CHAPTER 1: INTRODUCTION

The "ghostly, eerie" sounds, described by many whalers and fishermen on their various expeditions, were man's first encounters with whale vocality (Payne & McVay 1971). These reports and other statements made by observers in the field were discouraged by the prevailing scientific opinion that whales were mute, presumably because they were said to lack vocal cords. Sound is poorly transmitted between water and air, and because an immersed man's hearing is quite dull, it was not until World War II, when research sonar anti-submarine warfare provided the facilities for listening underwater, that it became generally known that many species of whales are vocal. It was at this time that the first recordings of whale vocalisations were made (Schevill 1964).

Sound is perhaps the major sense used by cetaceans. The physical properties of water allow sound to be propagated much more effectively than it can be in air. This advantage has been put to good use by cetaceans, who use sound in two main ways - for echolocation and for communication. Mysticetes (baleen whales) are not known to echolocate and, although short pulses resembling clicks have been heard in the presence of certain species, they are certainly not typically heard from such species (Evans 1987), and have not been demonstrated experimentally to have any echolocatory function.

In general the sounds made by odontocetes (toothed whales) can be classified into three general categories: tonal whistles, pulsed sounds of very short duration used in echolocation, and less distinct pulsed sounds such as cries, grunts and barks (Richardson, Greene Jr., Malme & Thomson 1995). Most odontocete whistles are narrow-band sounds - sometimes tones, having most of their energy below 20 kHz (Richardson et al. 1995). However, besides broad-band clicks, which can contain any frequency, marine odontocetes are not known to produce sustained frequencies much below 500 Hz, and most of their vocal activity is at frequencies above 20 kHz (Payne & Webb 1971). Pulsed calls are very complex with energy at 500 Hz to 24 kHz and pulse repetition rates up to 5000 per sec (Schevill & Watkins 1966; Ford & Fisher 1982).
Scientists are divided in their opinions about the site(s) of odontocete sound production. There are two schools of thought: those who predict its genesis from somewhere in the larynx (Lawrence & Schevill 1965), and those who predict its genesis from somewhere in the soft anatomy of the forehead (Evans & Prescott 1962; Norris 1964), especially from the region of the nasal plugs.

All the theories to date concerning the mechanism of delphinid sound production have implicated the larynx (arytenoepiglottic tube), the complicated diverticuli associated with the blowhole mechanism, the large muscular plugs that seal off the internal nares, or various combinations of these. The driving mechanism has been thought to be pneumatic, mechanical or both. Various combinations of internal sound transmission paths have been considered: air-muscle/fat-water; air-bone-water; tissue-water (Norris 1969; Evans 1973).

A group of mainly European advocates (Purves 1967; Purves & Pilleri 1983) claim that the sound source is located within the larynx and a second theory, favoured by mainly North American researchers places the sounds source in the nasal plug area just below the blowhole (Evans & Prescott 1962; Norris 1964; Norris 1969; Norris & Harvey 1973; Evans 1973; Evans & Maderson 1973; Amundin 1991). More recent studies have shown the upper nasal pathways to be the source of sound generation. Diercks et al. (1971, in Amundin 1991) used a multiple array of contact hydrophones, which was placed on the rostrum and the melon of dolphins to show that the location of the click sound source was in the nasal area. Cineradiographic evidence from live, phonating dolphins obtained by Norris et al. (1971, in Amundin 1991) and Dormer (1979) showed sound generation and air recycling taking place in the upper nasal pathways and diverticula. There has not, as yet, been any experimental demonstration that the larynx may be involved in sound production in odontocetes.

Mysticete sounds are varied and complex, consisting for the most part of lower and longer sounds than have yet been recorded from odontocetes. Clark (1990) describes mysticetes as producing vocal and non-vocal sounds. Non-vocal sounds include blow, slap and miscellaneous (rubbing against objects, flatulence or baleen rattle) sounds, while vocal sounds include calls and songs.
A broad classification of vocal mysticete sounds would consist of the following (Fish, Sumich & Lingle 1974; Winn & Perkins 1976; Thompson, Winn & Perkins 1979; Watkins 1981; Cummings, Thompson & Ha 1986):

1. Tonal pulses and typical low frequency moans, between 0.4 and 36 secs in duration and of 12 to 500 Hz frequency, but usually between 20 and 200 Hz. Moans may either contain strong harmonic structure or be pure tone, such as the 20 Hz signals recorded from fin whales (Schevill, Watkins & Backus 1964; Thompson, Findley & Vidal 1992). Clark (1990) describes the principal energy for these “simple” calls as being below 1000 Hz. All but the sei (Balaenoptera borealis) whale are known to produce these sounds.

