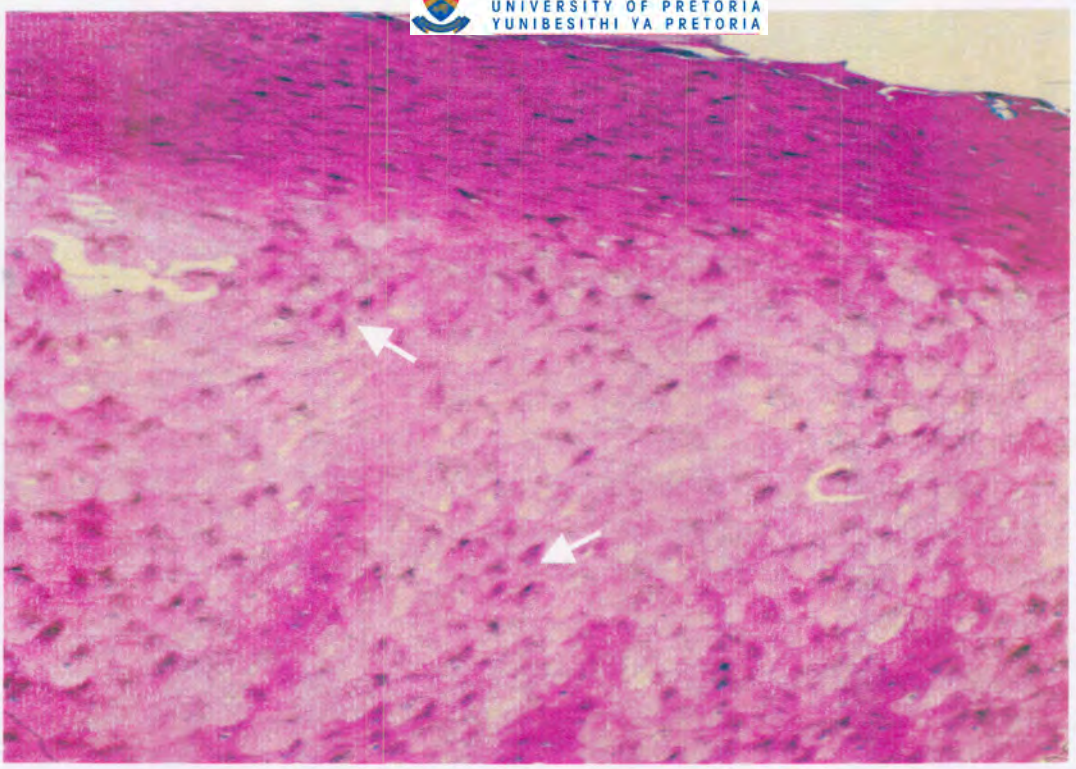

Plate 31: Stratum spinosum and stratum corneum cells of the epidermis of a "rough-skinned" southern right whale calf (00/09). Note reduced concentrations of melanin granules (arrows) compared to Figure 25. (H/E, Mag 100X).

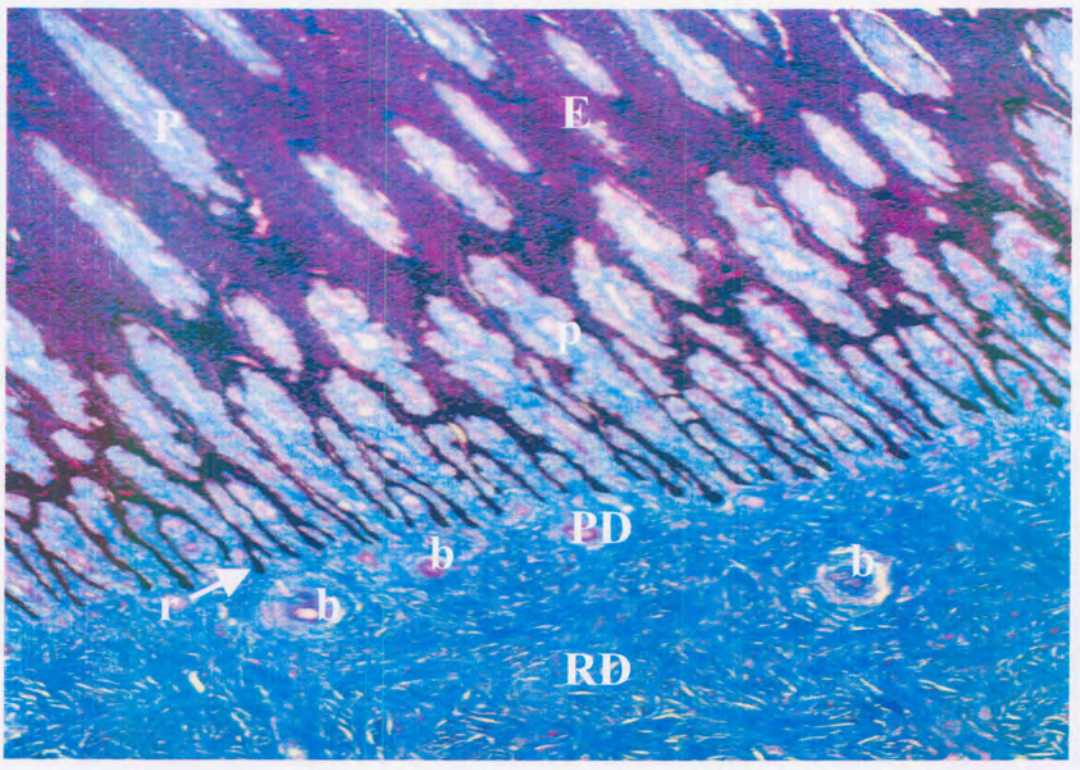

Plate 32: The skin of a neonatal southern right whale calf (98/09). Dermal papillae (p) reach from the base of the papillary dermis (PD) into the epidermis (E) and epidermal rete (r) interdigitate with the dermal papillae. The reticular dermis (RD) consists of dense collagen fibres (dark blue) with blood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

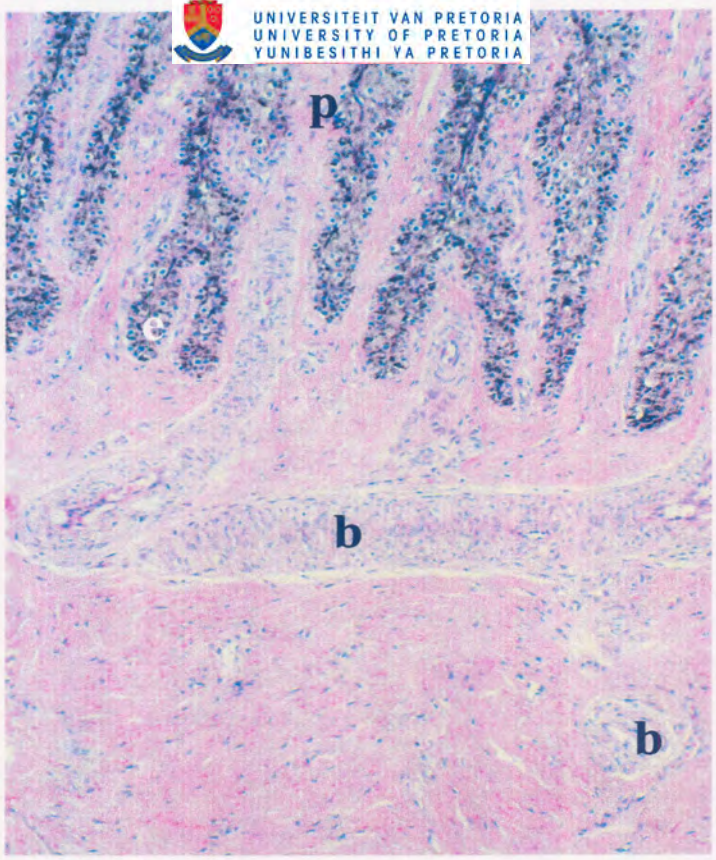

Plate 33: Blood vessels (b) extending from the papillary dermis into dermal papillae (p) and between epidermal rete (e) of the skin of a neonatal southern right whale (00/09). (H/E, Mag 100X).

