CHAPTER 4

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

4.1 Introduction

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

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

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

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

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

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

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

4.2 Materials and Methods

4.2.1 Sample collection

4.2.1.1 Study area

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

4.2.1.2 Sampling

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

4.2.1.2.1 Biopsies

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

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

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

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

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

Table 5: continued

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

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

4.2.1.2.2 Stranded animals

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

4.2.1.2.3 Scanning Electron Microscopy (SEM)

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

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

4.2.2 Determination of viable microorganisms

4.2.2.1 Sampling of skin

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

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

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

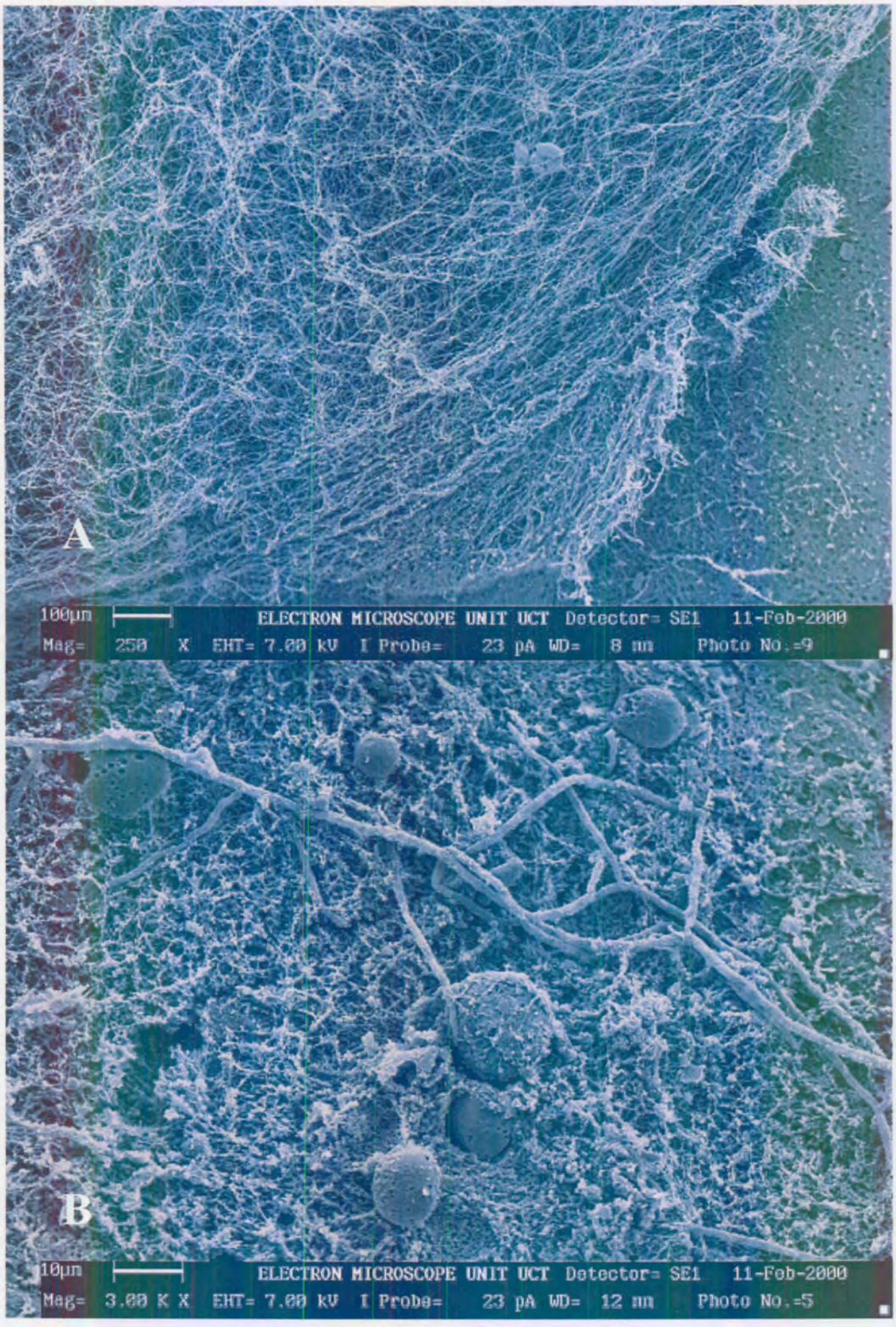

Plate 52: SEMs taken at the University of Cape Town's Microscopy Unit during random trial inspections showing microbial aggregations on the skin of a stranded neonatal southern right whale (99/05) . (Mag A 250X, Mag B 3 000X).

4.2.2.2 Culture techniques

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

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

4.3 Results

4.3.1 Microbial populations on the skin samples as revealed by SEM

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

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

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

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

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

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

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

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

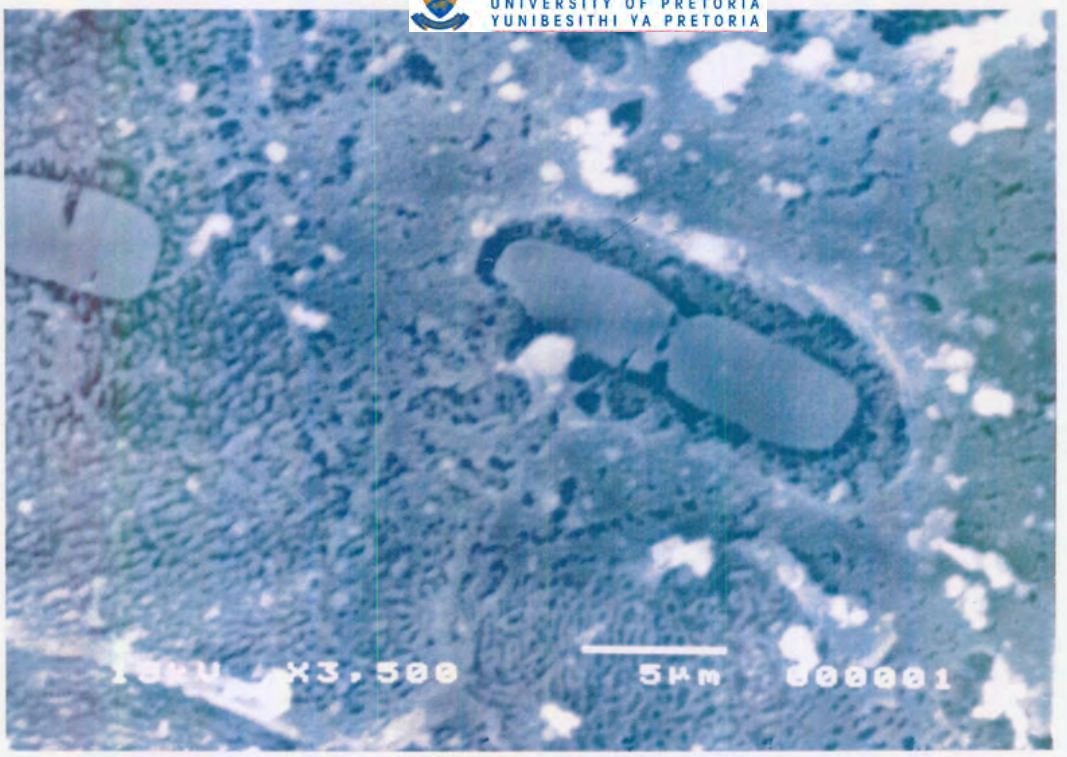

Plate 53: SEM of an undetermined species of cyanobacteria possibly forming crypts in the skin of a late season calf (# 90). (Mag 3 500X).

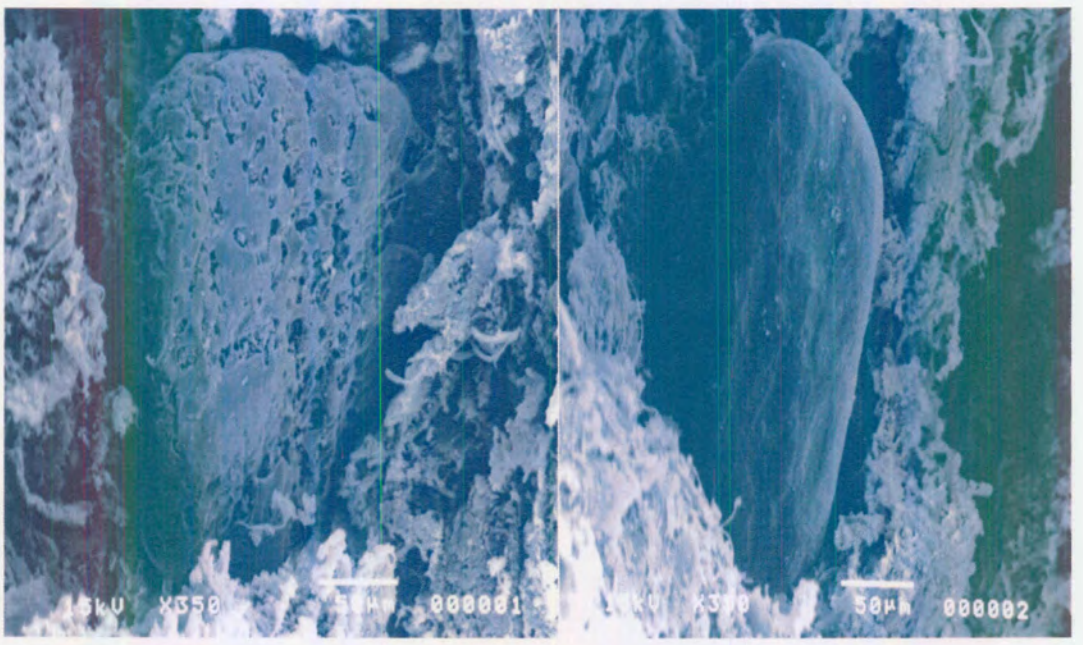

Plate 54: SEMs of "stonelike" micro-organisms deeply seated in the skin of a stranded neonatal southern right whale (99/05) (Mag 350X).

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

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

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

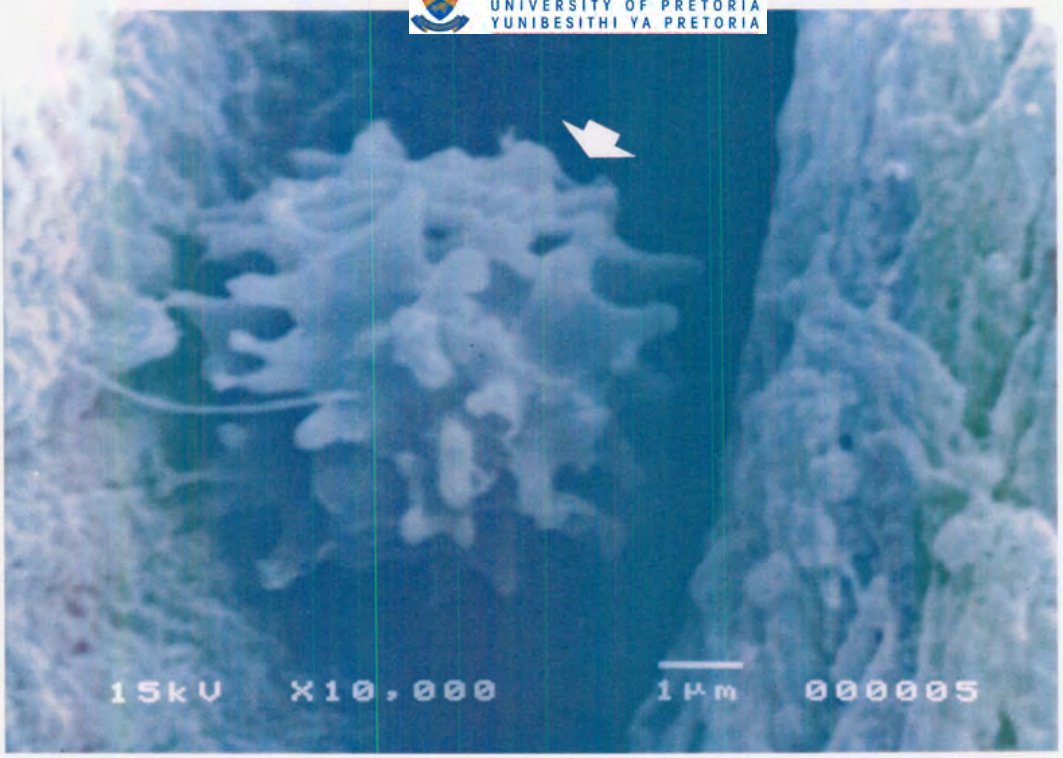

Plate 55: SEM of a spore with prosthecate appendages (arrow) found on the skin of a stranded neonatal southern right whale (99/05). (Mag 10 000X).

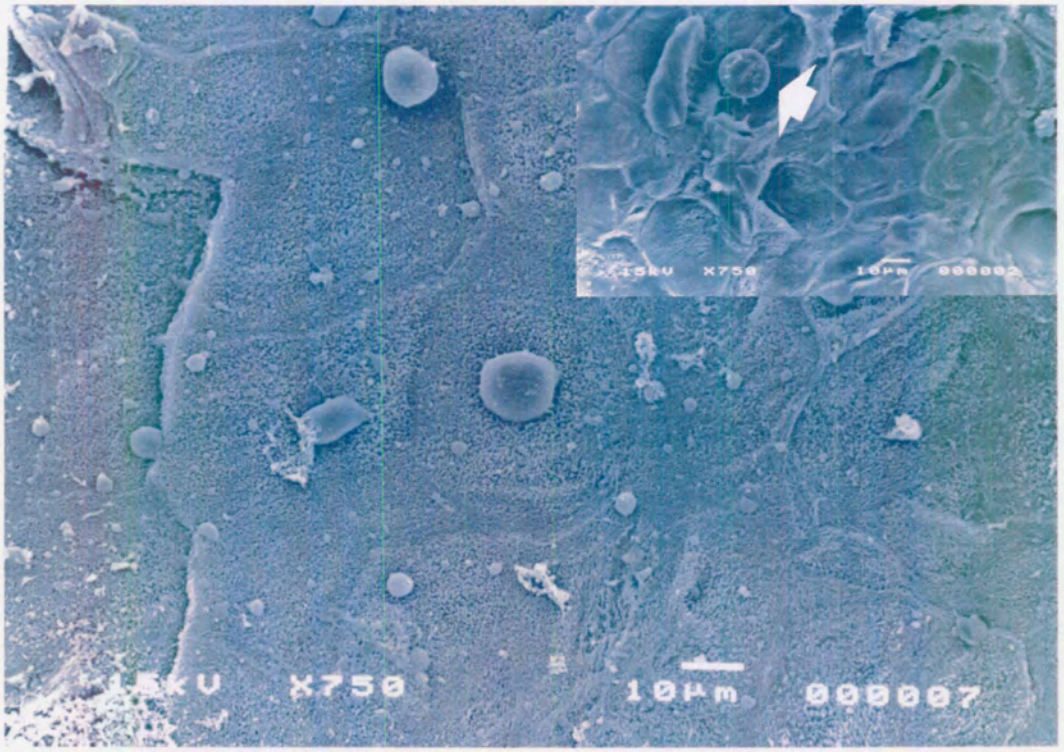

Plate56: SEM of spherical, smooth spores found on the skin of a late season southern right whale calf (# 61). (Mag 750X). Insert: SEM of smooth spore found on the skin of a stranded juvenile southern right whale (00/11) (Mag 750X).