2. Grunt-like thumps and knocks of shorter average duration than moans (50 to 500 msec), and between 40 and 200 Hz. Clark (1990) extended the average duration of these sounds to between 50 and 100 msec being in the 100-1000 Hz range. The humpback (Megaptera novaeangliae), southern (Eubalaena australis) and northern (E. glacialis) right, bowhead (Balaena mysticetus), gray (Eschrichtius robustus), fin (Balaenoptera physalus) and minke (B. acutorostrata) whales are all known to produce these sounds.

3. Chirps, cries and whistles, with frequencies above 1 kHz. Chirps are generally pulses producing short (50-100 msec) discrete tones which change frequency rapidly and are not harmonically related, whereas cries and whistles are pure tonal with or without harmonics.

4. Click-like sounds or pulses of a fraction of a second in duration (0.5 to 5 msec) with frequencies from 3 to as high as 30 kHz, recorded in the blue whale (Balaenoptera musculus) (Beamish & Mitchell 1971; Cummings & Thompson 1971). Click-like sounds of either pure frequency or broad band sounds are reported from B. acutorostrata, E. robustus, M. novaeangliae, B. borealis, Balaenoptera edeni, and B. physalus.

To this list, Clark (1990) adds what he refers to as complex calls. Complex calls are broadband, pulsive signals which consist of variable mixtures of amplitude, modulation of noise and/or a frequency-modulated fundamental. Typical bandwidths for complex pulsive signals are in the
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500-5000 Hz range. Complex calls are often described as sounding like screams, roars and growls. Complex calls have been recorded from *B. mysticetus*, *M. novaeangliae*, *E. australis* and *E. glacialis*.

Humpback whales produce a series of ordered themes containing a number of repetitive phrases in the form of a song, which may last 8-30 minutes (Payne & McVay 1971) and which may be repeated more or less exactly for several hours at a time, with no breaks longer than one minute (Payne & Webb 1971). Humpbacks are not the only baleen whales producing repetitive or monotonous vocalizations. According to Cummings and Philippi (1970) there is some evidence that northern right whales (*E. glacialis*) do so as well. But according to Clark (1990) southern right whales have been recorded extensively during their mating seasons but no sounds resembling song have been recorded and it is therefore assumed that this species does not sing. Songs have been reported for bowhead (Ljungblad, Thompson & Moore 1982; Würsig & Clark 1993) and fin whales (Watkins, Tyack, Moore & Bird 1987). The most precisely repeating sounds yet ascribed to a mysticete are probably the 20 Hz signals produced by fin whales (Schevill *et al.* 1964; Watkins *et al.* 1987; Thompson *et al.* 1992).

Many speculations have been made, but as yet, the mechanism by which mysticetes generate these sounds is unknown. Schulte (1916) mentions a “subcircular diverticulum from the dorsal wall of the respiratory passage” and a ‘spritzsack’ along the anterior wall, however, Howell (1970) did not find any true diverticula, only a slight folding and wrinkling of the rostral end of the mucosa.

Initial anatomical work on the laryngeal area of mysticetes was recorded from the late eighteenth to the late nineteenth centuries. In 1787, Hunter discovered the presence of a laryngeal sac on the ventral surface of the larynx of *Balaenoptera rostrata* (*B. acutorostrata*). The laryngeal sac was later recorded by Eschricht & Reinhardt (1866), Carte & Macalister (1868), Turner (1872), Watson & Young (1879), Beauregard & Boulart (1882), Dubois (1886), Benham (1901), Schulte (1916), Hosokawa (1950), Yablokov, Bel'kovich & Borisov (1974), Quayle (1991) and Haldiman & Tarpley (1993) for a variety of species including *B. mysticetus*, *B. acutorostrata*, *M. novaeangliae*, *Eubalaena australis*, *B. borealis*, *Delphinapterus leucas* and *Physeter catodon*. 
The muscular, "sac-like" structure connected to the ventral wall of the mysticete larynx has been referred to, by many authors, in a variety of ways i.e., "laryngeal pouch" (Turner 1872), "air bag/laryngeal sac" (Murie 1870, in Watson & Young 1879), "sub-laryngeal pouch" (Benham 1901), "ventral air-sac" (Watson & Young 1879), "saccus laryngis ventralis" (Hosokawa 1950) and "epiglottic cavity" (Yablokov et al. 1974). For the purposes of this study, this structure will be referred to as the "laryngeal sac".