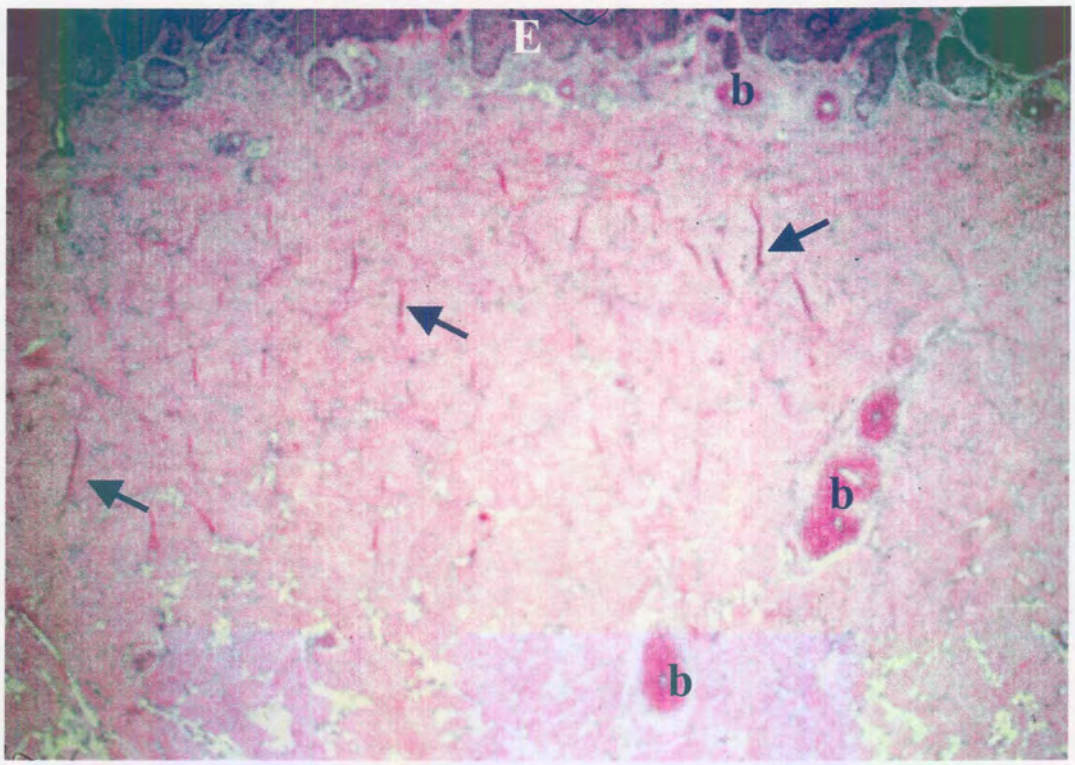

Plate 34: A skin sample from a neonatal southern right whale (00/10) showing the reticular dermis consisting of tightly packed collagen bundles (arrows) forming a "fat-free" zone. Blood vessels (b), shown in irregular cross-section here, course through this layer. Epidermis (E). (H/E, Mag 25X).

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

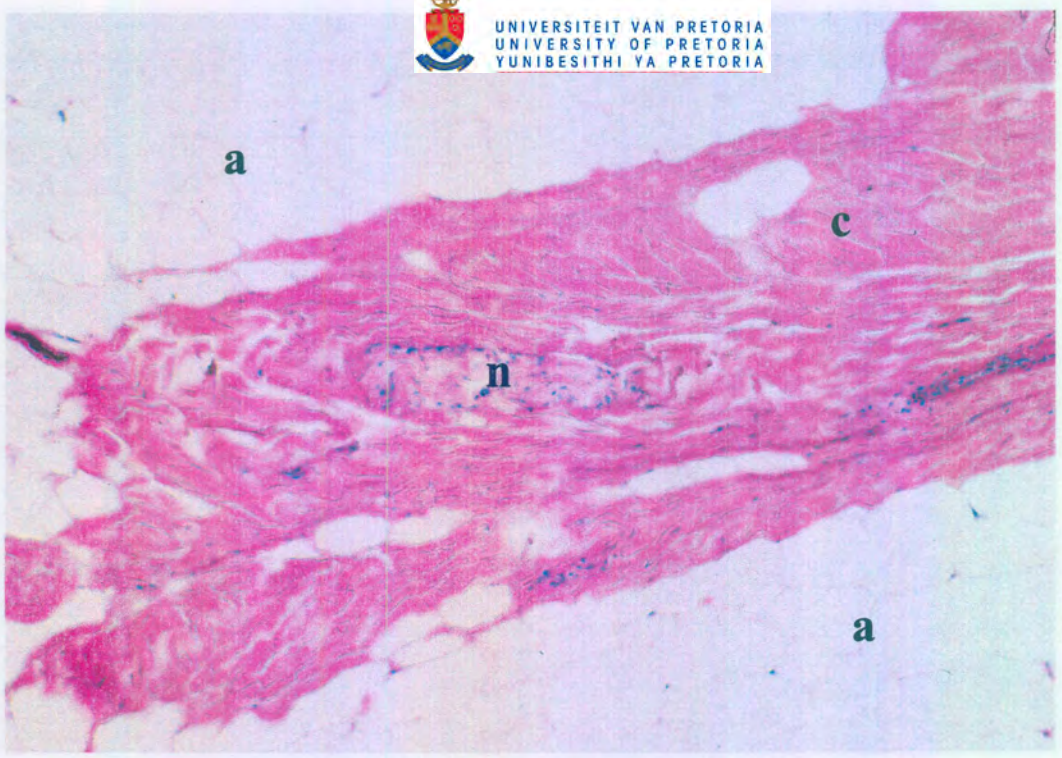

Plate 35: A nerve (n) extending through a collagen fibre bundle (c), surrounded by adipocytes (a), within the hypodermis of a neonatal southern right whale (00/09). (H/E, Mag 100X).