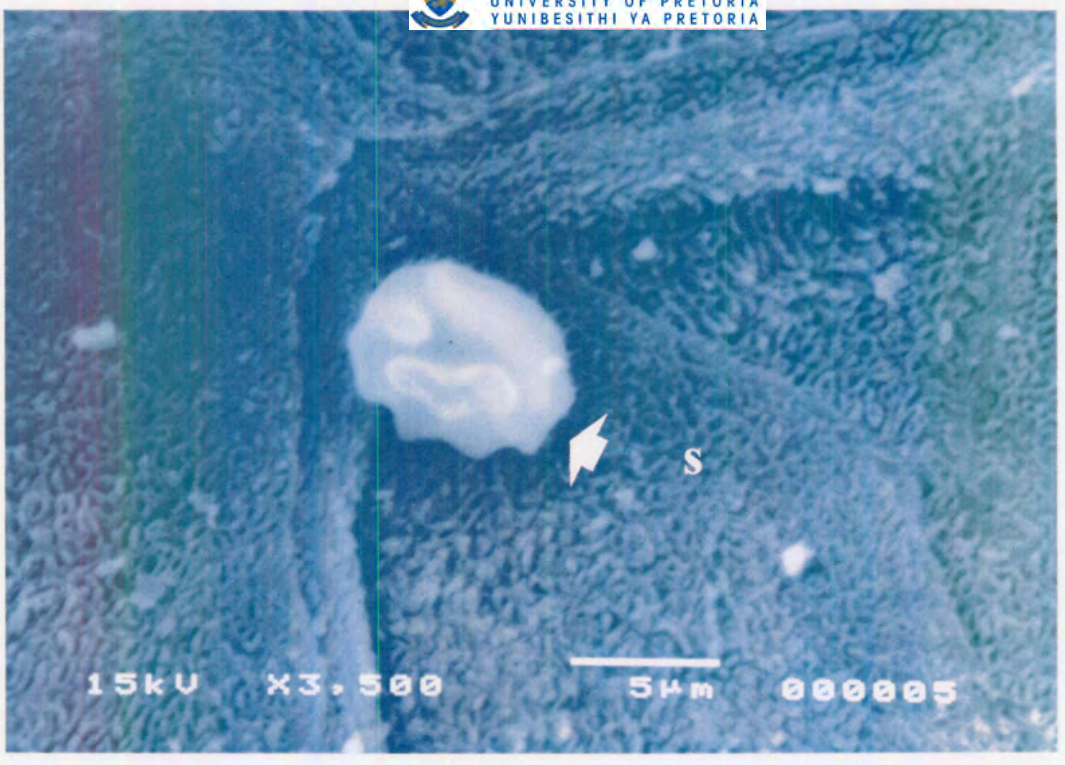

Plate 57: SEM of a unique spore, with corrugated edges (arrow), found on the exfoliated skin surface (s) of a late season southern right whale calf (# 84). Mag (3 500X).

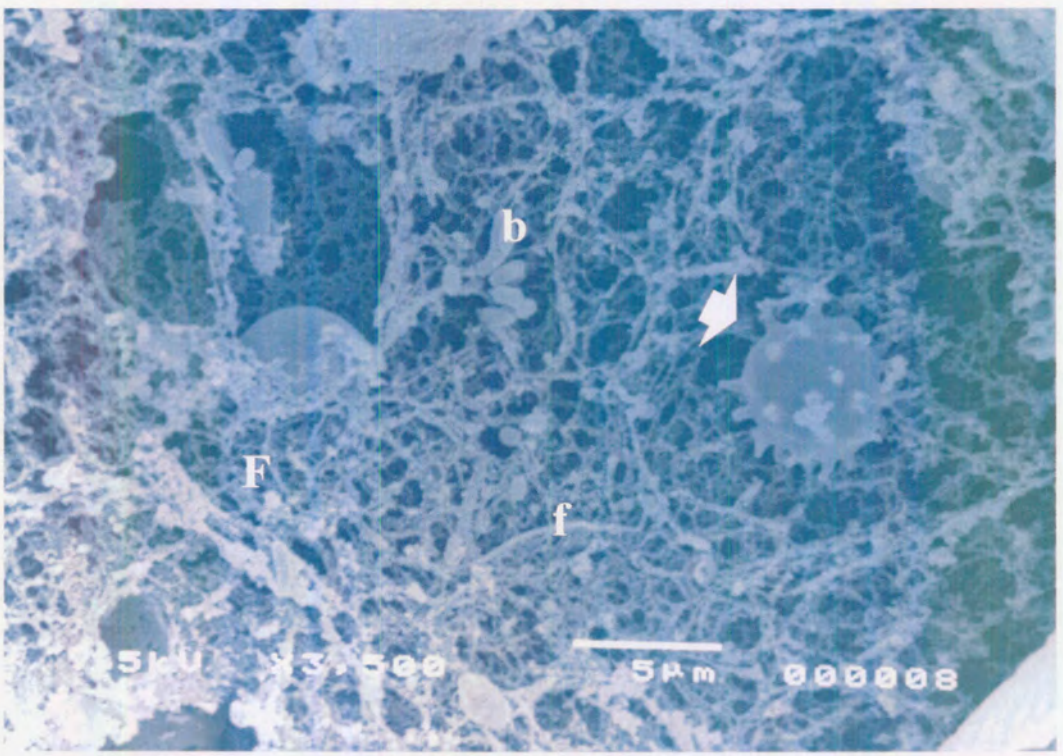

Plate 58: SEM showing a "spiky" spore (arrow) found on late season cows and calves, an early season calf and two non-calves sampled in the Antarctic. Bacterial cocci (b) and fungal mycelia (f) also present. (Mag 3 500X).

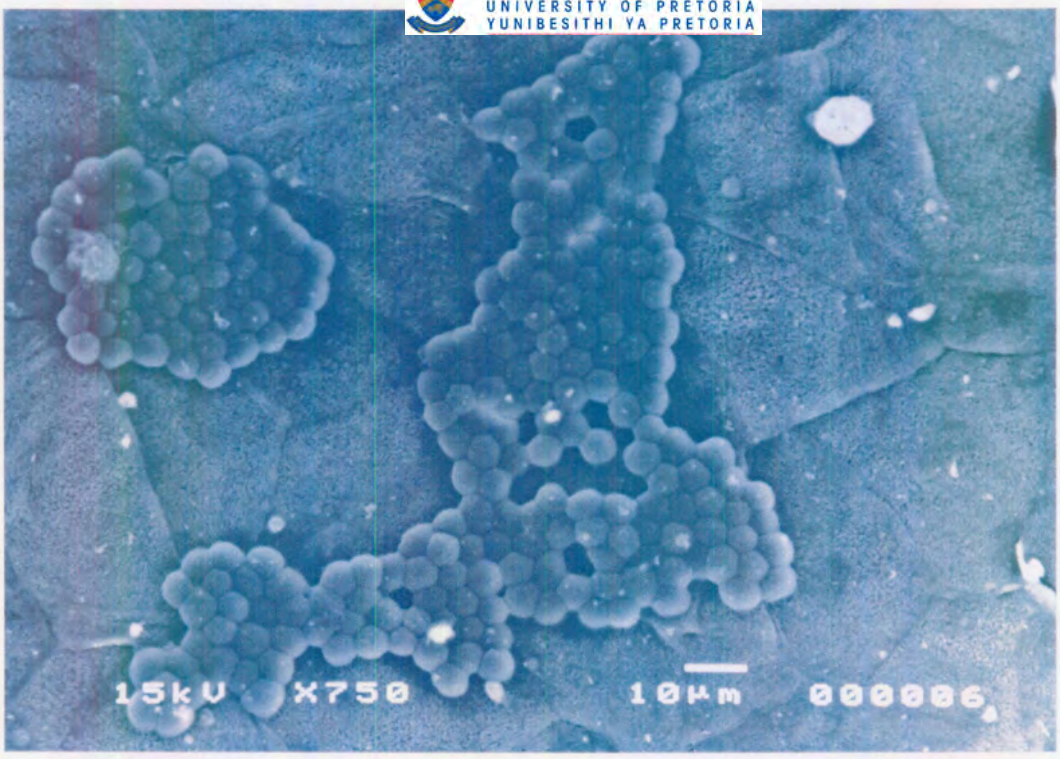

Plate 59: SEM showing yeast colonies on the surface of the skin of a late season southern right whale calf (# 73). (Mag 750X).

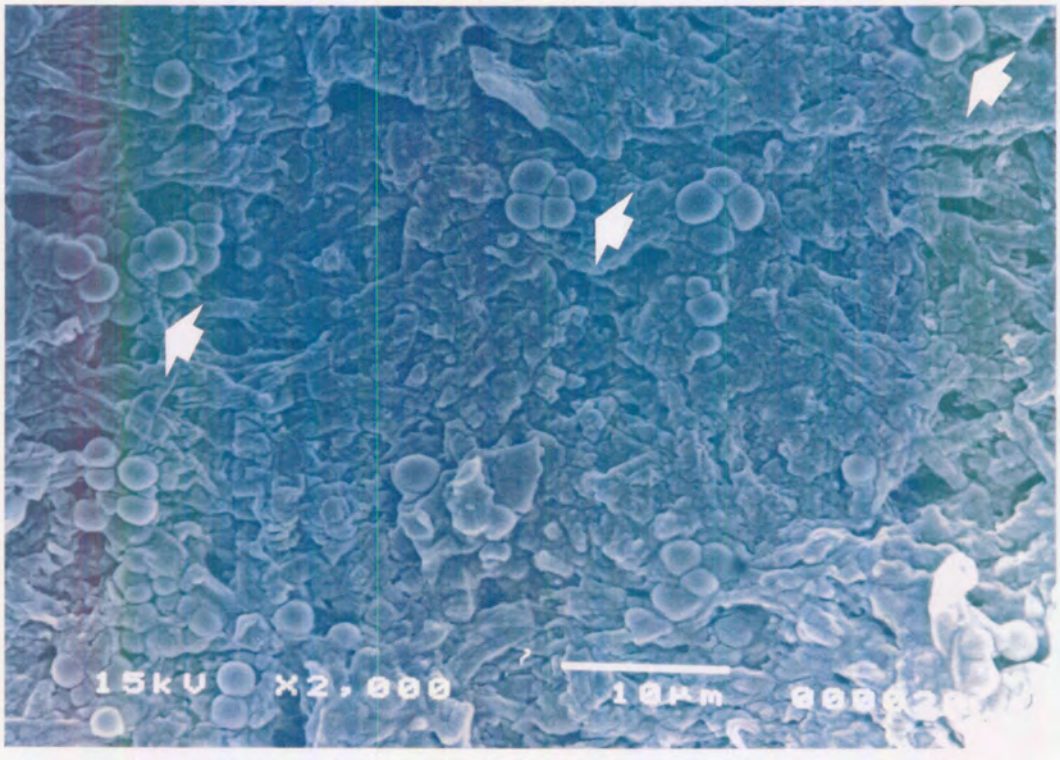

Plate 60: SEM showing actively dividing yeast cells (arrows) on the skin of a late season southern right whale calf (# 45). (Mag 2 000X).

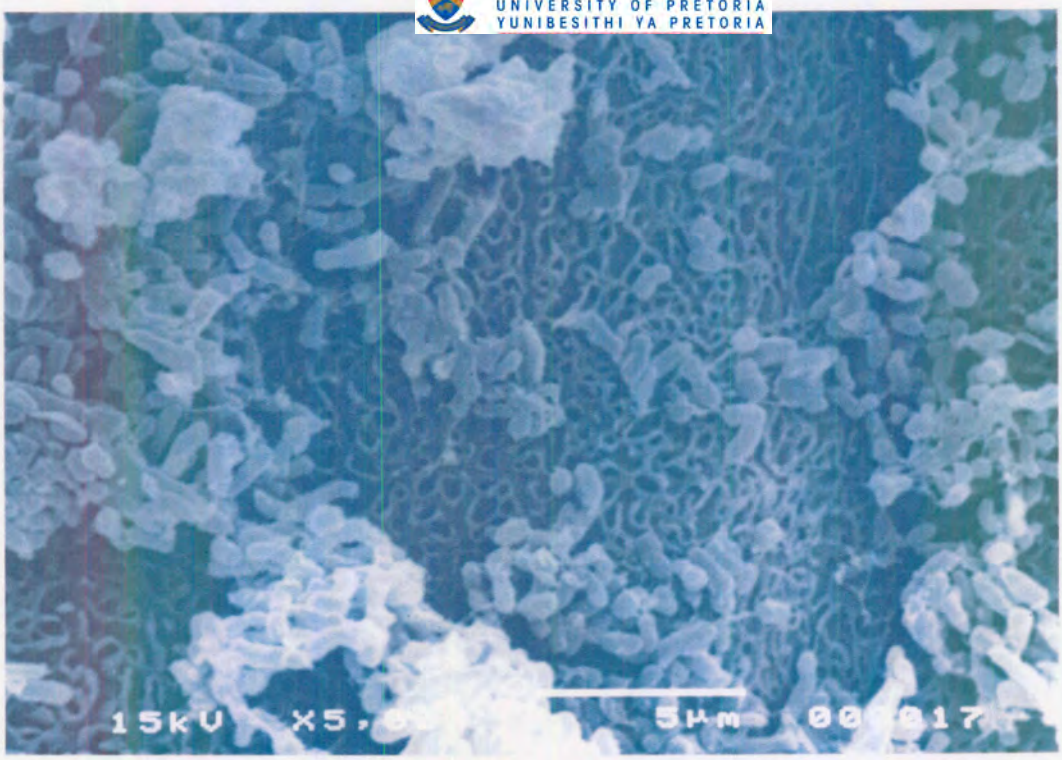

Plate 61: SEM showing the presence of bacterial cocci distributed on the sloughed surface of the skin of a non-calf southern right whale (# 140S) sampled in Antarctic waters. (Mag 5 000X).