The cetacean laryngeal apparatus is constructed, as in other mammals, of a cartilaginous framework and several muscles connecting the cartilages. These form as a whole a tubular organ with the laryngeal cavity in it. The inner surface is covered with a mucous membrane continuous with that of the pharynx upwards and of the trachea downwards (Hosokawa 1950).

The laryngeal cartilages are five in number, three of which (thyroid, cricoid and epiglottic) are unpaired and the other two (arytenoid) are paired (Carte & Macalister 1868; Turner 1872; Hosokawa 1950).

As in other mammals, the laryngeal muscles in mysticetes are classified into two groups, extrinsic and intrinsic. The extrinsic muscles are those connecting the larynx with neighbouring structures and the intrinsic muscles are those which begin and end within the larynx itself (Hosokawa 1950). Up to 17 different muscles (Carte & Macalister 1868; Benham 1901) have been described in the larynx of baleen whales (Yablokov et al. 1974).

Eschricht & Reinhardt (1866) pointed out that the most essential peculiarity of the larynx of mysticetes, as compared with that of the odontocetes, is in its allowing the mucous membrane of the respiratory canals, by means of an opening on the ventral surface, to appear in the form of a sac with an exterior covering of a strong layer of muscles. A similar sac is found in the respiratory canals of many terrestrial mammals, as is very well known. In most cases the sac appears between the hyoid bone and the thyroid cartilage, though in others, especially in some of the apes, it appears between the thyroid and cricoid cartilages, or between the latter and the first ring of the trachea; the last-mentioned case most resembles that of the mysticetes (Eschricht & Reinhardt 1866).
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In their studies of a juvenile and neonate female respectively, Carte & Macalister (1868) and Benham (1901) found no vestiges of vocal chords or of a ventricle, or a lateral laryngeal saccus in *B. acutorostrata*. However, situated in front of the cavity of the larynx, and opening by a wide orifice immediately at the root of the epiglottis, was a remarkable large musculo-membranous mesial sac or laryngeal pouch, which extended downwards and backwards in front of the trachea. Its walls, which were thick, were almost entirely composed of circular muscle fibres. The interior of the sac communicated directly with the central portion of the laryngeal cavity by a wide orifice and was lined by a continuation of the mucous membrane of the laryngeal cavity.

Likewise, in a 6 meter foetus of the blue whale, *Balaenoptera musculus*, Turner (1872) found that there were no true vocal cords passing from the thyroid to the arytenoid cartilages, or laryngeal ventricles. However, on each side within the aperture of the glottis, a short distance below the free edge of the aryteno-epiglottidean folds, a slight fold of the mucous membrane extended obliquely in the antero-posterior direction. These folds might be regarded as rudimentary false vocal cords. An interesting structure that was noticed by Turner (1872) was a "great laryngeal pouch or cul-de-sac" that was connected with the larynx. He describes the laryngeal sac as being 25.5 cm in length. In the same species, Beauregard & Boulart (1882) reported a female foetus of 3.6 m as having a laryngeal sac 21 cm in length (measured from anterior portion to laryngeal cavity).

Hosokawa (1950) conducted a detailed study of the laryngeal apparatus of two female sei whales (*B. borealis*), 12.7 and 13.9 meters in length. In both specimens he found a sac attached to the ventral wall of the larynx, elongated downwards through the whole length of the trachea. The cavity within the sac was elongated longitudinally and communicated with the proper cavum laryngis through a slit along nearly the whole length of the arytenoid cartilage. The inner surface of the sac had many folds and grooves and many granular prominences were visible on the mucous membrane. Judging from this structure, Hosokawa (1950) concluded that it seemed certain that this sac was capable of extension and contraction in the living whale.