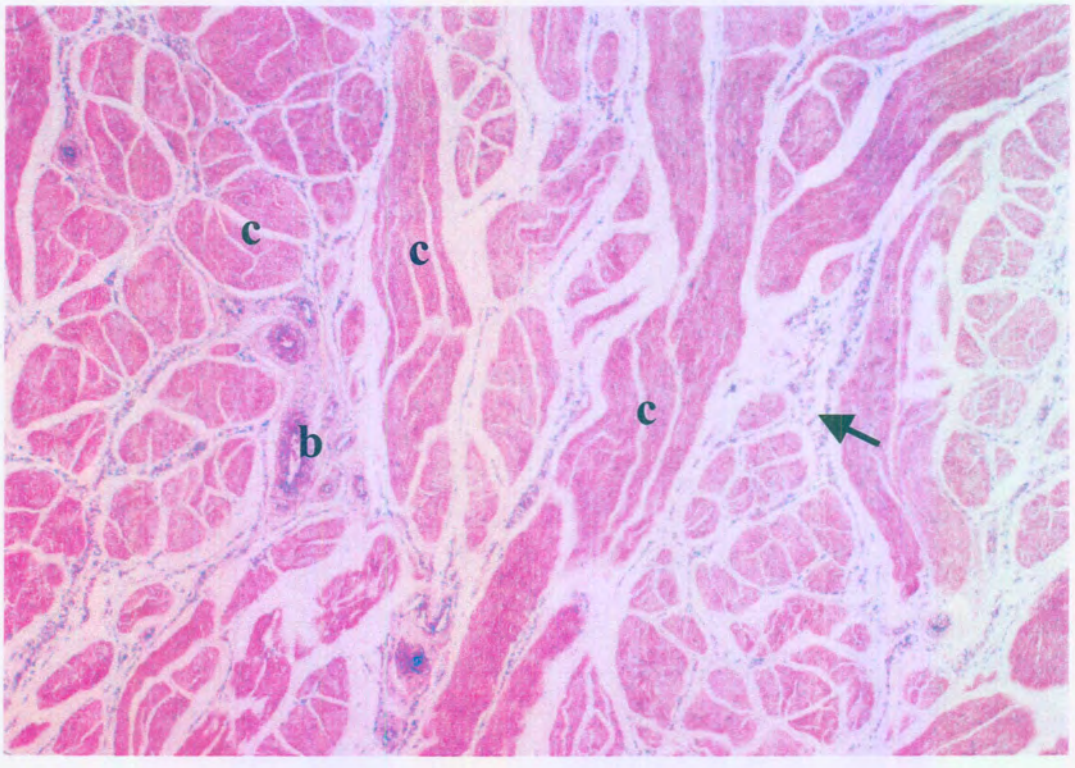

Plate 36: Vascularisation (b) is evident within collagen bundles (c) of the hypodermis of a juvenile southern right whale (00/11). Note the various orientations of the collagen bundles. Only remnants of adipocytes are visible (arrow) due to autolysis. (H/E, Mag 25X).

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).

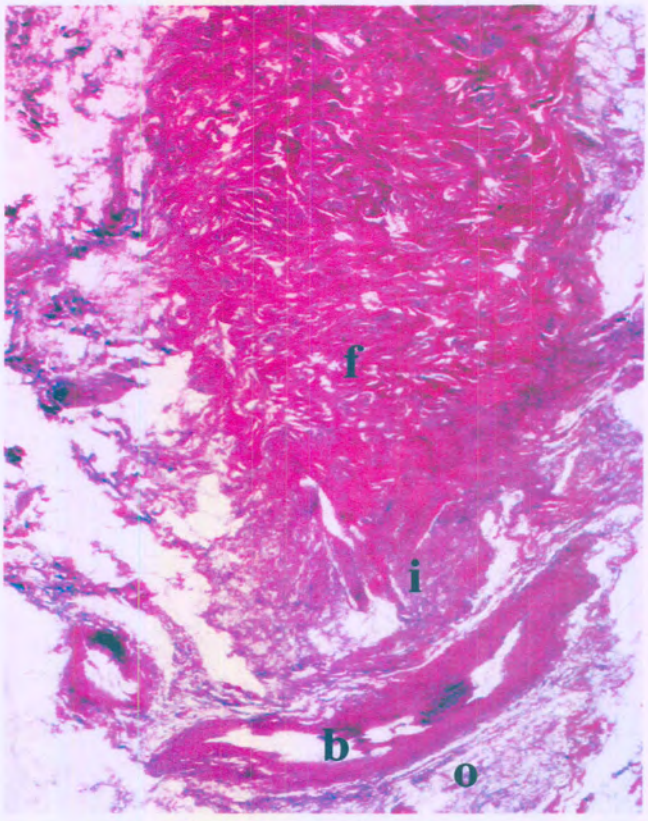

Plate 38: Photomicrograph of the base of the hair follicle (f) in Figure 37 showing the blood sinus (b) appendage between the inner (i) and outer dermal (o) connective tissue sheaths. (H/E, Mag 25X).

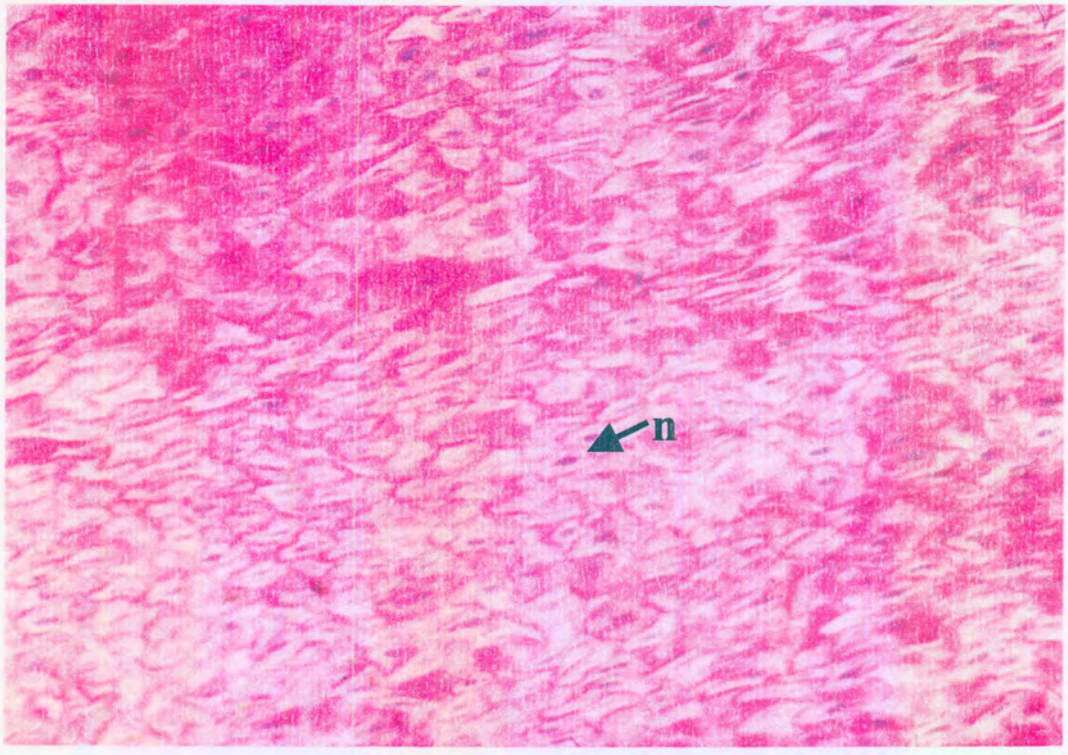

Plate 39: A longitudinal section through an epidermal "stalk", from the head of a stranded juvenile shows this structure consisting of viable, nucleated stratum spinosum cells (n). (H/E, Mag 200X).

