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Anatomy of the odontocete tympano-periotic complex in regard to sound conduction

A thesis submitted in fulfillment of the requirements for

the degree of Master of Veterinary Science

Faculty of Veterinary Science

University of Sydney

Duan March, August 2013

Statement of Originality

This thesis represents the original work of the author. It contains no work previously written or published unless due reference to this material is made.

Duan March

Ethics Approval

All necessary Ethical and Scientific permits required to complete this study were obtained.

Duan March

Acknowledgements

First and foremost thanks go to my supervisory team of Dr Damien Higgins, Dr Rachael Gray, Dr Ian Curthoys and Dr Daniel Brown. Without their patience, guidance and assistance this work would not have been possible. Your patience enabled me to explore possible "branches", your guidance kept me returning me to the "trunk" and your assistance ensured this work was completed.

Many thanks go to the Department of Primary Industries, Water and Environment including Dr Rosemary Gales, Dr Isabel Beasley, Drew Lee and Andrew Irvine in Tasmania for their fore-sight and dedication in obtaining specimens and computed tomography scans of stranded cetaceans. Thanks also the staff of the Office of Environment and Heritage, NSW, for the support provided to facilitate additional sample collection. Financial support from the Faculty of Veterinary Science, University of Sydney, was provided for further computed tomography scanning, general research support through the use of laboratory facilities for sample preparation and the use of the Microcomputed tomography machine.

Special mention to Dr Chris Wong for his time, expertise and assistance during the acquisition of Microcomputed tomography scans. Despite completing his own PhD during this period, Chris always made time to assist in the project.

Thank you to Dolphin Marine Magic, Coffs Harbour, who provided in kind support and the ongoing opportunity to work with these animals.

Finally to my family, who tolerated the time away and late nights required to complete this project and provided a welcome counterpoint that helped me to maintain a sense of balance throughout this project.

Thesis Summary

This thesis aims to examine the auditory anatomy of odontocetes, with a particular focus on Cuvier's beaked whale (*Ziphius cavirostris*) to determine if there are any anatomical features unique to this species that may be of functional significance.

Cuvier's beaked whale is the most commonly represented species at atypical stranding events associated with the use of naval sonar. This over representation may represent an acoustic sensitivity to the mid and low frequencies used during these naval exercises.

Chapter one is a review of the literature surrounding the atypical stranding events, including the acoustic parameters, location bathymetry and the pathology observed at necropsy. There is still no consensus on the causal factors required to precipitate such stranding events.

Chapter two is an examination of the gross structures of the tympanoperiotic complex using computed tomography. Morphological parameters are recorded and compared between species and the concept of cochlear stimulation via bone conducted vibrations is explored.

Chapter three is an examination of the acoustic windows of the cochlea using microcomputed tomography and the course of the cochlear aqueduct from the intracranial space to the peribullary sinus using computed tomography. The dimensions of the cochlear aqueduct are used in modelling equations to predict the impedance and the potential impact of this structure upon audition. The interaction between multiple routes of bone conducted vibrations and the potential for a new technique of sound localisation was explored.

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Chapter four is a general discussion of the results found, the potential implications of these results and recommendations for future study. A number of anatomical features that are unique to *Z. cavirostris* were identified and the thesis has created the opportunity to investigate the role that these features may play in sound reception following exposure to natural and anthropogenic frequencies.

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Abbreviations and Terminology

TPC	Tympano-periotic complex
MSMR	Medial sulcus of the mallear ridge
BCV	Bone conducted vibrations
NSW	New South Wales
OEH	Office of Environment and Heritage
DPIWE	Department of Primary Industries, Parks, Water and
	Environment
СТ	Computed tomography
MicroCT	Microcomputed tomography
MFAS	Mid frequency active sonar
Odontocete	Toothed whale
SPL	Sound Pressure level
SEL	Sound Exposure level
dB	Decibel
IID	Interaural intensity difference; a measure of the pressure
	difference between cochleae
ITD	Interaural time difference; a measure of the time difference
	between input into the cochleae
PTS	Permanent threshold shift; as defined by a permanent decrease
	in the sensitivity of the cochlea
TTS	Temporary threshold shift; as defined by a temporary decrease
	in the sensitivity of the cochlea
МАА	Minimum audible angle; a measure of the sensitivity of
	directional hearing
HRTF	Head related transfer function; the spectral filtering that occurs
	due to the shape of the head to enable monoaural directionality