Why this structure occurs in mysticetes, as well as the anatomical nature of the laryngeal sac, have been very differently answered by anatomists. Hosokawa (1950) proposed that a key for settling
the problem may lie in the fact that the laryngeal sac in the right and humpback whales is not as
large as those of the rorquals, showing an intermediate form between the laryngeal recess found
in odontocetes (Dubois 1886) and the laryngeal sac in the mysticetes (Eschricht & Reinhardt
1866; Carte & Macalister 1868; Benham 1901; Hosokawa 1950). Evaluation of Hosokawa's
hypothesis is complicated by the fact that the only references he cites refer to studies on juvenile
animals.

The laryngeal sac, according to Dubois (1886) and Benham (1901), is derived from the downward
"sagging" of the thyro-arytenoid muscle, so as to project between thyroid and cricoid cartilage
which has led to the oblique and nearly vertical position of the glottis.

When contemplating the origin of the laryngeal sac one is tempted to see some interrelation
between the laryngeal sac and the aryteno-epiglottideal tube of odontocetes - to assume in some
way that the function of the sac of the mysticetes is taken on by the glottideal tube and the
elaborate "spiracular saes" of the odontocetes. This leads us to look for any homologue in the
odontocete of the laryngeal sac of the mysticete (Benham 1901).

Eschricht & Reinhardt (1866) stated that a laryngeal sac was present only in mysticetes, but
Watson & Young (1879) point out that although the laryngeal sac is consistently recorded in
mysticetes, in which it attains its greatest development, this structure cannot be regarded as a
specific character of the mysticetes. An analogous sac has been described in *Grampus griseus*,
which, according to Murie (1870, in Watson & Young 1879) "fills in great part the angle of
junction between the enlarged epiglottis and the thyroid cartilage, but does not reach the posterior
border of the latter". The arrangement in *D. leucas* is similar to that described for *G. griseus* and
this fact corroborates Murie's assertion that the above apparent distinction between the mysticetes
and odontocetes is "one rather of degree than of kind" (1870, in Watson & Young 1879).

As previously stated, the laryngeal sac is a median, ventral evagination of the muscular wall of the
larynx, between the thyroid and cricoid cartilages which is post-thyroideal in position. In the
odontocetes, no such structures occur in the same relative position. A small median sac has been
described by various authors (Murie 1870, in Watson & Young 1879) in various odontocetes, but
this sac has glandular walls and occurs between the base of the epiglottis and the anterior border of the thyroid cartilage in a pre-thyroideal position (Benham 1901). Murie (1870, in Watson & Young 1879), Turner (1872) and Watson & Young (1879) regard this sac as homologous with that of the mysticetes, and Dubois (1886) supports their opinion. The latter author further includes in the homology, small lateral, glandular outgrowths (also known as Morgagni's ventricles) at the sides of the base of the epiglottis and projecting, more or less, over the upper margin of the thyroid; these ventricles are known in a great variety of mammals.

In all these cases, the sac or sacs lie above the thyroid cartilage. Of the mammals, it is only the mysticetes that possess a post-thyroideal pouch.

Benham (1901) states that it is unacceptable to assume that the median post-thyroideal laryngeal sac of the mysticetes is truly and genetically homologous with that of the odontocetes and other mammals - whether median or lateral - which is pre-thyroideal. Yablokov et al. (1974) concur with Benham (1901) and state that in referring to the comparative anatomic aspect, "this cavity cannot be homologised either with the small dilatations on the floor of the larynx of toothed whales as previously assumed by Murie (1870, in Watson & Young 1879) and Turner (1872) or to the known structures in terrestrial mammals".

As yet, the function or purpose of the laryngeal sac is uncertain. Several hypotheses have been put forward, of which three seem the most plausible.

Rawitz (1900 in Hosokawa 1950), suggested that the laryngeal sac acts as an apparatus for preventing the entrance of water and food into the respiratory canal. The contraction of the massive muscles of the sac would make the laryngeal sac, as well as the larynx itself, firm and solid, so as to avoid accidentally swallowing large quantities of food with water into the larynx and trachea. Alternatively, the blast of air produced by contraction of the laryngeal sac would prevent the entrance of water and food into the respiratory canal. Based on the laryngeal sac's position in B. mysticetus as an integral part of the ventral tracheal wall that bulges dorsally into the tracheal lumen, Haldiman & Tarpley (1993) found that enlargement of the sac should close off the trachea.
A second hypothesis proposes that the sac is concerned with complete utilization of oxygen in the inspired air. Schulte (1916) speculated that by the contraction and relaxation of the laryngeal sac during submergence, a circulation of air in the wide trachea and bronchi might be set up, which would favour the absorption of oxygen by bringing the air in these passages more rapidly into contact with the respiratory membrane than could be done by the usual diffusion currents. Alternatively, Negus (1962) suggested the "rebreathing of air", whereby the sac might act as an air-reservoir so that when the animal is submerged for a prolonged period of time, the used-up air which has been in contact with the pulmonary epithelium would mix with the relatively unused air which has lain in the sac. This mixed air, when blown back into the lungs, would provide a fresh supply of oxygen and take up CO₂ so that submergence times could be prolonged.