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

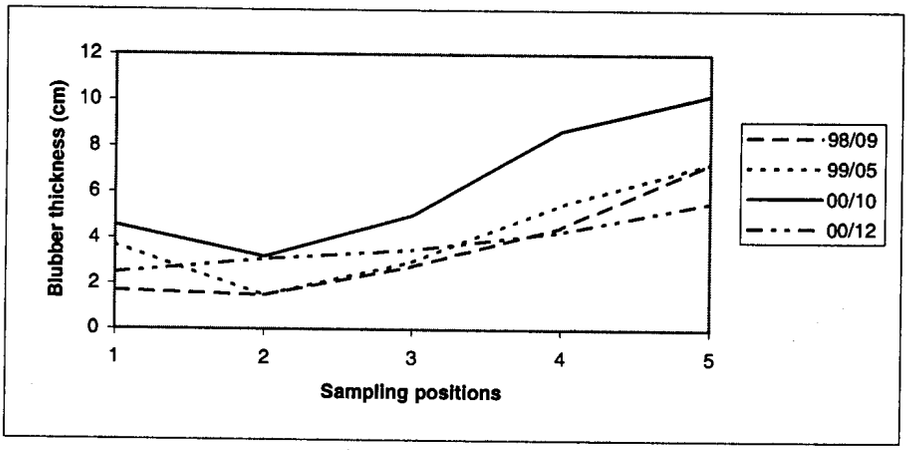

Figure 1: Blubber thickness measurements on the mid-dorsal plane along the body of neonatal southern right whales.

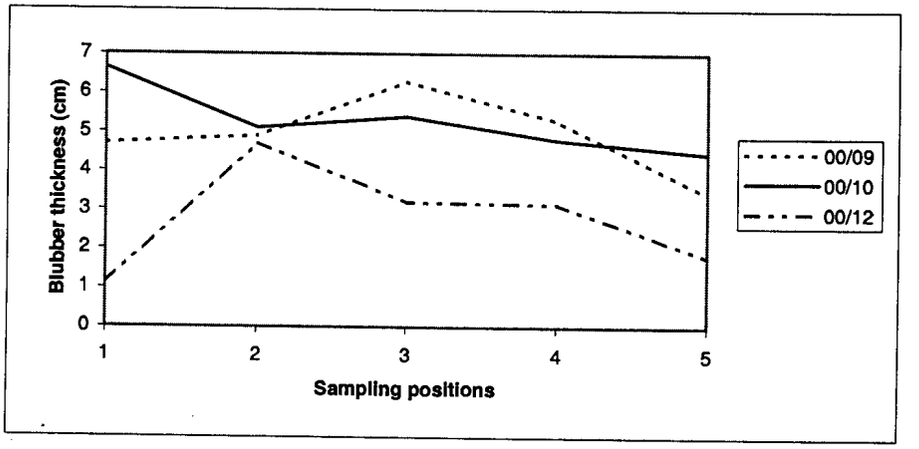

Figure 2: Blubber thickness measurements on the lateral plane along the body of neonatal southern right whales.

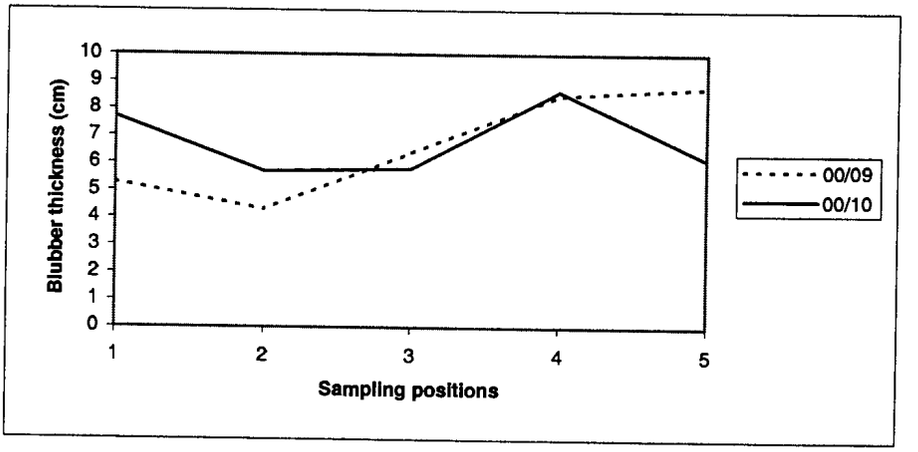

Figure 3: Blubber thickness measurements on the ventral plane along the body of neonatal southern right whales.

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

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

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,

* Whale Unit, Mammal Research Institute, University of Pretoria, c/o S A Museum, Cape Town, South Africa.

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).