Chapter 1: Literature Review

1.1 Abstract

The family Ziphiidae describes 6 genera and 21 known species of odontocete that are commonly known as beaked whales. These animals are some of the most prolific divers in the ocean and can reach depths of up to 1800 metres whilst foraging (Johnson *et al.* 2004; Tyack *et al.* 2006). There is scant biological data on these animals as *in situ* studies are inherently difficult due to the preferred pelagic habitat, extended dive times and the reclusive nature of the animals.

Beaked whales, in particular Cuvier's beaked whale (*Z. cavirostris*), have stranded in atypical patterns following naval exercises using low and mid-frequency active sonar (MFAS) (Evans & England 2001; Freitas 2004; Martín, Servidio & García 2004; Frantzis 2004). The exact causal factors for this relationship are unknown (Filadelfo *et al* 2009; D'Amico *et al.* 2009). The process to define this relationship is hindered by uncertainty surrounding the mechanisms by which odontocetes receive sound (Cranford, Krysl & Amundin 2010) and a paucity of biological data regarding the auditory biology and anatomy of *Z. cavirostris*. This thesis aims to investigate the auditory anatomy of *Z. cavirostris*, in comparison to other beaked whales and other odontocetes, in an attempt to identify anatomical features that may create a species-specific acoustic vulnerability to the low and mid frequency sounds used during naval events.

1.2 Atypical stranding events involving Cuvier's beaked whale associated with the use of mid frequency active sonar

The relationship between the use of mid frequency active sonar (MFAS) and atypical stranding events of beaked whales is unclear. Whilst several stranding events have coincided with naval exercises using MFAS, there are also many circumstances where this sonar was deployed and no stranding events occurred (Filadelfo *et al.* 2009). The correlation between exposure and stranding appears to vary geographically (D'Amico *et al.* 2009) and hence indicates that environmental factors may also be important in precipitating an atypical stranding event.

Recent studies examined the behaviour of Z. cavirostris following exposure to MFAS and recorded avoidance, extended dive durations and extended time between foraging dives (DeRuiter et al. 2013). However, there was no observed behavioural change that would explain the stranding events in Greece (Frantzis 2004), Madeira (Freitas 2004), the Canary Islands (Martín, Servidio & García 2004) and the Bahamas (Evans & England 2001). The locations all had similar environmental bathymetry (Zimmer 2003) and the reported findings at necropsy, where available, were considered consistent with acoustic or impulse trauma (Ketten 2005). There are a number of acoustic parameters that need to be considered when assessing the impact of sound exposure underwater. These include the sound pressure level (SPL), or loudness of the sound at the sound source; the sound exposure level (SEL), or loudness of the sound at the time the sound reaches the animal factoring in the dissipation of acoustic energy with distance, the frequency of the sound and the duration of the sound. The known acoustic parameters of the naval exercises involved with documented atypical stranding events comprised of frequencies ranging from 0.45-8.2 kHz, sound pressure levels (SPL) up to

230dB/uPa@1m and pulse durations of 2 seconds (Zimmer 2003). The potential effects of these parameters on *Z. cavirostris* are discussed below.

Exposure to high SELs can cause temporary or permanent hearing damage, described as temporary (TTS) or permanent (PTS) threshold shifts respectively. TTS and PTS are measured in dB and describe the resulting decrease in cochlear sensitivity following sound exposure. TTS have been recorded in Bottlenose dolphins (Tursiops truncatus) at SPL's as low as 160dB (Nachtigall, Pawloski & Au 2003), however this was with continuous exposure for a period of thirty minutes at a frequencies between 8-16 kHz. The relationship between SPL and time of exposure required to induce a TTS follows a logarithmic algorithm between increasing sound energy and decreasing duration of exposure (Mooney 2009). Using this relationship it was demonstrated that MFAS, at the acoustic levels used during these atypical stranding events, could induce TTS, however to do so, the animals exposed would need to remain within 40 meters of the sound source for approximately two minutes (Mooney 2009a). This scenario is unlikely to have occurred at these atypical stranding events as beaked whales are known to demonstrate avoidance behaviour following exposure to MFAS (Tyack et al. 2011; DeRuiter et al. 2013) and the geographic spread of animals at the Bahamas event indicated that some animals may have been as far as 25 kilometres away from the sound source at the time of exposure (Balcomb III & Claridge 2001).