In the third hypothesis, the sac is related to phonation. According to Turner (1872) the mechanism of phonation in Balaenidae (right whales) was such that the elongated caudal processes of the arytenoid cartilages would be drawn near to each other and vibrate from a strong expiration. If such a mechanism of phonation be true, we must bear in mind the possible utility of the laryngeal sac.

In 1950, Hosokawa reviewed the hypotheses that mysticete whales may recycle inspired air using the laryngeal sacs and that these sacs may possibly be involved in sound production. Apparently, he was unaware of the characteristics of mysticete sounds, which were not generally known at the time.

In studying the humpback whale, Quayle (1991) postulated that air forced from the laryngeal sac between the arytenoids causes the air column in the sac and perhaps the nasopharynx to vibrate. The resulting pressure fluctuations are transmitted through the soft tissues of the whale into the surrounding water. While the sac was compressed, the trachea would be occluded and gas exchange could presumably continue uninterrupted in the lungs. Conceivably the sac could be refilled from the thoracic air while the whale remained submerged.

The acquisition of laryngeal material from four pygmy right whales, *Caperea marginata*, (and photographs of the visceral anatomy of another - Plates 1 and 2) has revealed a marked
development of the laryngeal sac in adults compared to juveniles. The larynx of this species has
not been described previously, nor has the striking ontogenetic development of the laryngeal sac
in any baleen whale, although most dissections have been of foetal or juvenile material. To
investigate the ontogeny of the laryngeal apparatus in another mysticete, frozen material from four
minke whales, *B. acutorostrata* (an adult male and female and a juvenile male and female) has
been obtained.

*Caperea marginata* (Gray 1846) is the smallest mysticete, adult females reaching 6.5 m in length,
and is usually placed in a family of its own, the *Neobalaenidae*: it exhibits characteristics of both
balaenopterids and right whales.

*Balaenoptera acutorostrata* (Lacépède 1804) is the smallest of the balaenopterids, seldom
exceeding a length of 10.1 m.

Minke whale vocalisations have been described as a series of low-frequency grunts, thumps and
ratchets. Most are trains of sound at 100-200 Hz which seem to make the call of each individual
unique (Stewart & Leatherwood 1985). They also produce pure frequency pulsed sounds at 4-8
kHz involving series of clicks for 6-8 seconds at a time, possibly used for echolocation. Beamish
& Mitchell (1973) recorded short pulse length audio frequency sounds in the presence of a minke
whale. In another study conducted by Winn & Perkins (1976) grunts and thump-like sounds were
recorded. The grunt-like sounds had low, restricted frequencies with the greatest energy
somewhat variable between 80 and 140 Hz. The thump-like sounds had broadband energy from
below 100 Hz to at least 800 Hz with maximum energy between 100 and 200 Hz. Ratchet-like
pulses had an energy peak centred at about 850 Hz with harmonics extending up to at least 6 kHz
and single pulse durations between one and 6 msec. A few sounds were pulse-like and varied in
frequency from 3.8 to 12 kHz. Durations varied from one to 5 msec for the highest frequency
classes but were 16 to 20 msec long for the lower frequency class. A large number of high
frequency zip-like clicks were recorded with principal energy in the 5 to 6 kHz region but with
significant energy near 14 kHz and some energy beyond 20 kHz. These clicks were 0.5 to one
msec in duration.
Pygmy right whale sounds have only been recorded once, in Nov 1986-Feb 1987 when a juvenile spent more than 10 weeks in the harbour at Portland, Victoria (Dawbin & Cato 1992). The sounds recorded were intense, thump-like pulses, which occurred in pairs and in one sample a trio was recorded. The duration of single pulses varied from 140 to 225 msec and the number of cycles from 11 to 19. The frequency at the start of a burst varied from about 90 Hz to in excess of 135 Hz, but the final value was always about 60 Hz. The time from the start of the first pulse to the start of the second pulse in the pairs varied from 430 to 510 msec.