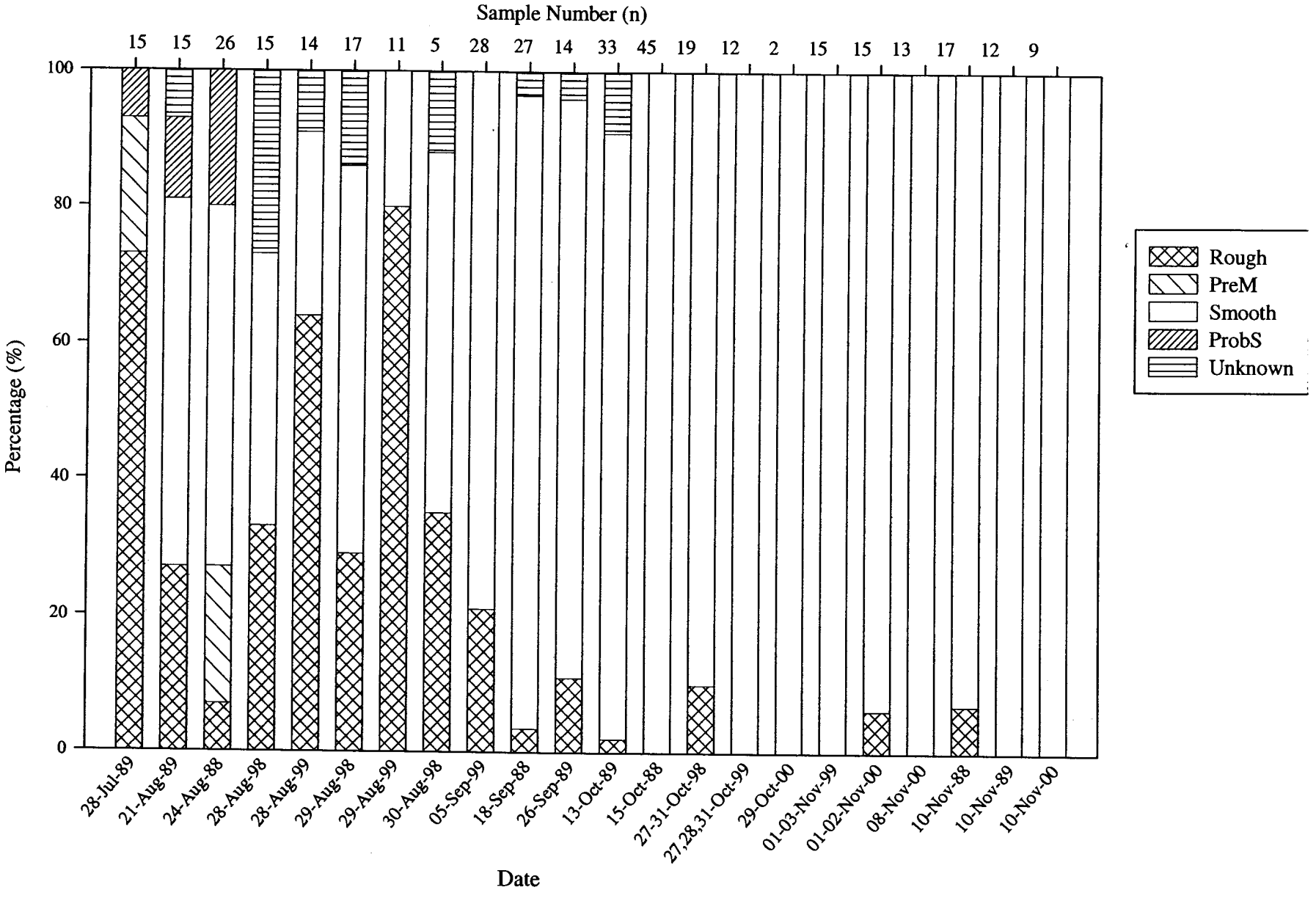

Figure 4: Frequency of different skin conditions seen in southern right whale calves during biopsy sampling and aerial surveys, South Africa 1988-1999

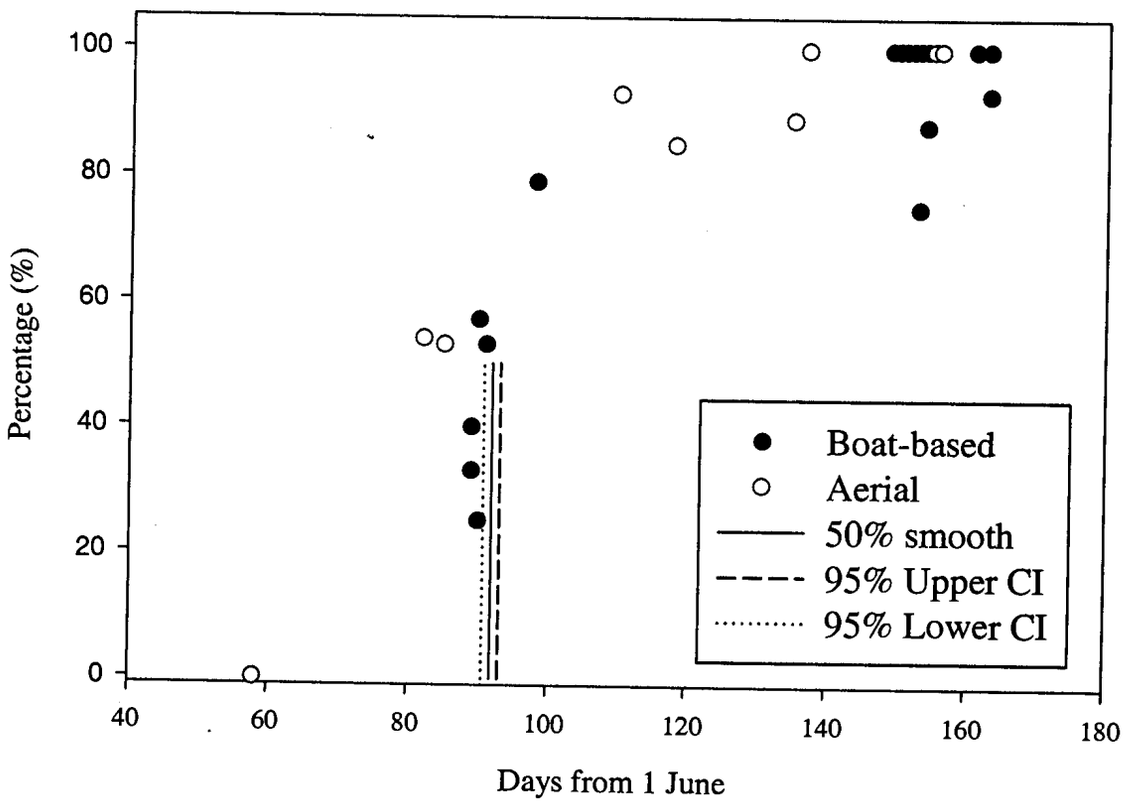

Figure 5: Percentage of smooth-skinned calves observed in boat-based and aerial surveys, South Africa 1988-1999 as a function of the number of days from 1 June (excluding "PreM, ProbS, Unknown" values). Solid vertical line represents the mean day at which 50% of the calves were smooth-skinned, broken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

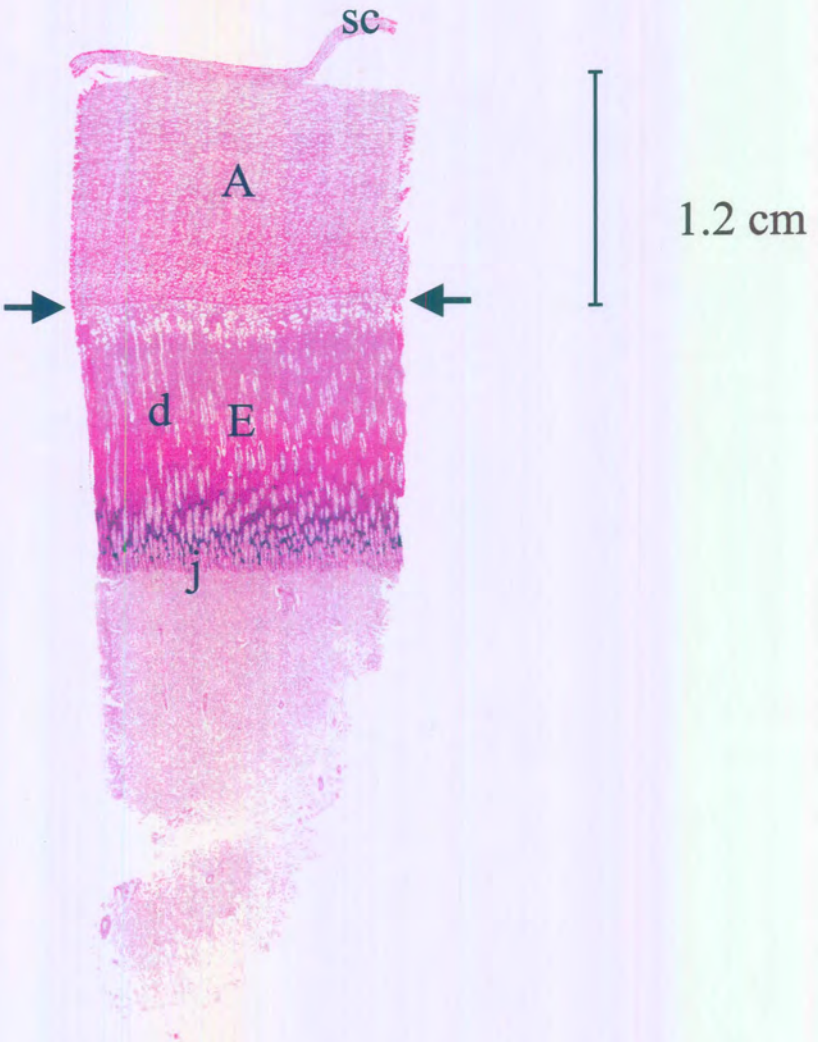

Plate 40: Longitudinal section through the mid-dorsal integument of a stranded southern right whale calf (98/09) showing a separate epidermal region (A) above the epidermis (E) and the distinct plane separating these regions (arrows). Note stratum corneum (sc) cells tearing away from the rest of the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