The dramatic bathymetry associated with all of the documented atypical stranding events has been suggested to be a contributing factor to these events (Evans & England 2001). Depths immediately off shore at these areas increase to greater than 1000m (D'Amico *et al.* 2009); such habitat is preferred by beaked whales (MacLeod & Zuur 2005) who are prolific divers and can descend to depths in excess of 1800

meters to forage for prey (Johnson et al. 2004; Tyack et al. 2006). Unfortunately, these canyons also provide ideal pathways for submarines trying to avoid detection and hence are the preferred training grounds for naval exercises using MFAS during Anti-submarine warfare training (D'Amico et al. 2009). Following exposure to MFAS, Z. cavirostris have been observed to stop foraging and leave the area with extended dives (DeRuiter et al. 2013). However, if an animal was on the shoreward side of the vessel at the time of exposure and attempted to flee, the steep bathymetry may result in the animals being driven ashore. The great depths within these canyons also result in oceanic layers of variable densities and strong currents, which may also play a role in the behaviour of both the animal and the sound underwater. It has been suggested that the interaction between these two variables may be one factor required to precipitate a stranding event (Ketten et al. 2003) however such interaction is difficult to predict and even more difficult to test experimentally. The pathological data collected during necropsies following these events suggests an acute sub-lethal event, with the ultimate cause of death being the physical stress of stranding (Evans & England 2001). Notable findings at necropsy of animals involved in the Bahamas and Madeira stranding events included atypical patterns of haemorrhage within the cochlea, cochlear aqueduct and the intracranial subarachnoid space (Ketten et al. 2003; Ketten 2005). These findings are consistent with an acoustic or impulse trauma and given the circumstances, the former was assumed to be more likely (Evans & England 2001). Necropsy findings from the Canary Island event included haemorrhage within intracranial spaces, the ocular region and within the acoustic fats, and the presence of fat emboli in the internal organs of animals; suggestive of a decompression sickness-type syndrome (Fernández 2004). No examination of the auditory structures was performed on the

animals involved in the Greece stranding event, however ocular or periocular hemorrhages were noted (Frantzis 2004).

Several possible causes for the observed haemorrhages were discussed, including concussive acoustic trauma, barotrauma, and spontaneous subarachnoid haemorrhage (Evans & England 2001). The SEL to which the animals were exposed are less than those used by Z. cavirostris whilst echolocating, which can reach 214 dB (Zimmer et al. 2005) and are not believed to be sufficient to cause concussive acoustic trauma (Mooney et al. 2009a; Foote et al. 2012). The frequencies used during MFAS are below 10kHz (Zimmer 2003) which is well outside the peak spectra, or range of peaks sensitivity, for Z. cavirostris. Z. cavirostris is predicted to have a peak spectra of between 40-50kHz (Cranford, Krysl & Amundin 2010; Zimmer et al. 2005), which is low compared with most other odontocetes, it is still markedly higher than the frequencies used during MFAS, and hence should not be capable of causing acoustic damage from normal pathways of sound reception. The effect of an uncontrolled ascent, driven by fear and steep bathymetry, with resulting decompression sickness and gas emboli has been strongly debated (Jepson et al. 2003; Piantadosi & Thalmann 2004) and to date no consensus has been reached. Studies have proposed the process of rectified diffusion, whereby nitrogen bubbles come out of solution within supersaturated tissues following esonification, and grow with the decreasing pressure that occurs during a rapid ascent (Zimmer & Tyack 2007). This hypothesis was rebutted by Saunders et al (2010) who demonstrated that static diffusion, or the general movement of gas along a concentration gradient, independent of esonification, is the primary mode of bubble growth in supersaturated tissues and is hence unlikely to be markedly effected by naval sonar. The uncharacteristically slow ascent exhibited by beaked whales

following exposure to naval sonar in controlled exposure experiments (Tyack *et al.* 2011; DeRuiter *et al.* 2013) would decrease the likelihood of decompression sickness, as would the absence of significant increase in blood nitrogen partial pressures following repeated dives (Houser *et al.* 2010). However the topic remains under debate.