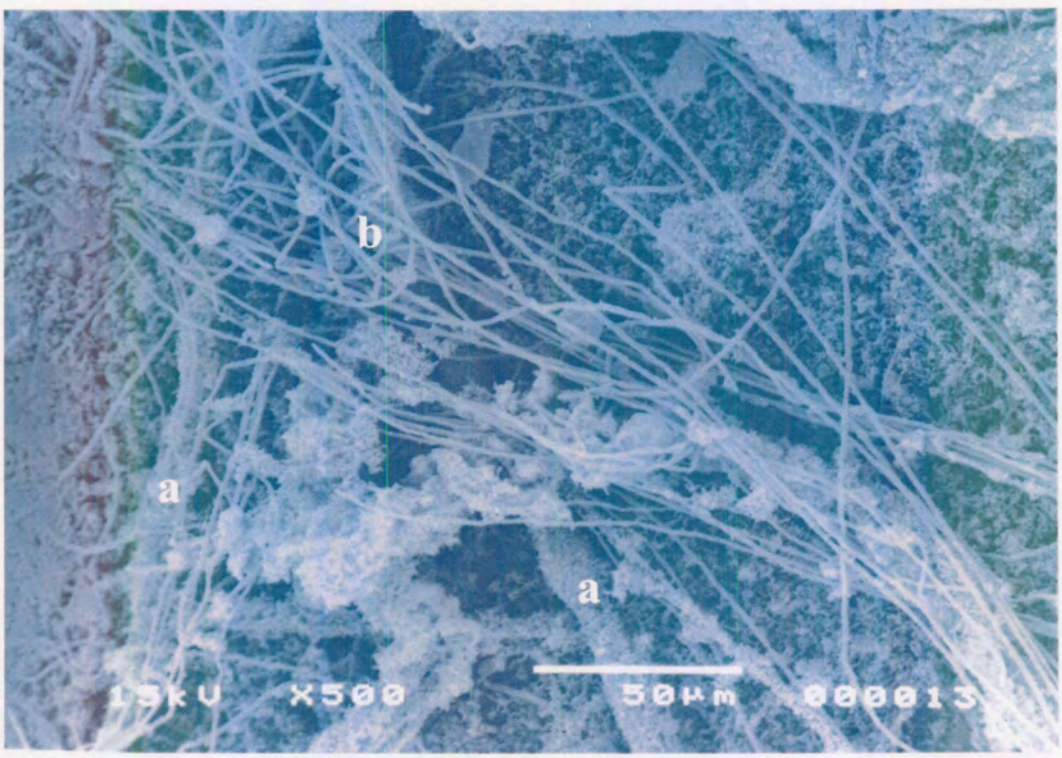

Plate 62: SEM showing the mycelia of different fungal species (a + b) on the skin of a non-calf southern right whale (# 146S) sampled in Antarctic waters. (Mag 500X).

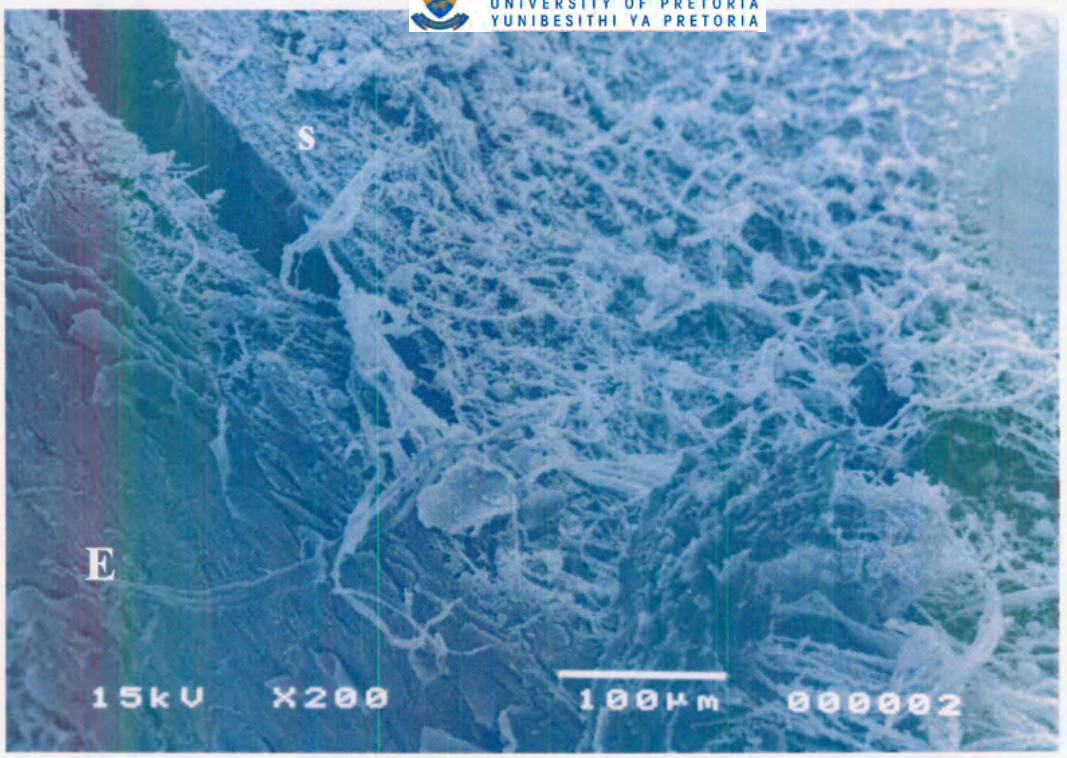

Plate 63: SEM showing the fungal aggregations on the sides (s) of a skin sample taken from a non-calf southern right whale (# 149S) in Antarctic waters. Epidermis (E). (Mag 200X).

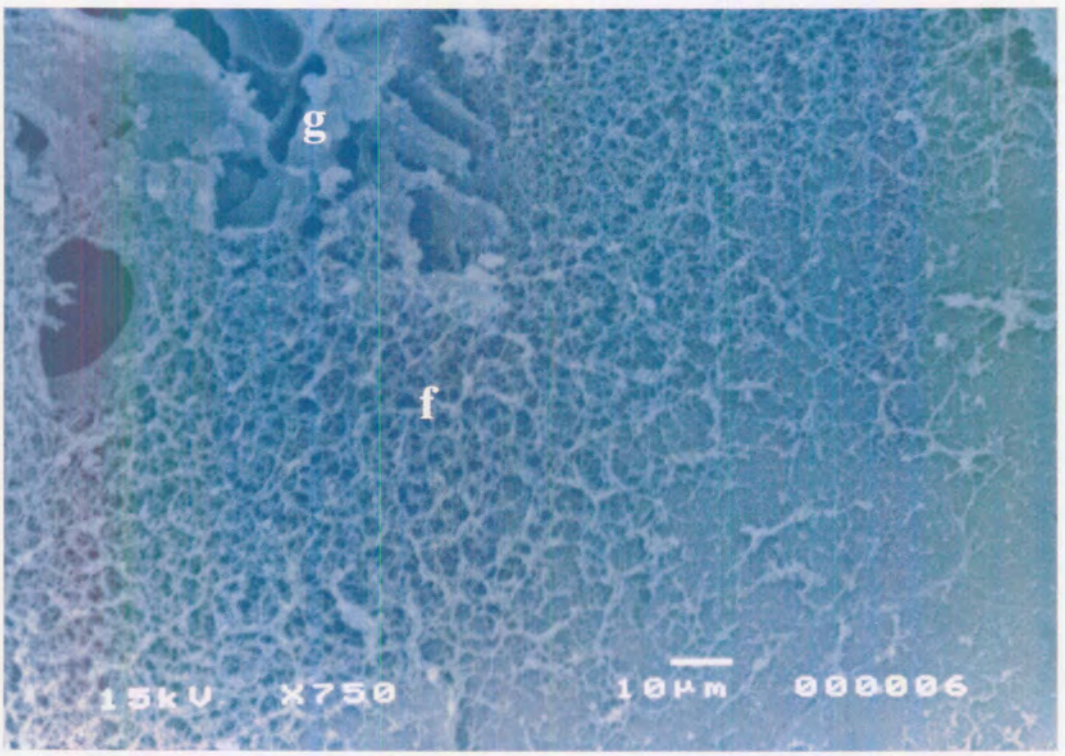

Plate 64: SEM of fungal mycelia (f) congregating around degenerating skin (g) of a stranded juvenile southern right whale (# 00/11). (Mag 750X).

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

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

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

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

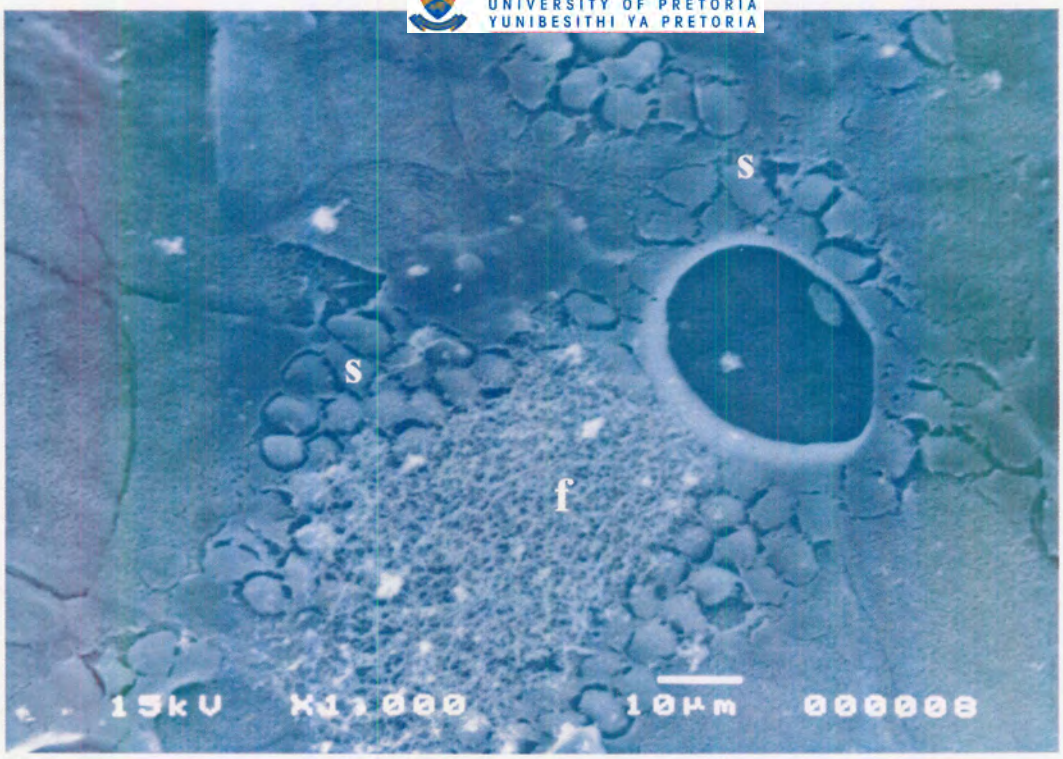

Plate 65: SEM of the skin of a late season calf (# 73) showing encrypted spores (s) in association with fungal mycelia (f). (Mag 1 000X).

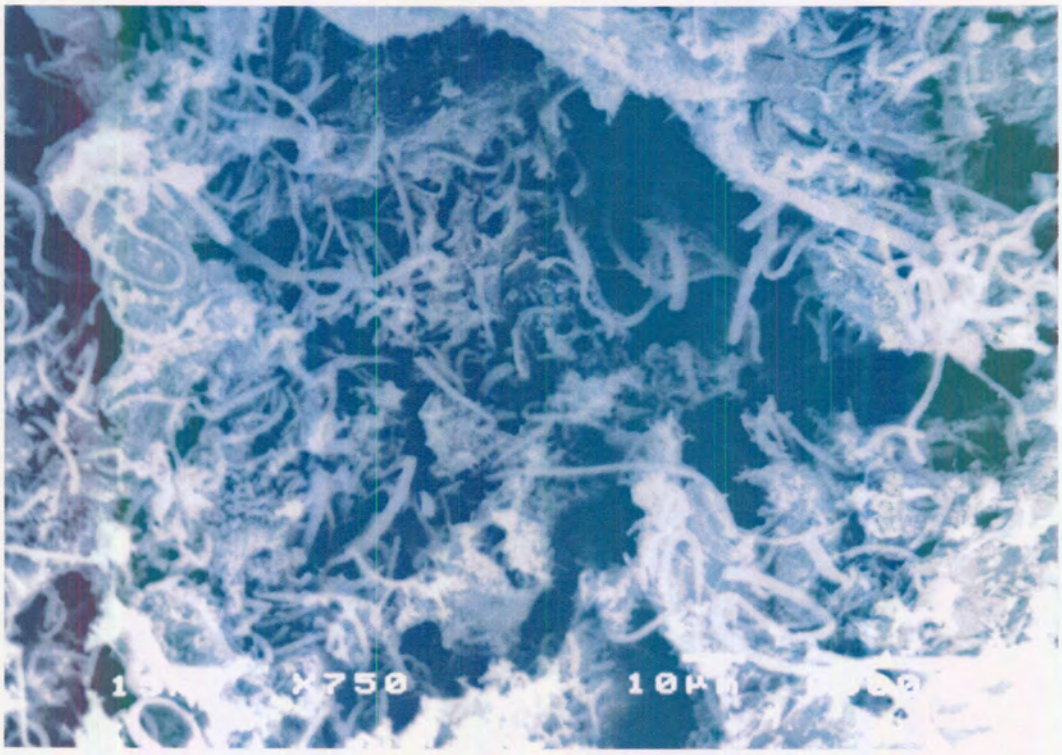

Plate 66: SEM of the skin of a stranded neonate southern right whale (99/05) showing invasive fungal growth. (Mag 750X).