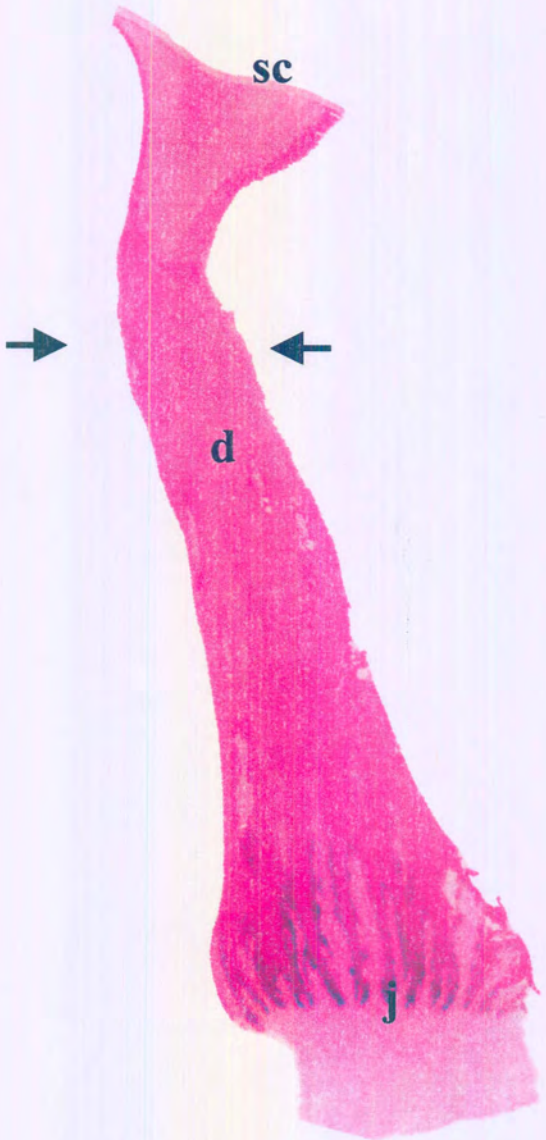

Plate 41: Longitudinal section through the integument of a "smooth-skinned" southern right whale calf. Note the absence of a second epidermal layer as well as the absence of a distinctive plane (arrows) above the dorsal tips of the dermal papillae (d). Epidermal/dermal junction (j), stratum corneum (sc).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

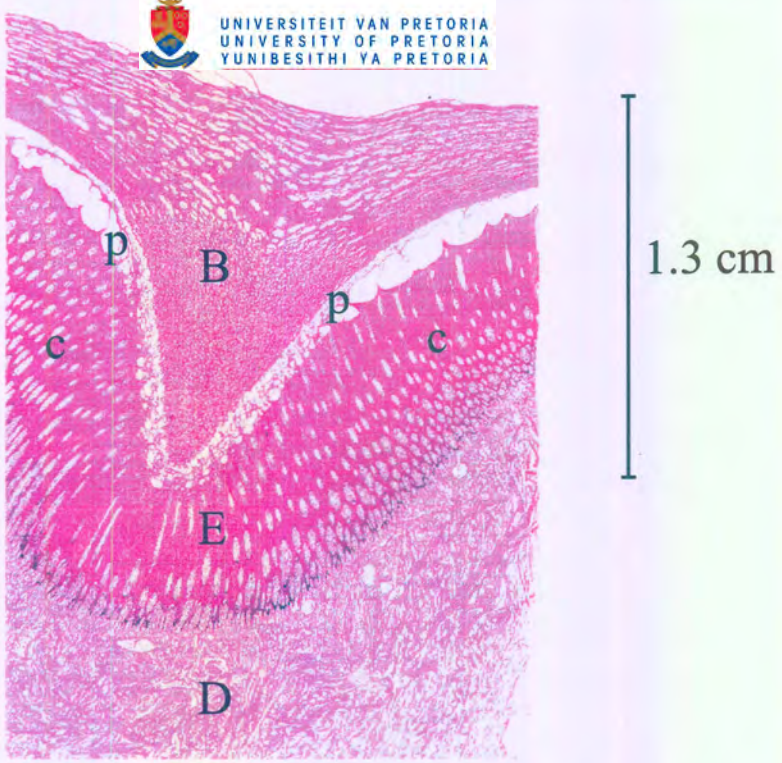

Plate 43: Longitudinal section through the integumentary layers between two rostral callosities (c) from a stranded southern right whale calf (98/09). Note the thickness of the outer epidermal layer (B) between the callosities (c), separation of the "baby skin" along the plane (p), epidermis (E), dermis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

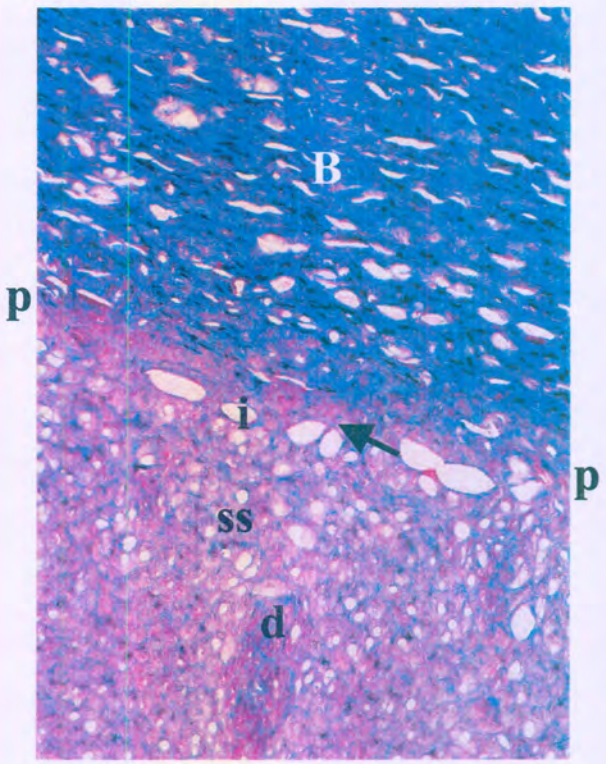

Plate 44: Longitudinal section through the epidermis of a mid-dorsal sample (Pos 4) from a stranded southern right whale calf (98/09). Note the stratum corneum cells (arrow) within the stratum spinosum (ss), inter-cellular oedema (i), "baby skin" (B) and cyto-keratin bodies (stained red) The plane (p)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