Some odontocetes have been shown to have unusual clotting pathways with an absence of Hageman's and Flectchor factors (Bossart 2001), which may result in a diathetic fragility. This may predispose odontocetes to spontaneous haemorrhage following exposure to impulse events (Ketten *et al.* 2003). No hemostatic studies have been performed on *Z. cavirostris* and hence it is unknown if they would be physiologically susceptible to haemorrhage. It has been suggested that aspects of the cranial anatomy may facilitate the resonance of soft tissue structures, which may precipitate a haemorrhage in this species more so than others (Rommel *et al.* 2006). Recommendations have been made to assess the resonance frequencies of the Eustachian tube (Evans, Lautenbacher Jr & Hogarth 2002), which is believed to be uniquely patent in beaked whales at depth (Ketten 1998), however to date this work has not been published.

There is an acknowledged correlation between the use of MFAS and beaked whale strandings (D'Amico *et al.* 2009). The inability of scientists to further define the causal factors in this relationship prevents the development of appropriate mitigation measures. The apparent supernormal sensitivity of *Z. cavirostris* to MFAS suggests an acoustic vulnerability and further investigation is required to clarify this.

1.3 Odontocete auditory anatomy

The auditory anatomy of odontocetes is markedly different from that of their terrestrial ancestors. The odontocete skull has telescoped cranially and caudodorsally (Miller 1923), creating a dorsal breathing apparatus, or blowhole, and the external pinnae have been reduced to vestigial cartilaginous rings embedded in subcutaneous tissue. The external auditory canal has been transformed into a debris-filled space terminating blindly prior to the tympanic membrane (Ketten 2000) and a pair of bones representing the middle and inner ears are isolated from the skull, suspended within sinus within a complex series of air sinuses derived from an anterior extension of the Eustachian tube, called the pterygoid sinus complex (Mead & Fordyce 2009). The sinus surrounding the bones is the peribullary sinus and is filled with a vascularised, spongy epithelium called the peribullary plexus. The pair of bones is called the tympanic bulla and the periotic bone, respectively, and together is known as the tympanoperiotic complex or the TPC (Mead & Fordyce 2009). The



Figure 1: Transverse section of a CT scan of the head of *Mesoplodon hectori* showing the extra-cranial location of the tympanoperiotic complex (TPC) showing and surrounding structures involved in sound conduction in *a*) grey scale and *b*) coloured for identification. The TPC (pink) sits within the peribullary sinus (orange), which is lined with a fibrous venous plexus (green). The mandible (yellow) flares posteriorly and is filled with specialised acoustic fats (purple) that communicate with the lateral plate of the tympanic bulla at the Medial sulcus of the mallear ridge (black). The internal acoustic meatus (blue) houses the eighth cranial nerve and the cochlear aqueduct and travels through the basicranium between the intracranial space and the TPC. Scale bar is 5 cm.

The functional equivalent to the external auditory canal of terrestrial mammals is a set of specialised acoustic fats that lay within the posteriorly flared region of the mandible, called the pan bone (Norris 1968), which has a thin bony wall laterally and is open medially. The middle ear equivalent is the tympanic bulla, which has a thickened bony scroll medially called the involucrum and a thin dorsolateral wall called the tympanic plate. Cranially the tympanic bulla tapers into the anterior spine. The tympanic bulla terminates caudally with medial and lateral bulbous prominences protruding ventrally and the dorsal surface extends caudally as the posterior tympanic process.