The sounds reported for the pygmy right have most similarity to those of a minke whale recorded in the Antarctic. In both cases the sounds show a downsweep in frequency over much the same range for about the same duration, but the minke whale sounds occurred individually rather than in pairs or trios (Schevill & Watkins 1972; Dawbin & Cato 1992). This would seem to indicate a common method of sound production and similar acoustic structures (Watkins 1981; Dawbin & Cato 1992).

In this project, the morphology and anatomy of the laryngeal apparatus of *C. marginata* will be described, and compared with that of *B. acutorostrata*, with special emphasis on the ontogeny of the laryngeal sac in both species and its possible significance in phonation. This study adds to the previous descriptions of the laryngeal apparatus of minke whales by including both adult and juvenile specimens of both sexes, and by adding descriptions of the fine anatomy.
Plate 1: Ventral view of the viscera and laryngeal apparatus of an adult male *C. marginata*, stranded at Bordjies Drif, Cape Point Nature Reserve on 25/05/82 - (# 82/11). Photographs taken by PBB. Note laryngeal sac lying above the heart (arrow).

Plate 2: Ventral view of the laryngeal sac of an adult male *C. marginata*, details as above.
1.1 PROBLEMS WITH NOMENCLATURE

Because most of the anatomical papers referred to in this study were written over a century ago, the nomenclature used to describe various species of cetaceans sometimes differs from that in current usage and it is not always clear to which species they refer. To this end, the table below summarises the nomenclature used by various authors to describe their specimens, together with the presently accepted taxonomy and revised identifications where necessary (together with the justification therefor).

Table 1: List of nomenclature used by various authors with current interpretation of the species involved and justifications therefor.

<table>
<thead>
<tr>
<th>Authors and associated nomenclature</th>
<th>Present identifications and justifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carte &amp; Macalister (1868)</td>
<td></td>
</tr>
<tr>
<td>1. <em>B. rostrata</em></td>
<td><em>B. acutorostrata</em> -♀ - 5.6 m - synonymised by author.</td>
</tr>
<tr>
<td>Turner (1872)</td>
<td></td>
</tr>
<tr>
<td>1. <em>B. sibbaldii</em></td>
<td><em>B. musculus</em> -♀ approx. 23.9 m (measured along the mid-line of the back from the tip of the lower jaw to the end of the tail) - dark steel grey / almost black in colour, with a generally black ventral surface being mottled with white silvery grey patches. Black baleen.</td>
</tr>
<tr>
<td>Beauregard &amp; Boulart (1882)</td>
<td></td>
</tr>
<tr>
<td>1. <em>B. sibbaldii</em></td>
<td><em>B. musculus</em> -♂ foetus, 3.60 m long - D Robineau (pers. comm.). Authors state that this animal is the same species as Turner’s specimen.</td>
</tr>
<tr>
<td>2. <em>B. musculus</em></td>
<td><em>B. physalus</em> -♀, 12 m long - D Robineau (pers. comm.)</td>
</tr>
<tr>
<td>3. <em>B. antipodum</em></td>
<td><em>E. australis</em> -♀ foetus, 55 cm long -according to Hershkovitz (1966).</td>
</tr>
<tr>
<td>Dubois 1886</td>
<td></td>
</tr>
<tr>
<td>1. <em>B. sibbaldii</em></td>
<td><em>B. musculus</em> -♀ foetus, 2.27 cm long - Author states that this animal is the same species as Turner’s specimen.</td>
</tr>
</tbody>
</table>
Largely on morphological grounds, four "forms" of minke whales have been recognised, one in the North Atlantic and one in the North Pacific (referred to *B. a. acutorostrata* and *B. a. davidsoni* respectively by Omura 1975), and two forms in the Southern Hemisphere. One of the latter was recognised as *B. a. bonaerensis* by Omura (1975) but the second, a smaller "dwarf" form was only described in 1985 (Best 1985; Arnold, Marsh & Heinsohn 1987), and has not yet been given a scientific name. On genetic grounds, the *bonaerensis* form could be considered specifically distinct from that in the North Pacific (Wada & Numachi 1991; Hoelzel & Dover 1991).