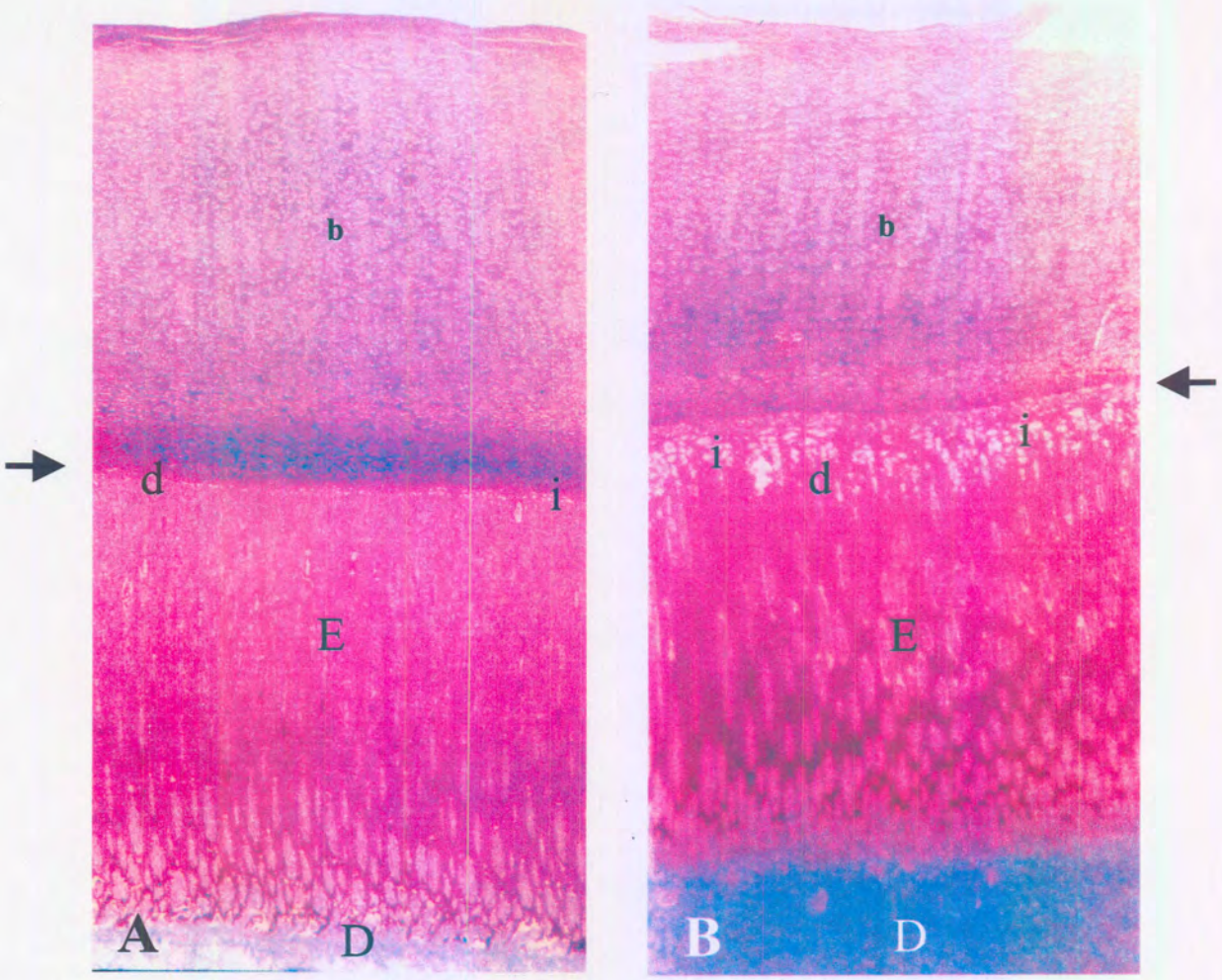

Plate 45: Longitudinal section through the integumentary layers of mid-dorsal samples from Pos 1 (A) and Pos 4 (B) along the body of a stranded southern right whale calf (98/09). Note the conspicuously porous plane (arrows) in B compared to A. Dermal papillae (d), intercellular oedema (i), "baby skin"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

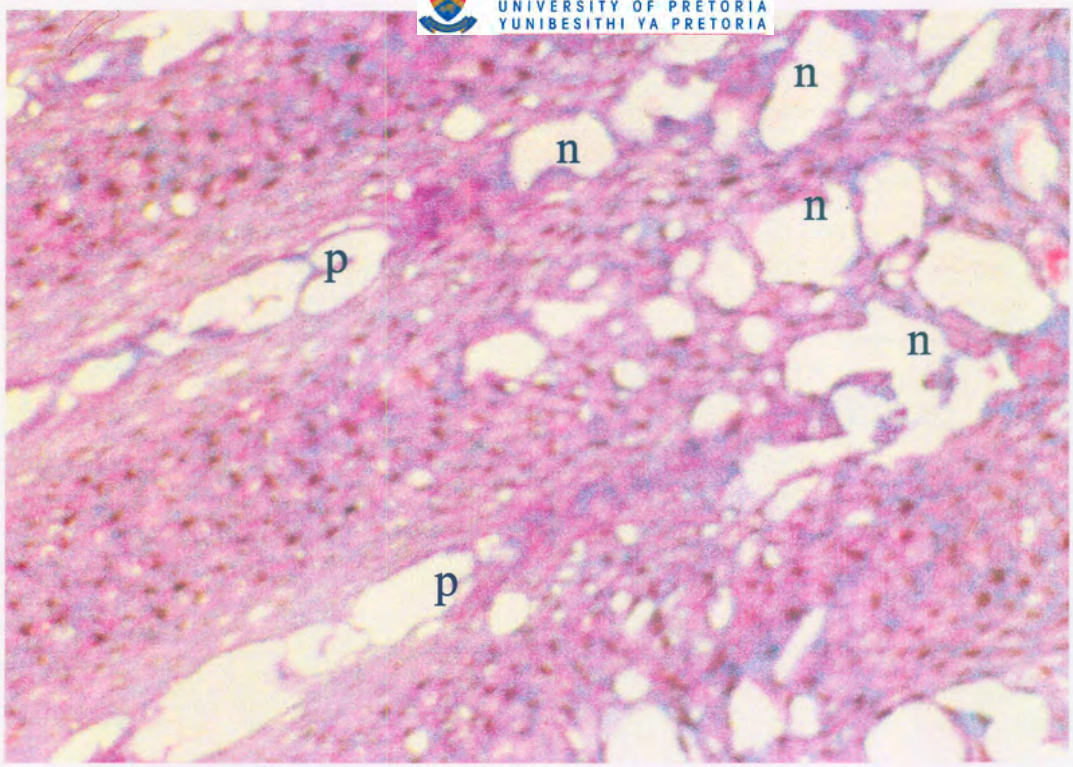

Plate 46: A (rotated) longitudinal section through the stratum spinosum of a mid-dorsal sample (Pos 1) taken from a stranded southern right whale calf (98/09), showing necrotic cells (n) at the distal tips of the dermal papillae (p). (Ayoub-Shklar, Mag 100X).

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.