The periotic bone sits on top of the tympanic bulla and houses the inner ear including the cochlea and vestibular apparatus. The posterior tympanic process articulates with the posterior periotic process and comprises a point of attachment between these two bones. Laterally the periotic bone and tympanic bulla are in contact for the extent of the periotic bone, with the process sigmoideous of the tympanic bulla protruding across this boundary dorsally. The medial sulcus of the mallear ridge (MSMR) is an indentation along the dorsolateral wall of the tympanic bulla just cranial to the process sigmoideous and houses a dorsal extension of the mandibular fat body. The MSMR is believed to serve as the functional equivalent to the tympanic membrane, transferring vibrations from the acoustic fats through the ossicular chain (Cranford, Krysl & Amundin 2010). The point of origin of the ossicular chain is immediately opposite the MSMR, within the tympanic cavity, and it traverses the tympanic cavity to connect with the oval window of the periotic bone as shown in Figure 2.



Figure 2: Transverse view of the TPC of *Physeter Macrocephalus*. The TPC is oriented with the periotic bone (pb) on the top of the image and the thickened medial involucrum (tbi) on the right. Note the ossicular chain (oc) communication between the lateral edge of the tympanic bulla (tb) near the medial sulcus of the mallear ridge (msmr) and the oval window (ow). Scale bar is 1 cm.

The tympanic bulla is lined with a vascularised mucosa called the corpus cavernosum. The role of this tissue is not certain; however it is possible that it is involved in regulating the space within the middle ear whilst diving, to maintain a similar impedance value of the round window (Ketten 2000). CT scans have demonstrated that there is air in the middle ear at sea level; however it is not clear if this remains the case as the animals dive. Beaked whales have a unique bony strut at the opening of the Eustachian tube, assumed to allow the flow of air into the sinus, thereby helping to equalize the pressure in this cavity and maintain consistent cochlear impedance at depth.

The periotic bone sits tightly on top of the tympanic bulla and is in close contact with the ventral surface of the skull. The shape of the periotic bone varies between species (Kasuya 1973) but is basically globular and dense, with the central component, called the pars cochlearis, housing the cochlear and vestibular systems. The morphology of the odontocete cochlea varies between species, depending upon the range of hearing frequencies used (Ketten 1992). Animals using high frequencies have a greater degree of osseous support for the basiliar membrane, with well-developed inner and outer osseous laminae within the cochlea. These morphological observations have seen odontocete cochlea classified as Type 1, where the peak spectra of sensitivities are greater than 100kHz; and Type 2, where the peak spectra is less than 100kHz.

The semicircular canals of the vestibular apparatus are also housed in the pars cochlearis. These structures are disproportionately small in odontocetes (Spoor 2003; Gray 1907). This diminution is demonstrated by the marked contrast in the size of the semicircular canals between odontocetes and humans as shown in Figure 3. Attempts were made to standardise the comparison and both specimens are

taken from the left hand side inner ear, however the *Z. cavirostris* labyrinth was reconstructed from a specimen lacking the internal membranous structures due to an inability to obtain a freshly fixed periotic bone. Given the close association between the membranous structures and the osseous compartment typical of these structures, the differing preparatory methodologies are not assumed to create a significant difference.



Figure 3: Three dimensional reconstruction of the cochlea and vestibular apparatus of a) *Ziphius cavirostris* and a b) Human. The reconstruction is of the left inner ear and was performed on a sample with no membranous structures. Note the unique diminution of the semicircular canals in odontocetes relative to the size of the cochlea

The TPC communicates with the skull via a number of routes. Firstly, the Eustachian tube exits the tympanic bulla and extends anteriorly to communicate with a series of sinuses, known as the pterygoid sinus complex, which line most of the basicranium (Mead & Fordyce 2009). The anatomy of *Z. cavirostris* in this regard is unique in that a bony sigmoid strut is present at the entry of the Eustachian tube into the tympanic cavity to prevent closure (Ketten 2000). Secondly, the internal auditory meatus communicates between the dorsal surface of the periotic bone and the subarachnoid space intracranially. This structure houses the eighth cranial nerve and the cochlear aqueduct (Evans & England 2001). Thirdly, whilst the TPC is usually suspended via a ligamentous arrangement within the peribullary sinus, some species of odontocete also have an osseous connection between the posterior process of the tympanic bulla and the squamous bone at the caudolateral border of the peribullary sinus (Kasuya 1973).