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

4.3.2 Viable microbial populations as revealed by culturing

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

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

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

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

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

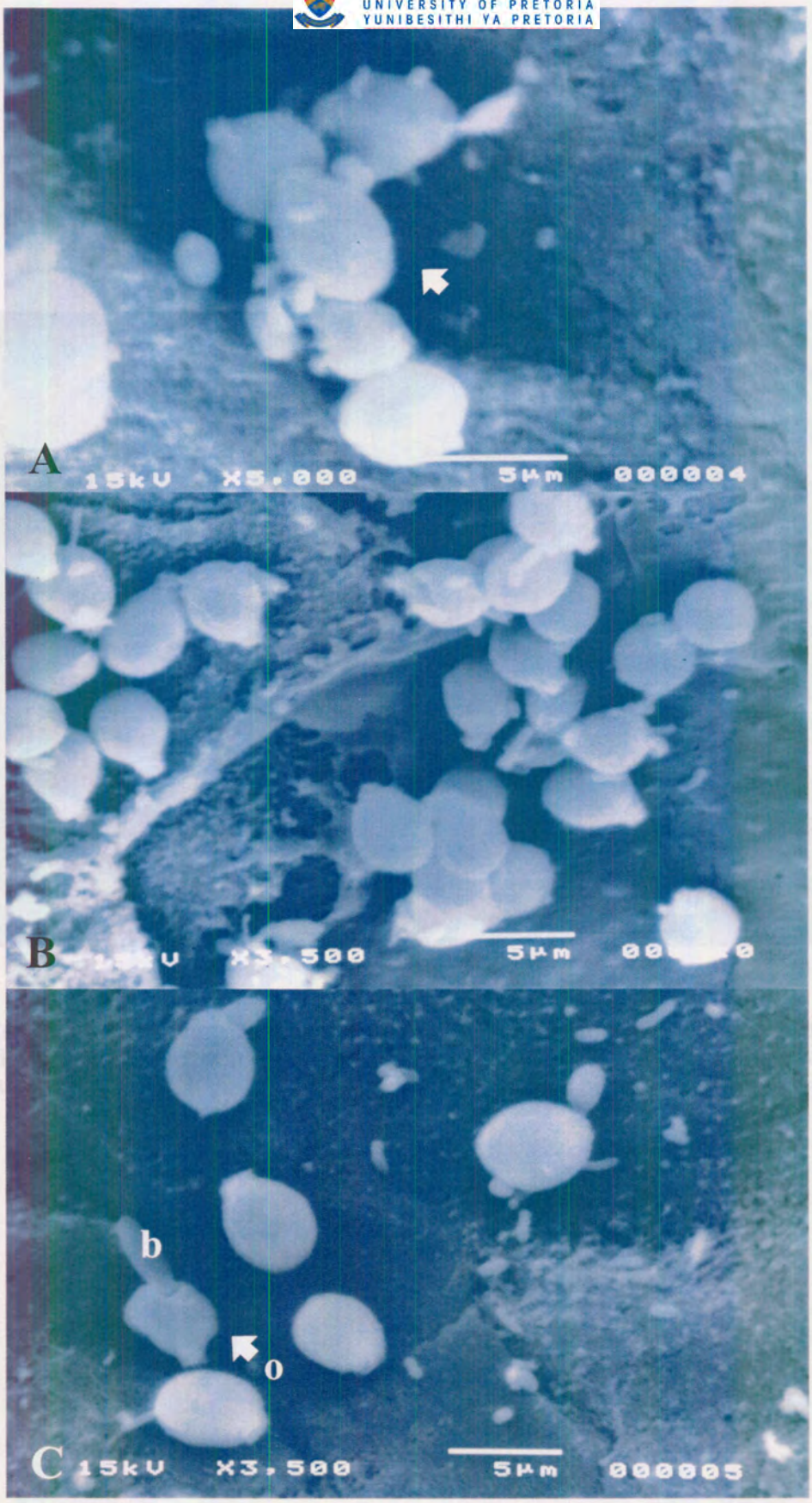

Plate 67: SEMs of yeast cells found on the surface of the skin of a stranded neonatal southern right whale (99/05). A: stalks (arrow), B: clumps and C: budding (b) yeast cells. Blastospore (o) (Mag A, 5 000X; Mag B + C, 3 500X).

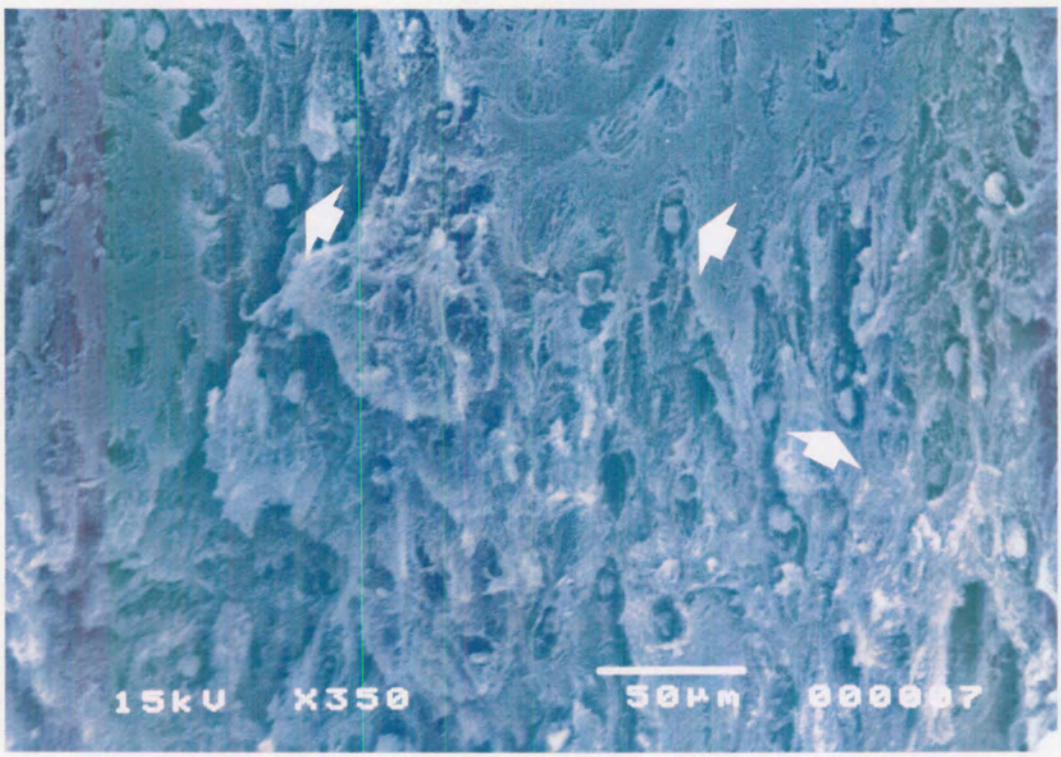

Plate 68: SEM showing small, spherical spore-like structures nestled within the epidermal tissue (arrows) of a stranded neonatal southern right whale (99/05). (Mag 350X).

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

4.4 Discussion

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

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

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

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

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

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

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

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

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

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

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

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

4.5 Conclusion

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

CHAPTER 5

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

5.1 Introduction

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

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

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

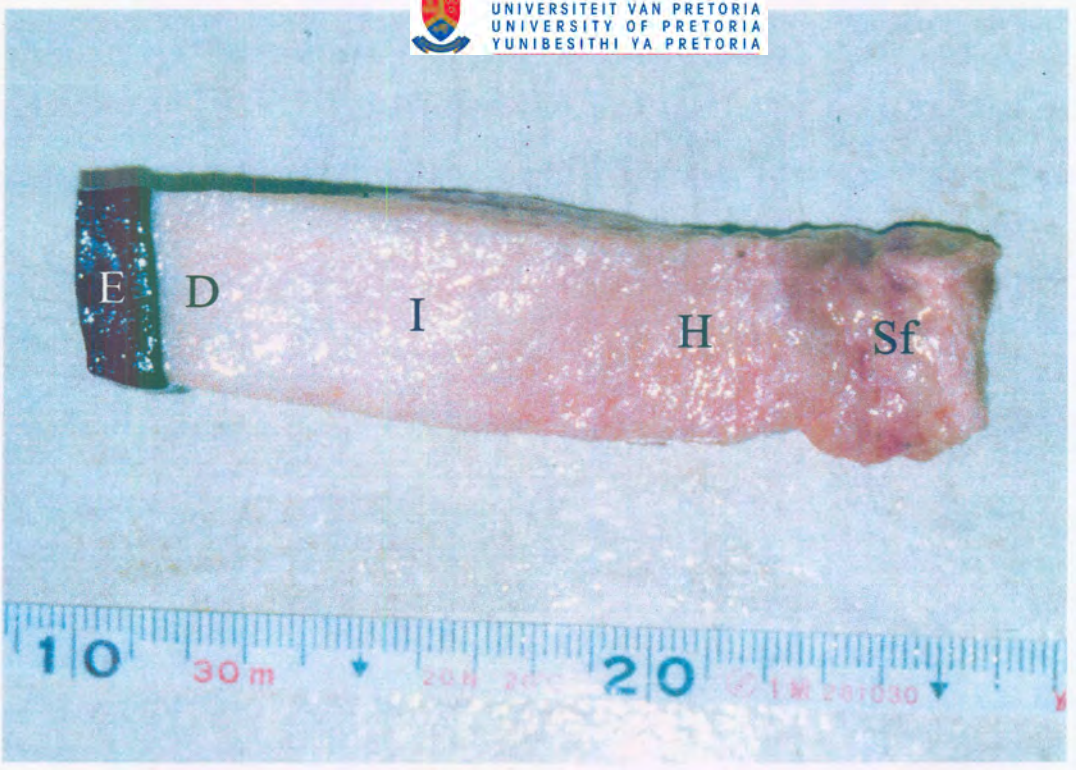

Plate 69: The integument of a neonatal southern right whale. The separation of the layers for lipid analysis is possible on gross inspection of the integument, dermal layer (D), intermediary fibrous/fatty layer (I), fatty hypodermal layer (H), superficial fascia (Sf), epidermis (E).

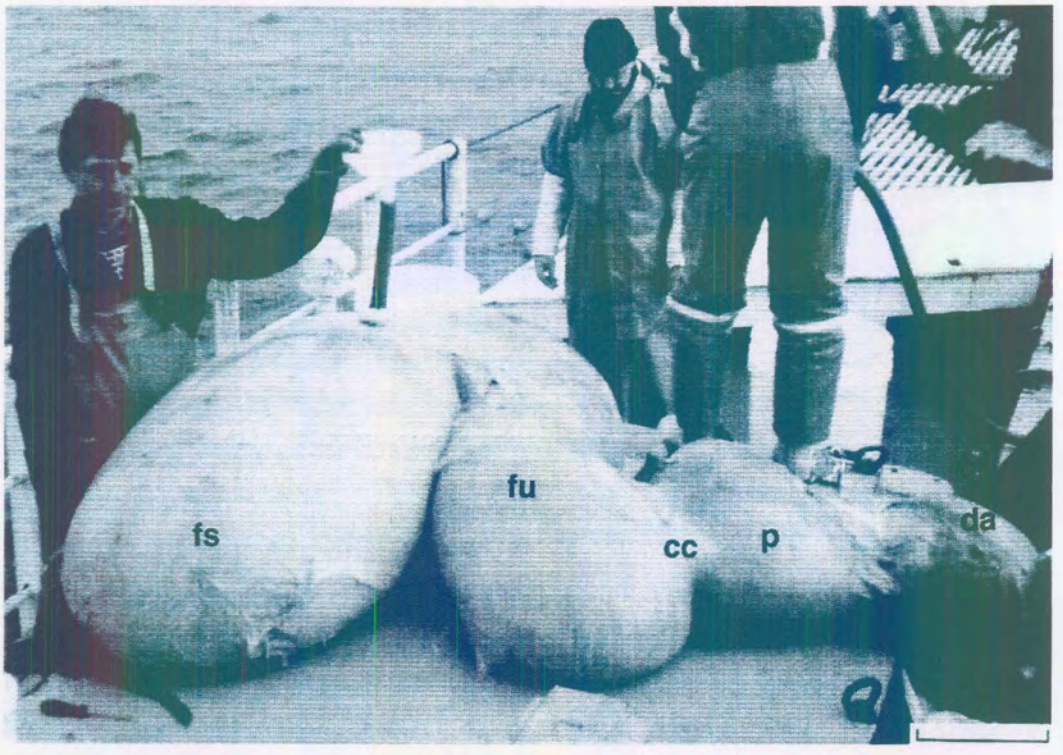

Plate 70: A maximally expanded minke whale (*Balaenoptera acutorostrata*) stomach and proximal duodenum, showing the forestomach (fs), the fundic chamber (fu), the pyloric chamber (p) and the duodenal ampulla (da). The connecting channel (cc) is situated between the fundic and the pyloric chamber. Scale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