If this is accepted, the laryngeal material examined in this study should be regarded as referring to *Balaenoptera bonaerensis* Burmeister, 1867, making this study the first description of the laryngeal anatomy of this species. But, due to the continuing confusion surrounding the nomenclature of the minke whale, the specimens are referred to as *B. acutorostrata* in this text.
CHAPTER 2: MATERIALS AND METHODS

Laryngeal material from a juvenile female, juvenile male, an adult female and an adult male (specimen numbers 44, 159, 15 and 13, respectively) *B. acutorostrata* was obtained from the 1993/1994 Japanese Whale Research Programme under special permit in the Antarctic (JARPA) (Table 2), and was frozen immediately after collection.

Table 2: Collection details of *B. acutorostrata* specimens during the 1993/94 season.

<table>
<thead>
<tr>
<th>Details (specimen #)</th>
<th>Juvenile ♂ (13)</th>
<th>Adult ♂ (159)</th>
<th>Juvenile ♀ (44)</th>
<th>Adult ♀ (15)</th>
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<tbody>
<tr>
<td>Total length of animal (m)</td>
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<td>8.5</td>
<td>5.7</td>
<td>8.9</td>
</tr>
<tr>
<td>Time of capture</td>
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<td>11:28, Jan 18</td>
<td>15:19, Dec 14</td>
<td>10:22, Dec 9</td>
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<tr>
<td>Time of treatment</td>
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<td>12:30, Jan 18</td>
<td>16:20, Dec 14</td>
<td>12:30, Dec 9</td>
</tr>
<tr>
<td>Post-mortem times (hr-min)</td>
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<td>1-00</td>
<td>1-00</td>
<td>2-10</td>
</tr>
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<td>Age (years)</td>
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<td>31</td>
<td>2</td>
<td><em>-</em></td>
</tr>
<tr>
<td>Locality</td>
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<td>60-69 S 70-130 E</td>
<td>60-69 S 70-130 E</td>
<td>60-69 S 70-130 E</td>
</tr>
</tbody>
</table>

* Age undetermined due to broken ear plug

Laryngeal material from two juvenile females, one juvenile male and one adult male (specimen nos 89/3, 90/12, 21/27 and 93/07, respectively) *C. marginata* was available (Table 3). All were strandings on the coast of Southern Africa. All of the specimens, except one juvenile female (specimen no 89/3) were preserved in 10% formalin. Specimen 89/3 was frozen.

Radiographs of the whole laryngeal apparatus of the adult male *B. acutorostrata*, as well as of the juvenile male and female *C. marginata* were taken using a Shimodzu Medical X-ray Unit at the South African Museum. The KV's ranged between 52-70 using Trimax mammography film. Manual processing was undertaken using Polycon developer, with a development time of 4 minutes, and Perfix fixer, with a fixing time of 2 minutes.
Materials and Methods: Collection details of *C. marginata* specimens.

<table>
<thead>
<tr>
<th>Details (specimen #)</th>
<th>Juvenile ♀ (89/3)</th>
<th>Juvenile ♂ (90/12)</th>
<th>Juvenile ♂ (91/27)</th>
<th>Adult ♂ (93/07)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total length of animal (m)</td>
<td>3.3</td>
<td>3.7</td>
<td>3.2</td>
<td>5.9</td>
</tr>
<tr>
<td>Date of stranding</td>
<td>17/02/89</td>
<td>04/03/90</td>
<td>30/03/91</td>
<td>15/05/93</td>
</tr>
<tr>
<td>Post-mortem times</td>
<td>12-24 hours</td>
<td>24-48 hours</td>
<td>24-48 hours</td>
<td>several days</td>
</tr>
<tr>
<td>Locality</td>
<td>Walvis Bay, Namibia</td>
<td>Murdock Valley, Simons town</td>
<td>Salt works, Walvis Bay, Namibia</td>
<td>Buffels Bay, Cape Point Nature Reserve</td>
</tr>
</tbody>
</table>

All frozen material was stored at -18 °C. Before dissection, each specimen was placed in a cold room (2 °C) where it was left to defrost. The juvenile material took approximately 3 days to defrost while the adult material required approximately 4 days to defrost. Once defrosted, the material was moved to a wet laboratory for dissection. After each session the material was returned to the cold room.

All formalin-fixed material was soaked in fresh water before dissections were undertaken. The adult *C. marginata* material was soaked in fresh water for 24 hours, while that from the juveniles was soaked overnight.