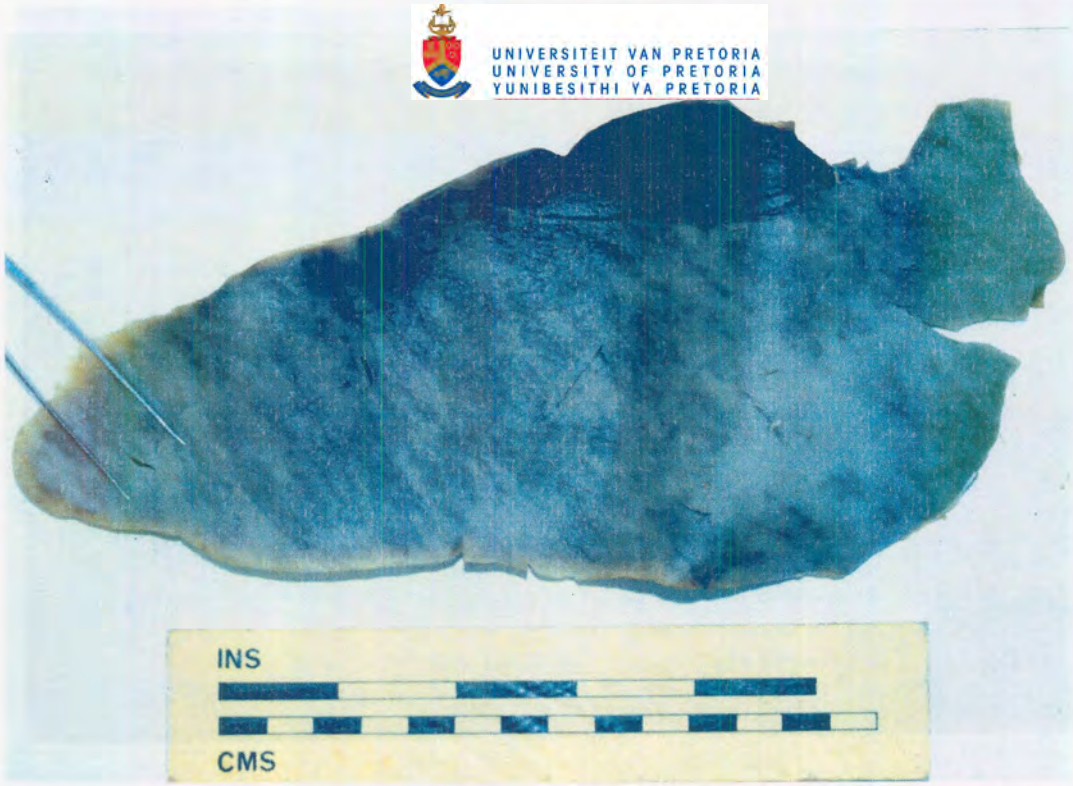

Plate 48: A piece of sloughed skin recovered at sea from an apparently abandoned neonatal southern right whale.

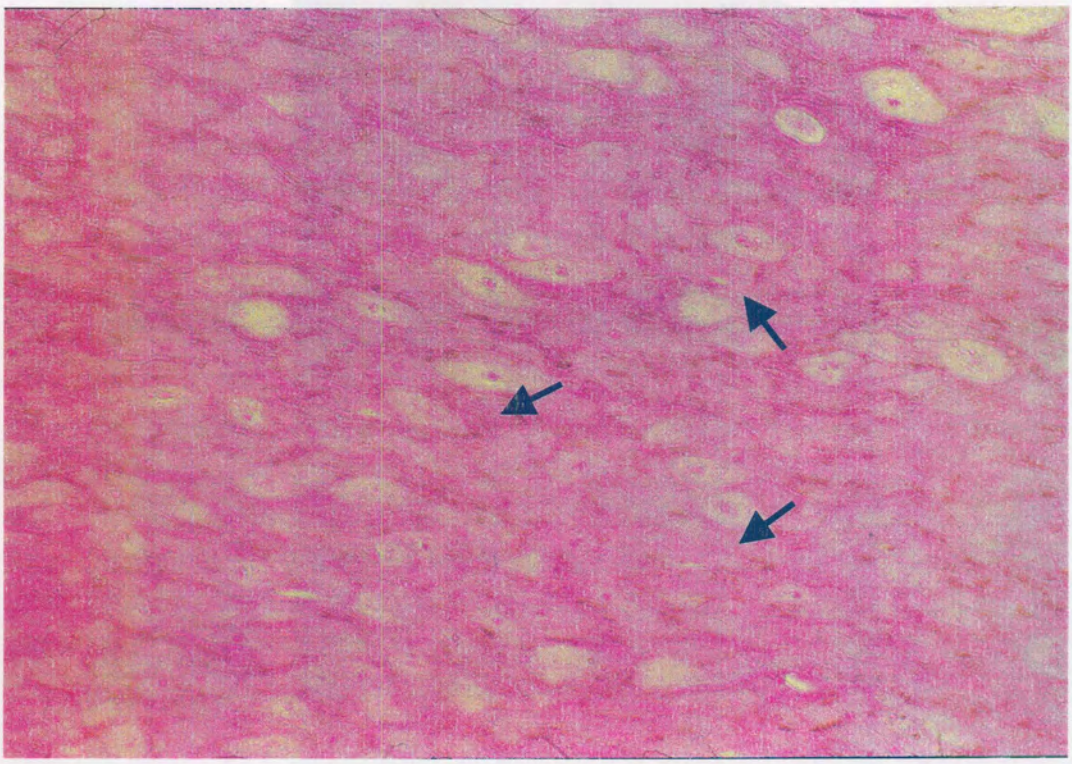

Plate 49: Longitudinal section through a piece of sloughed skin recovered at sea from a neonatal southern right whale. Note the spindle-shaped spinsosal cells possessing only ghost nuclei (arrows). (H/E, Mag 200X).

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).

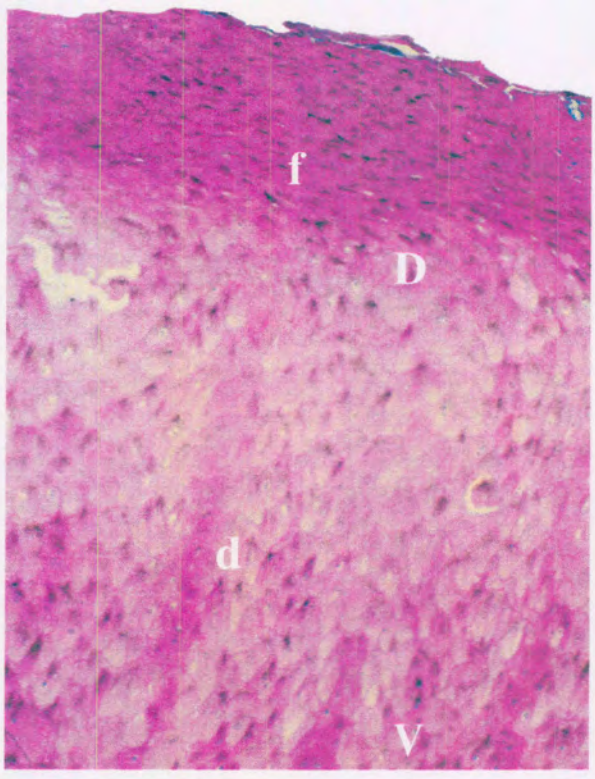

Plate 51: Longitudinal section through the upper epidermal layers of an apparently "rough-skinned" southern right whale calf (00/09). Note the flattened stratum spinosal cells (f) replacing a true stratum corneum and the absence of an ecdysal plane at the distal tips of the dermal papillae (d). (H/E,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