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

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

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

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

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

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

5.2 Materials and Methods

5.2.1 Study area

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

5.2.2 Sample collection

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

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

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

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

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

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

* Layer analysis was done these samples

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

5.2.2.1 Biopsies

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

5.2.2.2 Stranded animals

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

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

5.2.3 Lipid analysis

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

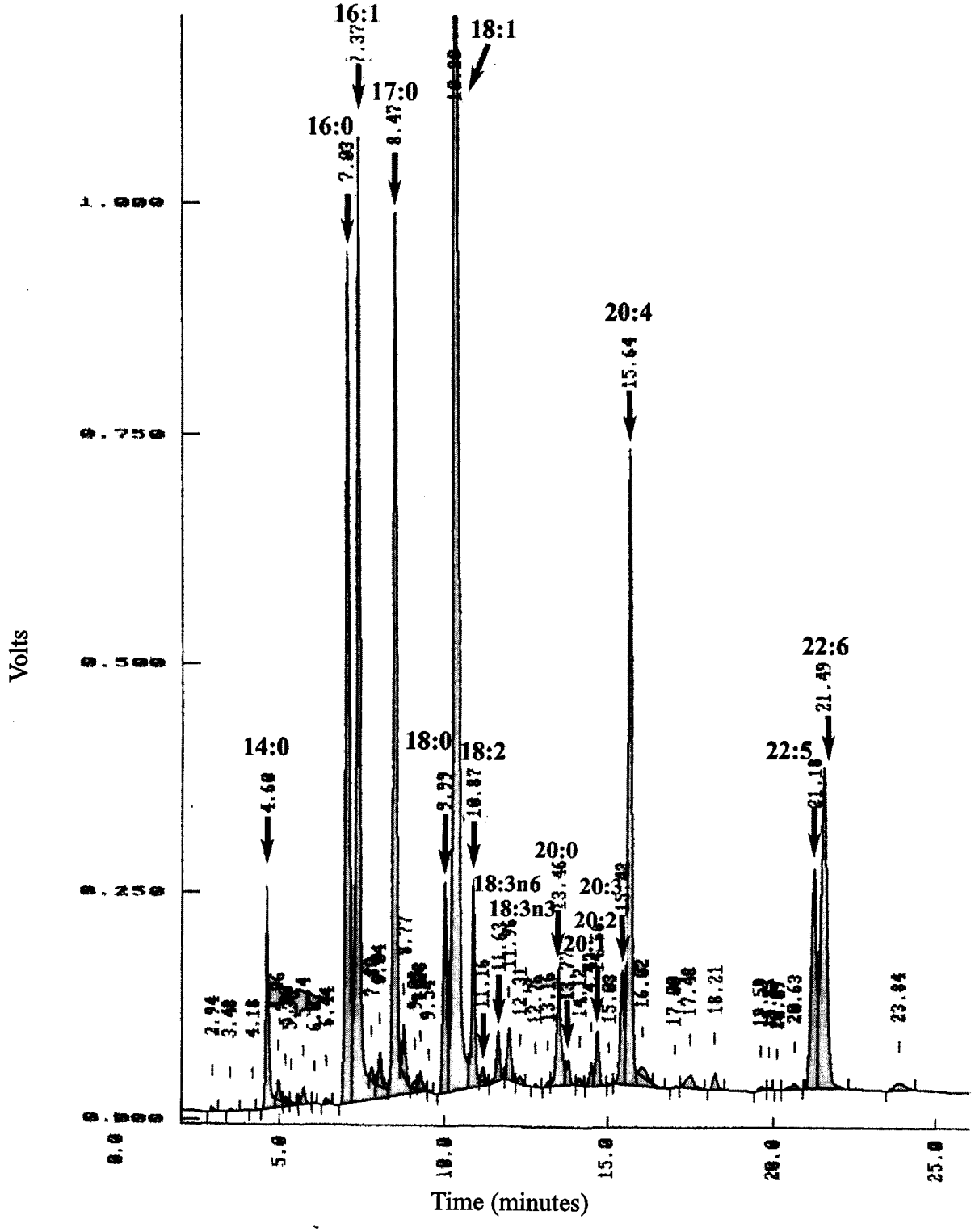

Figure 6 : Chromatograms showing the fatty acid profile from the blubber of a southern right whale calf (# 87), indicating the major fatty acids as well as unidentified peaks, detected using gas chromatography (MRC).

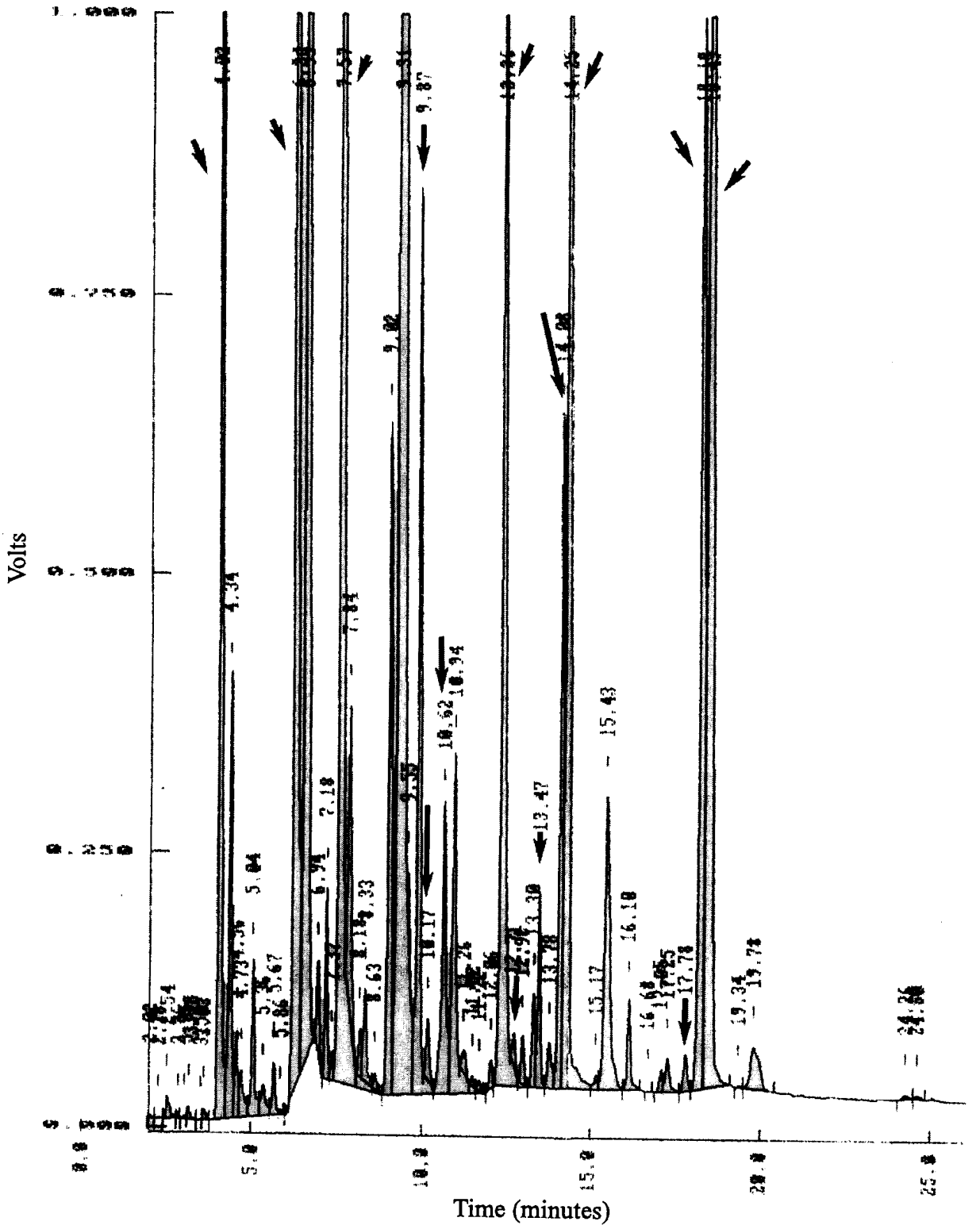

Figure 7: Chromatograms showing the fatty acid profile from the blubber of a southern right whale mother (# 86), indicating the major fatty acids as well as unidentified peaks, detected using gas chromatography (MRC).

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

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

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

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

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

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

5.2.3.3 Standard for composition analysis

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

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

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

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

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

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

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

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

5.2.4 Data Analysis

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

5.2.5 Statistical Analysis

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

5.2.6 Proximate analysis on faecal sample (AOAC, 1984)

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

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

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

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

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

5.2.6.1 Determination of crude fat (ether extract)

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

5.2.6.2. Determination of crude protein (Macro Kjeldahl method)

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

The following was added to the Kjeldahl flask:

a) 350 ml distilled water

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

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

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

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

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

Where F = 0.1432
Blank = 0.01

% crude protein = % N x 6.25

5.2.6.3 Determination of dry matter

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

5.2.6.4 Ash Determination

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

5.3 Results

5.3.1 Fatty acid composition of southern right whale blubber

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

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

5.3.1.1 Effect of position on body

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

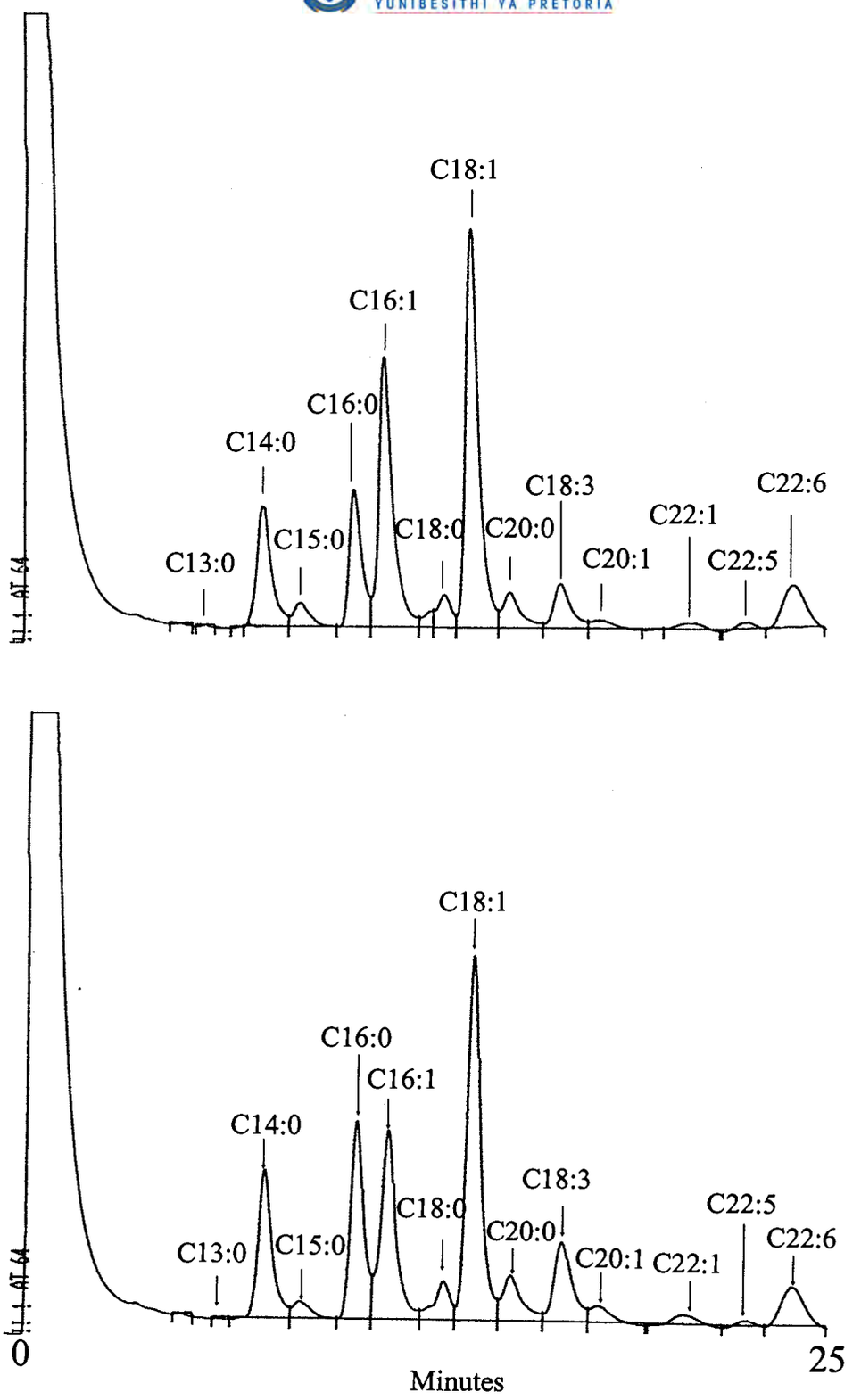

Figure 8: Chromatograms showing the fatty acid profile from the blubber of a southern right whale calf (# 30, top) and mother (# 29, bottom) pair indicating the 13 major fatty acids detected using gas chromatography.

Neonates (n = 4):

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

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

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

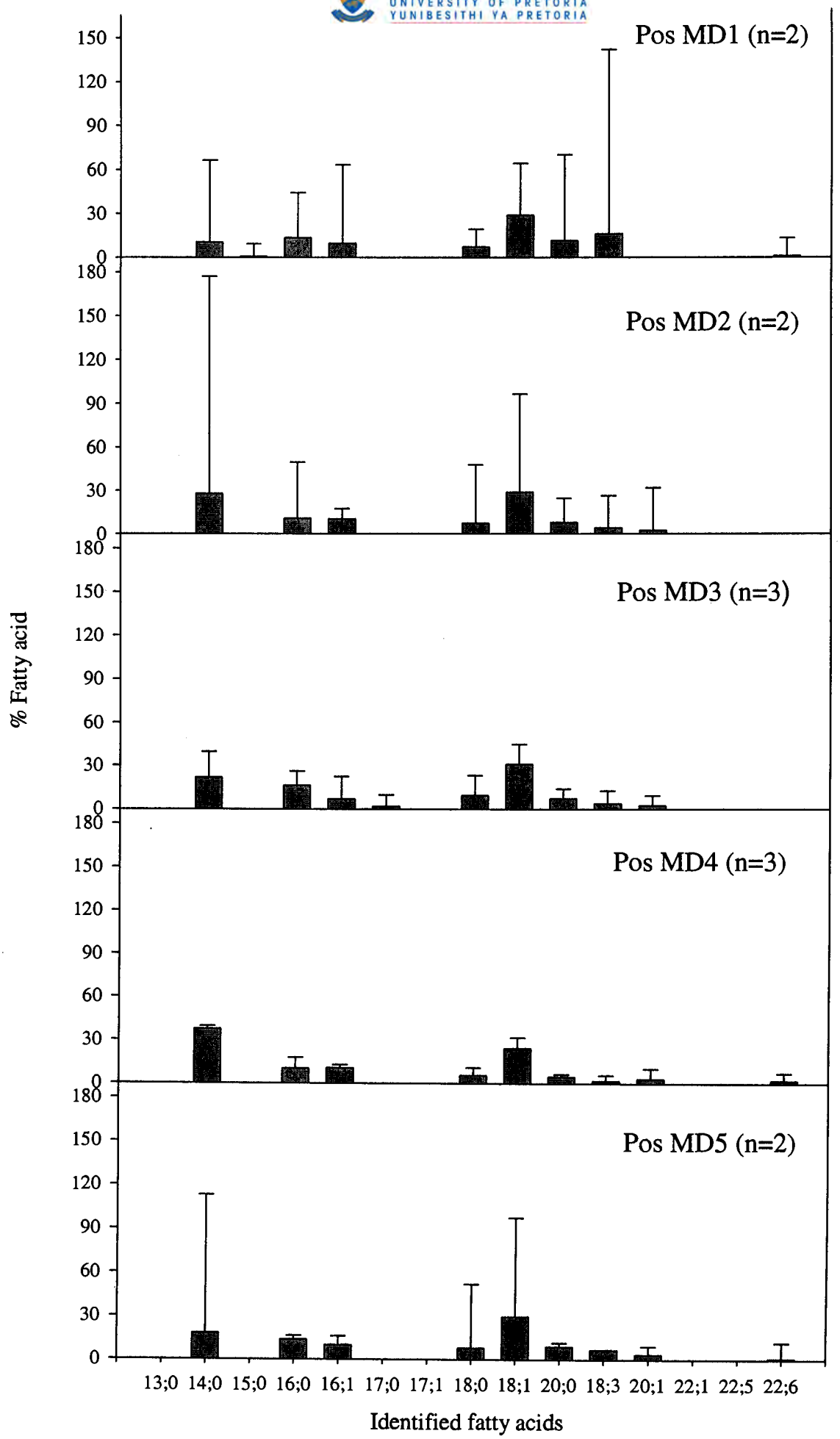

Figure 9: Proportions of fatty acids in positions 1-5 on the mid-dorsal plane along the bodies of neonatal southern right whales (mean ± S.E.).