Each step of the dissections was recorded on film, using a Nikon F-601 Quartz Date camera with Fuji colour slide, 400 ASA film. Observations were also recorded as physical notes and diagrams.

The dissections of the minke whale material were carried out according to the techniques and diagrams of Benham (1901).

The frozen juvenile female specimen of *C. marginata* consisted not only of the laryngeal apparatus, but the viscera as well. This provided an opportunity to test whether the introduction of water or air into the trachea would cause any reaction in the laryngeal sac.
A 1 m airhose attached to a pump was placed in the pharynx, held at the *aditus laryngis* (Benham 1901) and then air was introduced into the laryngeal area. Observations taken before air was introduced, during the inflow and after the airflow was cut-off, were recorded on film, using a Nikon F-601 Quartz Date camera with Fuji colour slide, 400 ASA film.

Tissue samples from the various muscles, mucosas and epithelia of the laryngeal apparatus of both species were taken using scalpels and were preserved in 10% buffered formalin solution in 125 ml sample bottles.

Each sample was dehydrated and embedded in wax, following standard histological procedure. Sections between 2 μm and 5 μm thick were cut on a rotary microtome, mounted onto slides and stained. Three slides were prepared of each sample; the first was stained with haemotoxylin and eosin, using standard histological procedure (Drury & Wallington 1967; Bancroft & Stevens 1982), the second with Masson's trichrome (procedure described below) and the third with Victoria Blue (procedure described below).
PROCEDURE FOR MASSON'S TRICROME STAIN (Drury & Wallington 1967; Bancroft & Stevens 1982)

1. Bring sections to distilled water
2. Stain in Weigert's Iron haematoxylin for 20-30 minutes
3. Wash in water
4. Differentiate in 1% acid-alcohol until only the nuclei are stained
5. Wash in water until sections are blued - 5 minutes
6. Stain in 1% Ponceau 2R in 1% Acetic acid - 5 minutes
7. Rinse rapidly in distilled water
8. Mordant and diffuse in 1% aq phosphomolybdic acid until collagen is decolourized, muscle, red blood cells and fibrin remaining red - 3 minutes
9. Drain slide and counterstain with 2% Light green in 2% acetic acid - 2 minutes
10. Rinse, dehydrate, clear and mount

RESULTS:
Nuclei - blue-black
Cytoplasm, muscle, Acidophil granules - red
Collagen, cartilage, mucin, Basophil granules - green

PROCEDURE FOR AN ELASTIN STAIN WITH VICTORIA BLUE (Lustgarten 1886, in Bronte Gatenby & Beams 1950); Drury & Wallington 1967; Bancroft & Stevens 1982)

Victoria Blue 4R 1 gm
New fuchsin 1 gm
Crystal violet 1 gm
Dissolve in 200 ml of hot distilled water then add in the following order -
Resorcin 4 gm
Dextrin 1 gm
30% ferric chloride 50 ml (freshly prepared)

Boil for 5 minutes then filter when hot. Transfer precipitate plus filter paper to original beaker
Materials and Methods: 19

and redissolve in 200 ml of 95% alcohol. Boil on a hot plate or in a water bath for 15-20 minutes. Filter and make up to 200 ml with 95% alcohol. Finally add 2 ml of concentrated HCl.

1. Take sections to water.
2. Stain in 0.5 % potassium permanganate - 5 minutes
3. Rinse well in distilled water then 0.5 % Oxalic acid
4. Rinse in water - 95 % alcohol - 2 minutes
5. Use freshly filtered stain in Coplin jar for 1-3 hours or overnight in 0.5 stain and 0.5 96 % alcohol
6. Rinse in several changes of 96 % alcohol
7. Wash in Distilled water
8. Counterstain 2-3 minutes with von Gieson
9. Take through absolute alcohol and xylol - mount

For Australian Antigen stain overnight.

RESULTS:

Elastin - Black
Australian Antigen - Grey-black

Each stain was used in order to highlight specific characteristics of the various tissue samples i.e. Masson's trichome stain for muscle and connective tissue; Victoria Blue for elastic fibres and mucous cells; Haemotoxylin and Eosin for general histology. Once prepared, the slides were examined using a Zeiss light microscope (magnifications are indicated on the various plates).

Slides were photographed using a Zeiss Axiophot photomicroscope, loaded with 35mm 100 ASA colour slide film which was processed by and at The University of Cape Town's Medical School.