The odontocete auditory anatomy is a remarkable example of evolution. The role of the structures remains the same; to receive and conduct vibrations. However the properties of sound underwater alter the methods in which this outcome can be achieved. Odontocetes have evolved an auditory system from a terrestrial blue print that enables this function and have expanded their auditory capabilities to enable echolocation. This has enabled navigation and foraging behaviours in often light poor, object dense environments to the extent that odontocetes are able to occupy and thrive in every aquatic niche on earth, using audition as their primary means of navigation and communication (Ketten 1992).

1.4 Odontocete auditory physiology

The bulk of scientific data regarding sound conduction in odontocetes supports the "pan bone" theory of sound conduction (Ketten 2000) first proposed over 40 years ago by Norris (1968). This theory describes the transmission of vibrations from the specialised acoustic fats of the "pan bone", and the "gular region" under the tongue (Cranford *et al.* 2008), to the TPC. These structures are in contact with the thin ventrolateral wall of the tympanic bulla, which acts as a receiving plate and transfers the vibrations through the ossicular chain to the cochlea. A schematic demonstration of this pathway is demonstrated in Figure 4.

The acoustic fats, which lie within the pan bone and extend medially to contact the lateral wall of the tympanic bulla. A dorsal branch of this fat body has recently been identified and connects with the TPC at the dorsolateral edge in the MSMR. This sulcus is immediately adjacent the point of commencement of the ossicular chain, which consists of three articulating bones. The vibrations are then transferred through the ossicular chain to the cochlea, which is housed within the periotic bone and articulates with the tympanic bulla.



Figure 4: Schematic version of the structures involved in sound conduction in the odontocetes. Pressure waves, as symbolized by the speaker icon, enter the head via the pan bone, which is the posteriorly flared region of the mandible and the gular pathway, which is between the mandibles and under the jaw. Vibrations are transferred to the associated acoustic fats, which lie within the pan bone and extend medially to contact the lateral wall of the tympanic bulla. A dorsal branch of this fat body has recently been identified and connects with the TPC at the dorsolateral edge of the tympanic bulla at the MSMR. This point of commencement of the ossicular chain within the tympanic cavity lies directly underneath the MSMR. The ossicular chain transfers the vibrations to the oval window and through the cochlea, which is housed within the periotic bone

Though widely adopted now, the pan bone theory, and the role of the odontocete middle ear in general, has long been the topic of debate. McCormick et al (1970) hypothesized an alternative pathway of cochlear stimulation involving boneconducted vibrations (BCV). In this model, cranial vibrations generated after exposure to sound are transferred to the TPC creating a gross vibration of the complex and subsequent motion between the oval window and the stapedal footplate, due to the inertia inherent within the ossicular chain. This model was based on experimental data, which showed that the application of tension to the ossicular chain resulted in a marked decrease in hearing sensitivity, whilst surgical removal of the first ossicle, the malleus, had minimal effect (McCormick et al. 1970). Fleischer (1978) debated this theory and maintained that the ossicular chain, including the malleus, played an active role in the transmission of vibrations from the tympanic bulla to the periotic bone. This dispute was based on the fact that gross vibrations of the cranial tissues would not enable directional hearing and that the surgical technique employed by McCormick may have damaged the auditory structures and given false results.

A series of theoretical modelling papers (Nummela 1999; Hemila, Nummela & Reuter 1999) supported an ossicular pathway and demonstrated how the ossicular chain would function within the odontocete middle ear. The role of the ossicular chain in the terrestrial middle ear is to act as an impedance mismatch device to compensate for the low impedance of air and the much higher impedance of the fluid filled cochlea. The terrestrial mammalian ear achieves this via the concentration of vibrations from a large receiving plate, or tympanic membrane, onto a small end plate, the oval window and by the leverage generated through the ossicular chain (Nummela & Sirpa 1995). However, impedance matching requirements of the