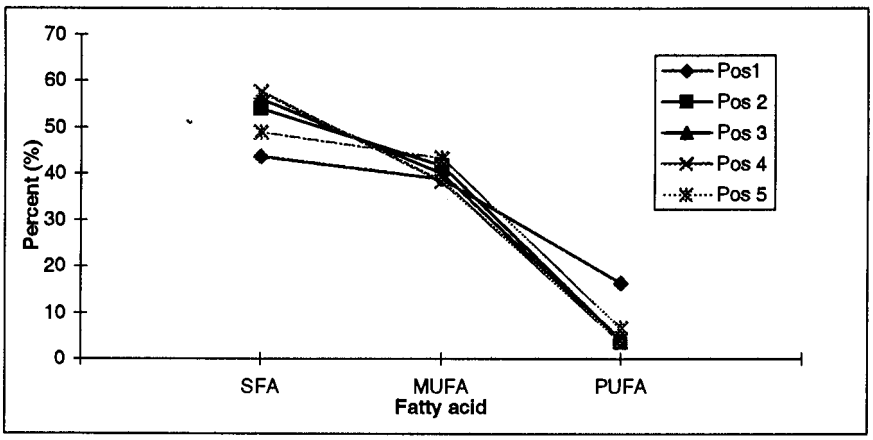

Figure 10: Proportions of fatty acids on the mid-dorsal plane along the body of a male neonatal southern right whale.

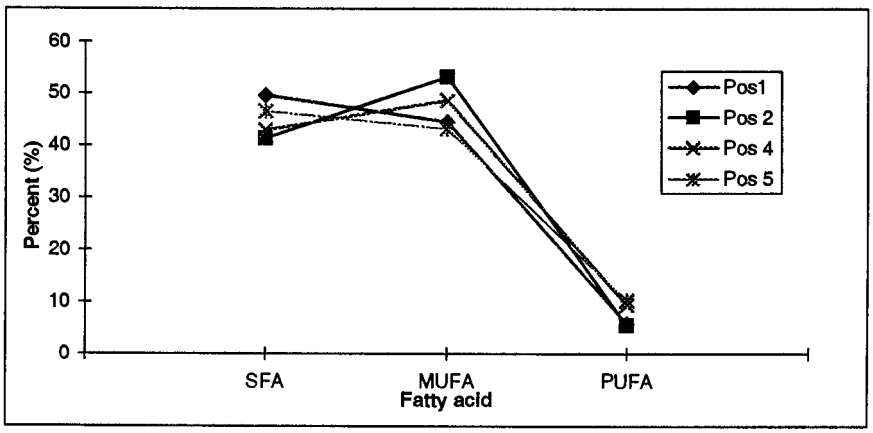

Figure 12: Proportions of fatty acids on the mid-ventral plane along the body of a male neonatal southern right whale.

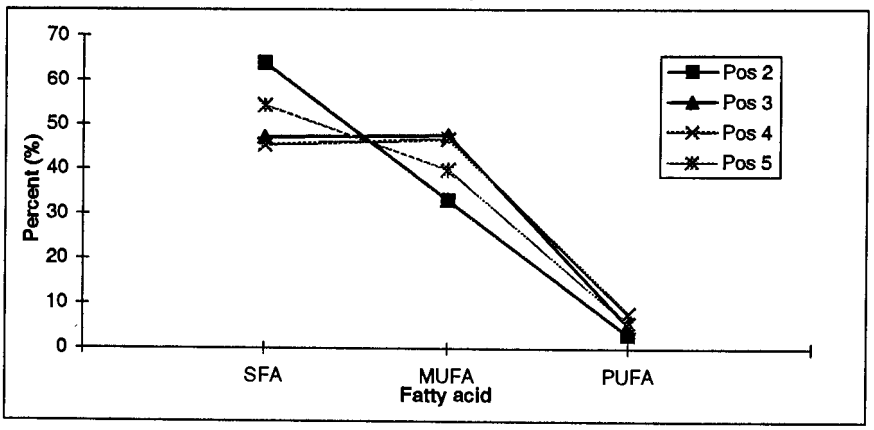

Figure 14: Proportions of fatty acids on the lateral plane along the body of a male neonatal southern right whale.

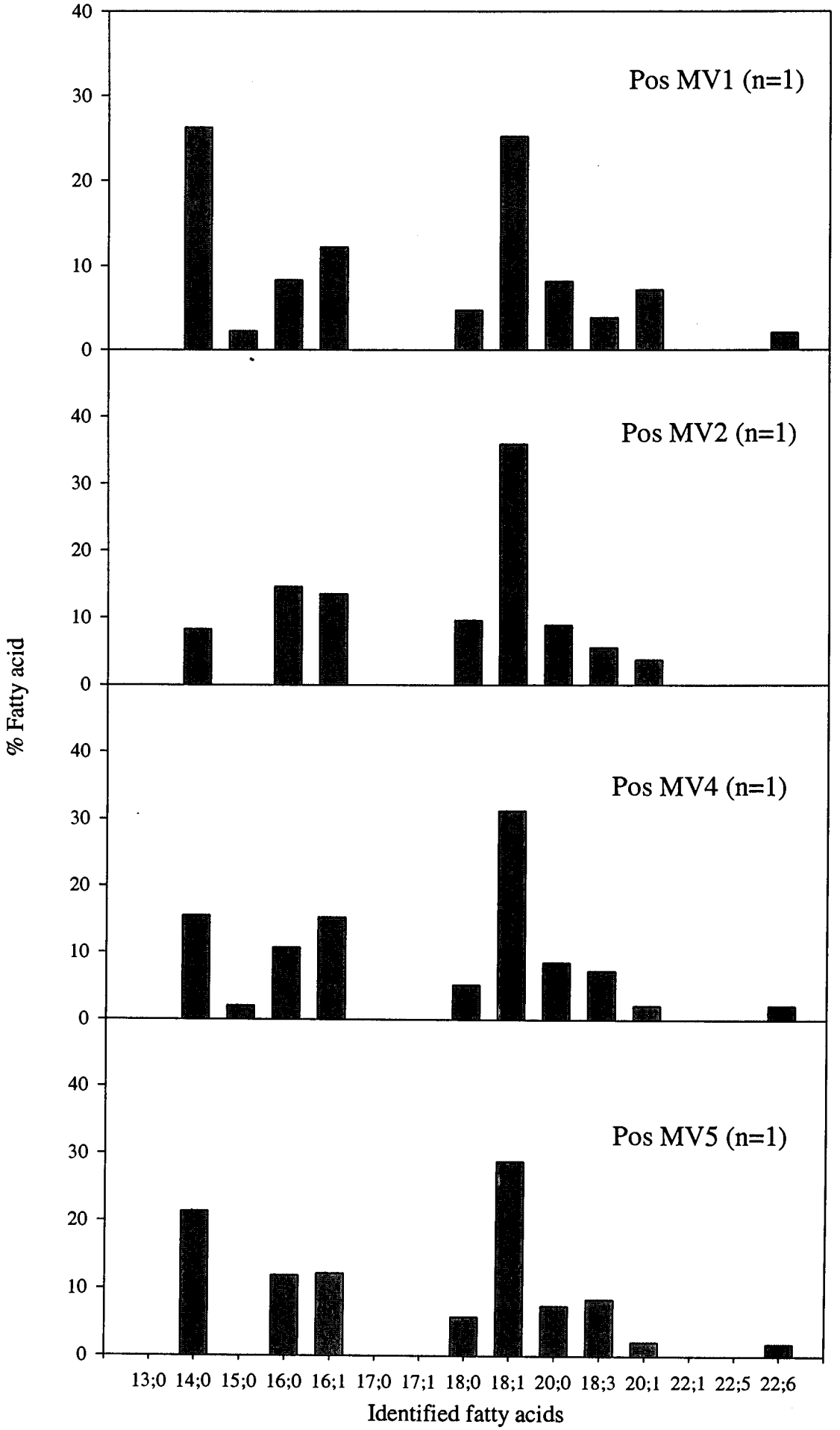

Figure 11: Proportions of fatty acids in positions 1,2,4 and 5 along the mid-ventral plane of a neonatal southern right whale.

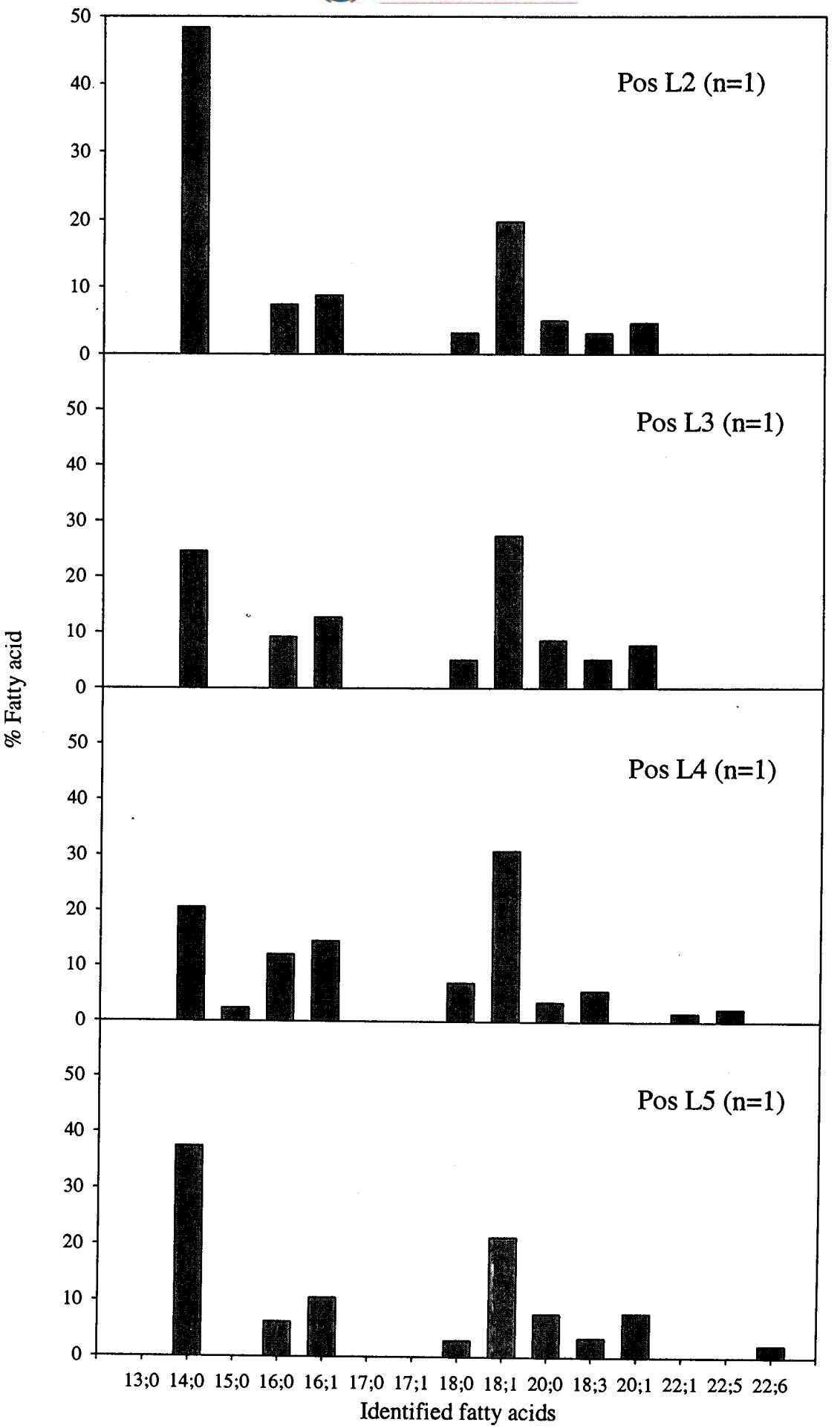

Figure 13: Proportions of fatty acids in positions 2-5 along the lateral plane of a neonatal southern right whale.

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

Juvenile (n = 1):

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

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

Subadult (n = 1):

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

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

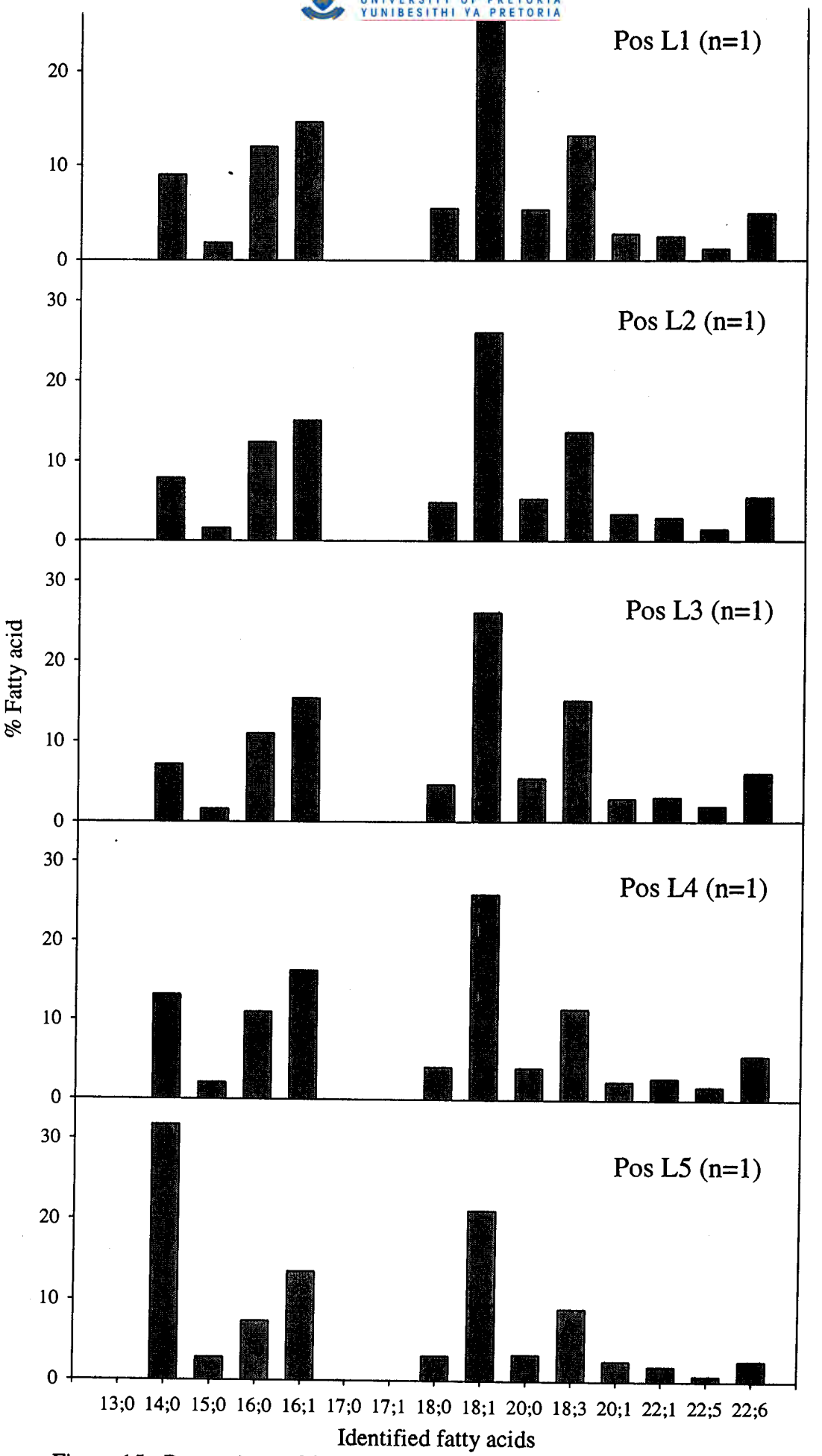

Figure 15: Proportions of fatty acids in positions 1-5 along the lateral plane of a juvenile southern right whale.

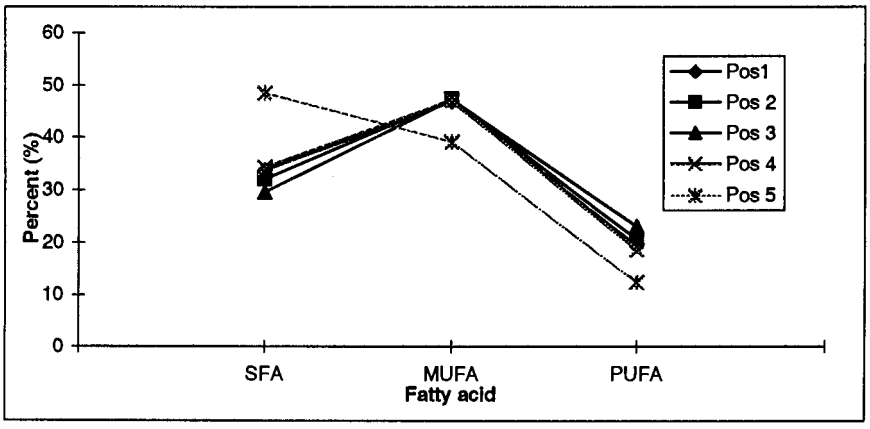

Figure 16: Proportions of fatty acids on the lateral plane along the body of a juvenile male southern right whale.

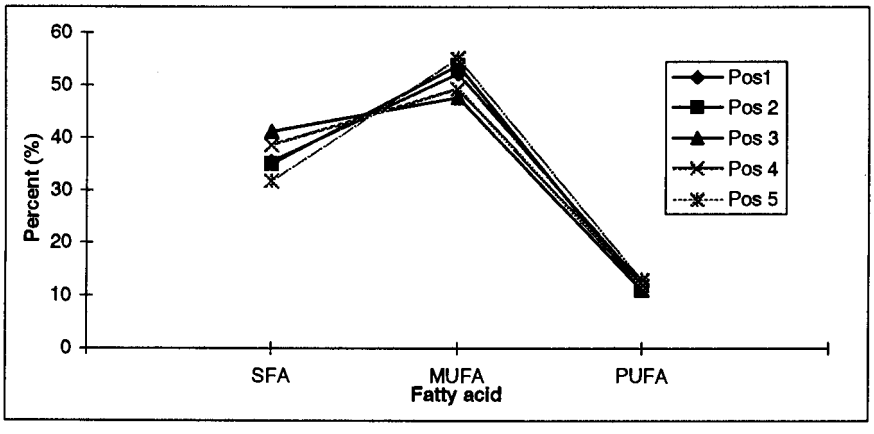

Figure 18: Proportions of fatty acids on the dorso-lateral plane along the body of a subadult male southern right whale.

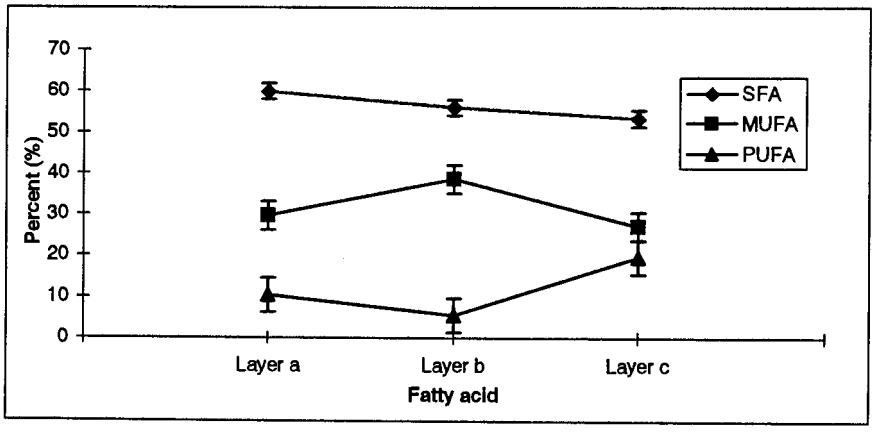

Figure 19: Average proportions of fatty acids in the layers of mid-dorsal position 3 samples from 4 neonatal southern right whales.

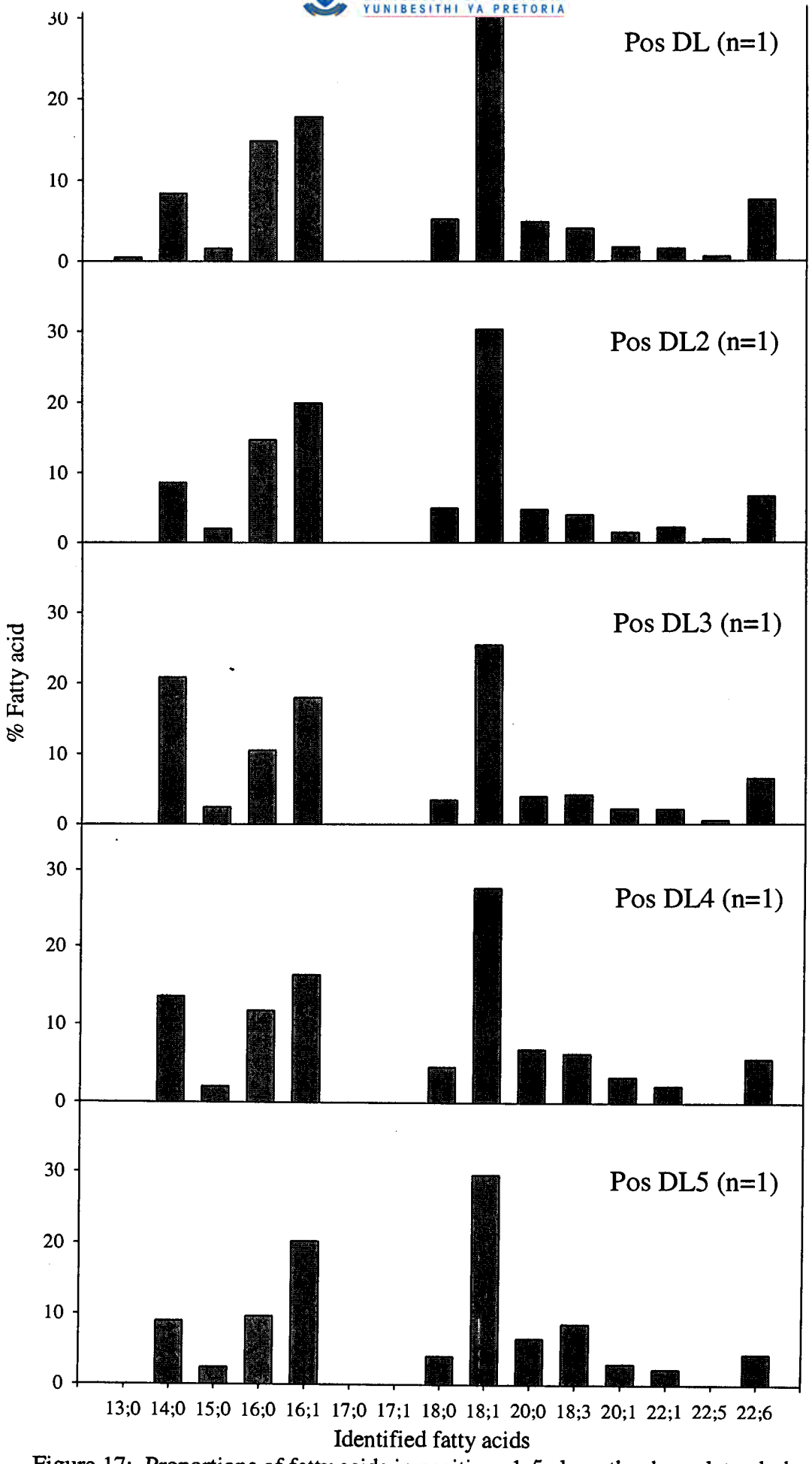

Figure 17: Proportions of fatty acids in positions 1-5 along the dorso-lateral plane of a subadult southern right whale.

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

5.3.1.2 Effect of layer in blubber

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

Neonates (n=4):

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

Juvenile (n=1):

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

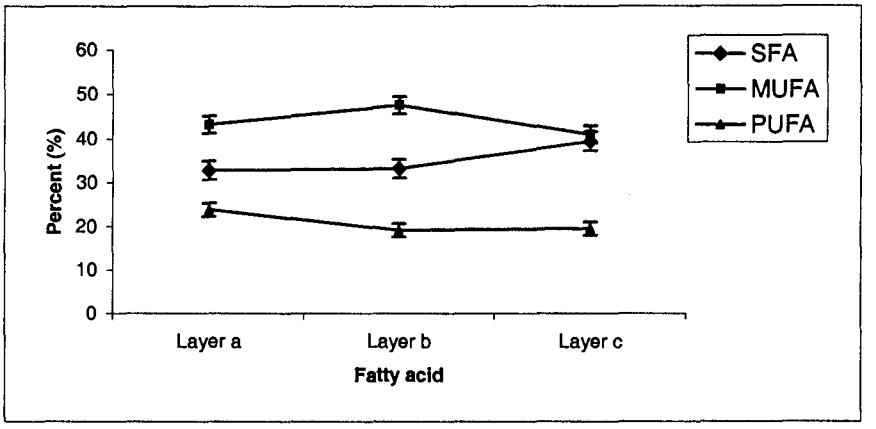

Figure 20: Average proportions of fatty acids in the layers of lateral positions 1-5 from a juvenile southern right whale.

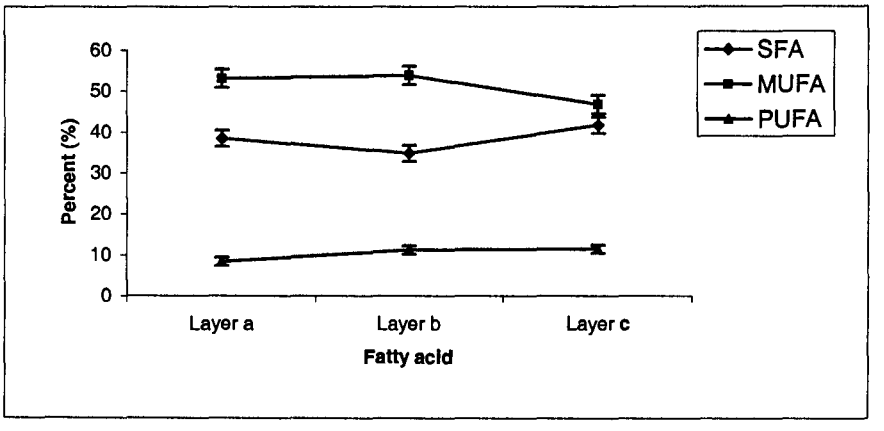

Figure 21: Average proportions of fatty acids in the layers of dorso-lateral positions 1-5 from a subadult southern right whale.

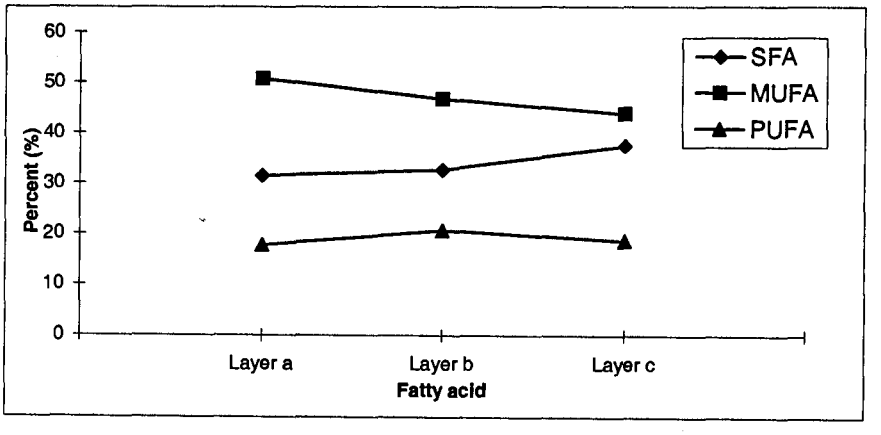

Figure 22: Proportions of fatty acids in the layers of a mid-dorsal position 3 sample from an adult male southern right whale.

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

Subadult (n=1):

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

Adult (n=1):

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

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

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

5.3.1.3 Age and seasonal effects on the proportions of fatty acids

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

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

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

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

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

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

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

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

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

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

5.3.1.4 Seasonal trends in lipid composition between different age groups

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

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

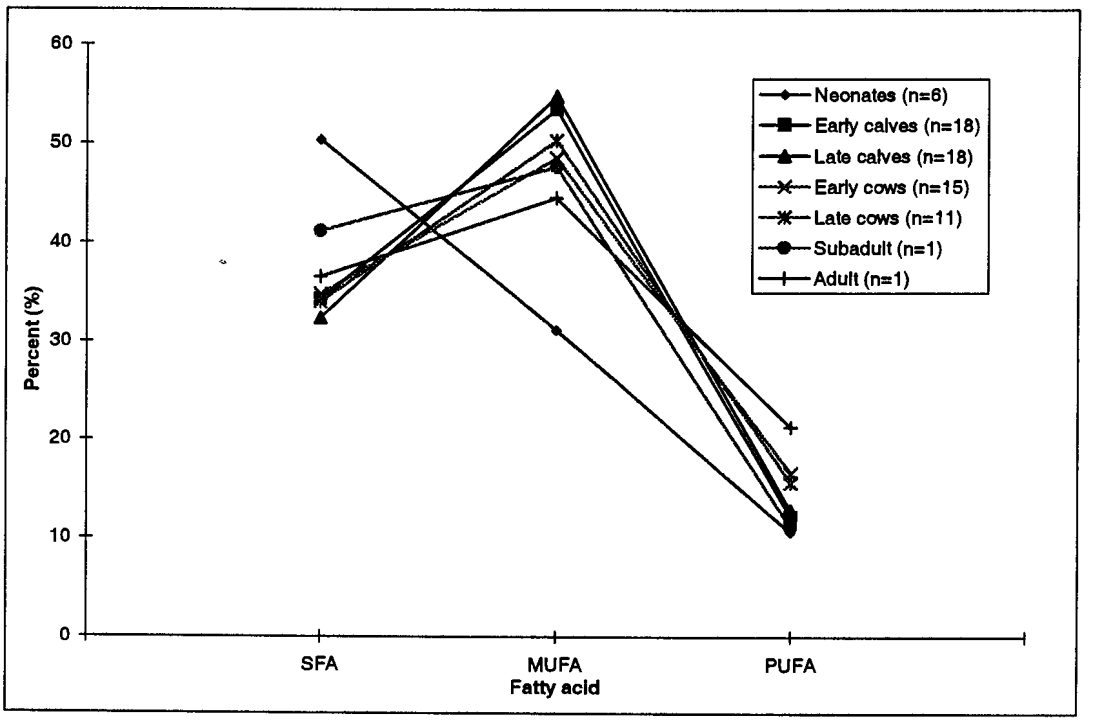

Figure 23: Proportions of saturated (SFA), Monounsaturated (MUFA) and Polyunsaturated (PUFA) fatty acids in various age and seasonal groups.

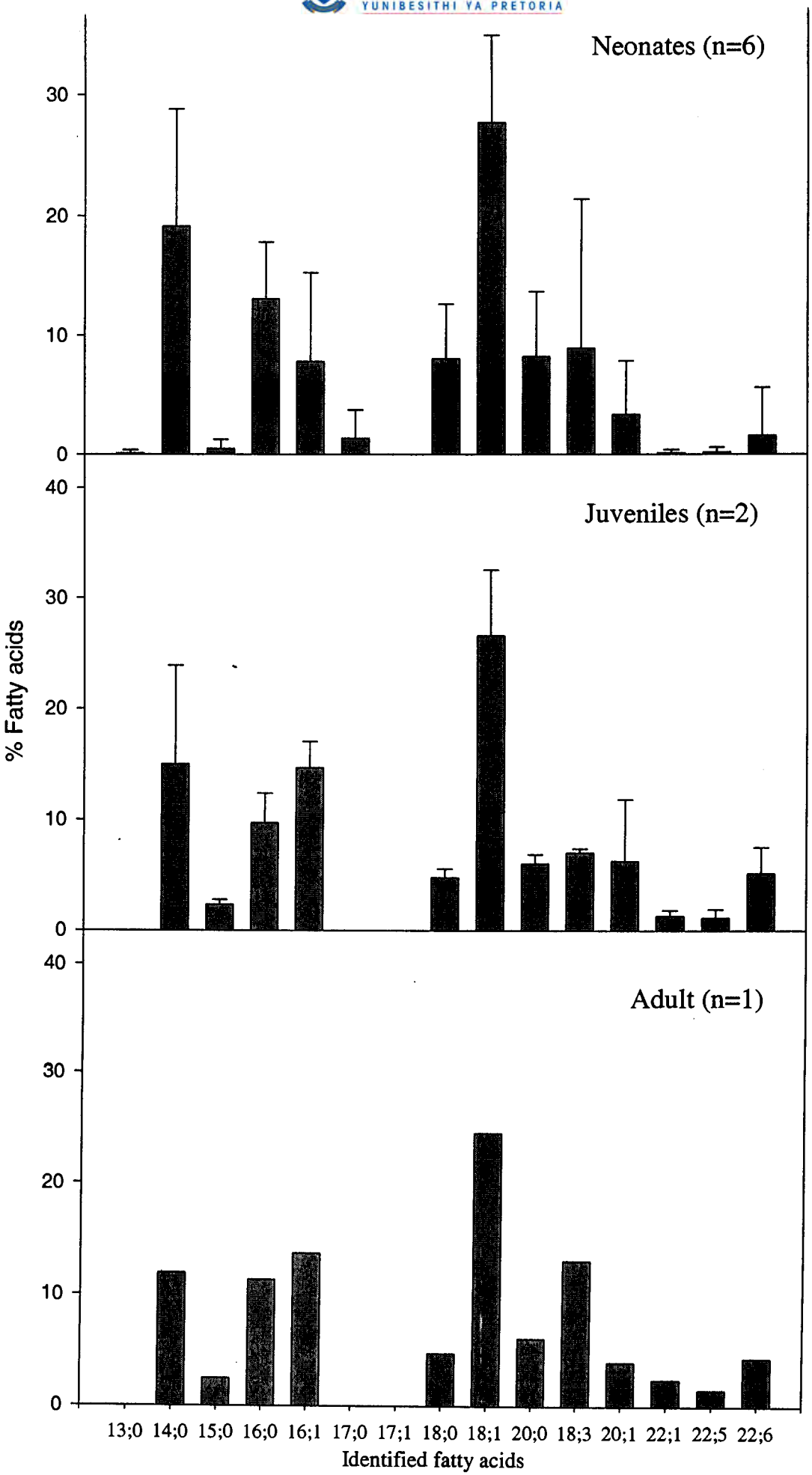

Figure 24: Proportions of fatty acids in various age groups of southern right whales (Mean ± S.E.).

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

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

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

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

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

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

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

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

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

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

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

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

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

5.3.3 Quantitative fatty acid analysis

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

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

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

5.3.4 Proximate analysis of faecal sample

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

5.4 Discussion

5.4.1 Fatty acid composition of southern right whale blubber

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

5.4.3 Quantitative lipid values of southern right whale blubber

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

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

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

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

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

5.4.4 Microbial fermentation in southern right whales

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

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

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

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

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

5.5 Conclusion

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

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

CHAPTER 6

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

6.1 Introduction

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

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

6.2 Materials and Methods

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

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

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

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

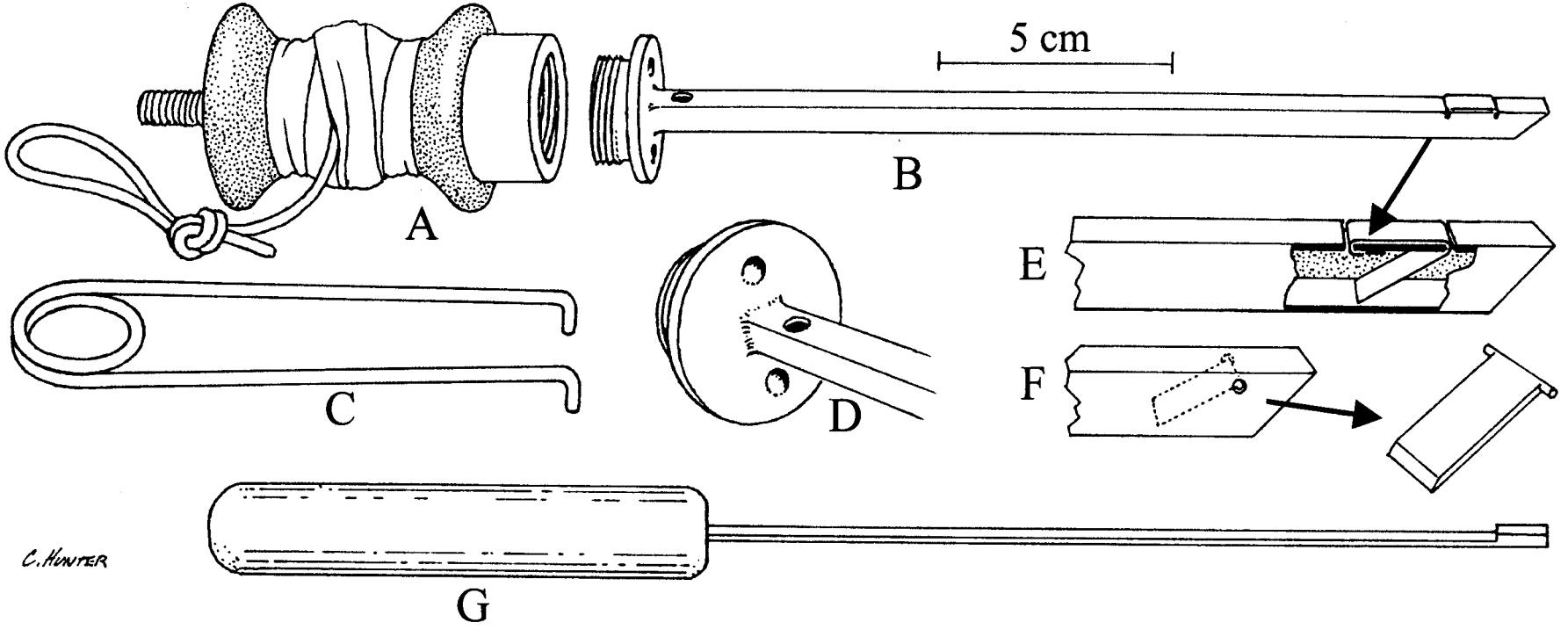

Plate 71: Sketches of the hand-held biopsy system and tools. (A) rubber bush fitted with a brass coupling and nylon cord; (B) brass coupling and stainless steel needle, note the bevelled tip of the needle; (C) spring-steel tool for tightening the biopsy head; (D) brass coupling at the base of the biopsy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

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

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

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

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

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

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

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

6.3 Results

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

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

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

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

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

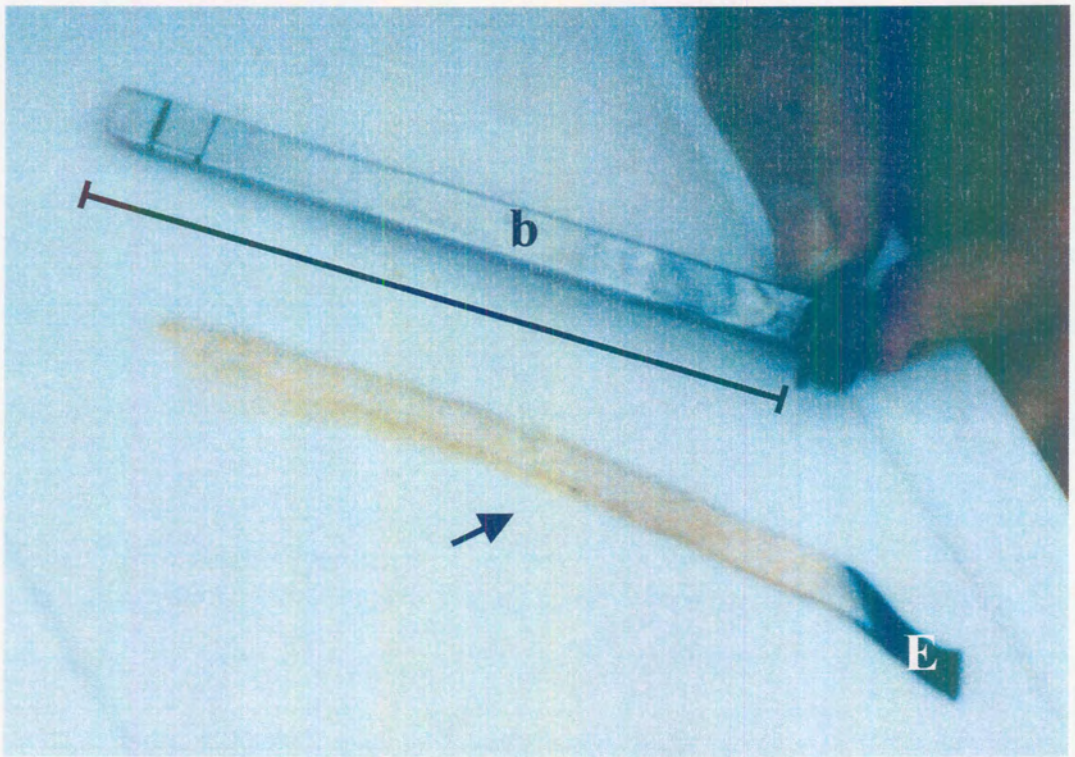

Plate 72: Integument of a southern right whale calf (arrow) and the biopsy head (b) used for sampling (11 cm x 0.8 cm x 0.4 cm). Epidermis (E).

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

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

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

6.4 Discussion

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

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

6.5 Conclusion

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