odontocete middle ear are markedly different because of the aquatic environment in which whales receive sound. The impedance of water and the odontocete cochlea, and the particle velocity of a sound wave underwater, are such that the odontocete middle ear is required to generate small increases in pressure and a marked increase in particle velocity of the incident sound wave as it travels from the tympanic plate to the oval window (Hemila, Nummela & Reuter 1999). This model is supported by the ability of models described to predict, with reasonable accuracy, audiograms for a number of odontocetes (Hemilä, Nummela & Reuter 2001). The two competing theories were amalgamated by a proposal that described a multimodal mode of cochlear stimulation (Cranford, Krysl & Amundin 2010). Cranford proposed that high frequency sounds would travel through the ossicular chain, as per Norris's theory, whilst low frequency sounds would grossly vibrate the TPC and achieve cochlear stimulation via McCormick's model. This hypothesis was based on the decreasing ability of long wavelength, low frequency sound to generate complex movements at the MSMR. However, despite these advances there is still uncertainty surrounding the pathways by which odontocetes receive sound (Cranford, Krysl & Amundin 2010).

1.5 Odontocete Sound Localisation

The localisation of sound in both vertical and horizontal planes is an essential requirement of the odontocete auditory system. Odontocetes need to be able to communicate with conspecifics, avoid predation, and interpret returning sonar signals whilst echolocating. Terrestrial animals achieve directional hearing through the interpretation of Inter-aural Time Differences (ITD) and Interaural Intensity Differences (IID). These terms describe the difference between the arrival times of the sound to each ear (ITD) and the differences in the loudness of the sound between each ear (IID) and enable animals to determine sound direction in the horizontal plane (Mooney, Yamato & Branstetter 2012). The sensitivity of these techniques is referred to as the Minimal audible angle (MAA), which describes the animal's ability to locate a sound source to the nearest degree. Due to the altered physics of sound underwater, the information marine mammals receive differs from terrestrial mammals. The IID is created in terrestrial mammals when the acoustic energy is reflected from the animal's head due to the difference in the impedance values of the air and the animal's head. This results in an acoustic shadow being cast over the far ear and a decrease in the received acoustic energy. This is reduced in odontocetes due to the similarity in impedance of the aquatic environment and biological tissue, which allows the acoustic energy to travel through the animal's head and thus decreases the intensity of the acoustic shadow on the far side of the head. The IID in odontocetes is created by internal structures such as the acoustic fats and the peribullary sinus, which serve to conduct sound to the TPC, whilst isolating the TPC from the general cranial vibrations (Mooney, Yamato & Branstetter 2012). This arrangement has been demonstrated to create an IID of up to 20dB between cochleae (Supin & Popov 1993) which is well in excess of the 1 dB

sensitivity measured in T. truncatus (Moore, Pawloski & Dankiewicz 1995). The ITD is also reduced in marine mammals, due to the accelerated speed of sound underwater, which is approximately five times faster than in air. This is overcome by the remarkably well-developed cochlear nerve of odontocetes (Ketten 2000), which is transmitting neural impulses at speeds that enable the animal to detect differences as small as 7 microseconds (Moore et al. 1995). These adaptations enable odontocetes to determine direction underwater with remarkable accuracy. The determination of sound in the vertical plane by terrestrial mammals is achieved by position-dependent spectral filtering, or head-related transfer function (HRTF) (Branstetter & Mercado III 2006). This function is performed by the external pinnae in terrestrial mammals and allows monoaural directionality to be achieved. There has been no demonstration of HRTF in odontocetes; however the cranial asymmetry, the acoustically isolated TPC and the high frequencies that odontocetes use to echolocate would all theoretically enhance the sensitivity of this function. These techniques contribute to allow the dolphin a MAA of 0.9 and 0.7 in the horizontal and vertical planes, respectively (Renaud & Popper 1975) which facilitates a successful life underwater, guided by audition.

1.6 Knowledge Gap

The exact mechanisms via which odontocetes receive sound and the combined effect of these acoustic inputs are unclear. This knowledge gap directly hinders the investigation into the causal relationship between MFAS and the atypical strandings involving *Z. cavirostris*.

The inertial mode of BCV has been discussed in regard to odontocete hearing; however there are additional routes that have been shown to activate the cochlea in terrestrial mammals (Tonndorf 1966) which have not yet been explored fully in odontocetes. These include the compression and relaxation of the cochlear shell following exposure to a sound wave and the resulting disturbance of intracochlear fluid (Tonndorf 1966) and the transfer of audio-frequency pressure waves from the intracranial space to the cochlea via the cochlear aqueduct (Freeman, Sichel & Sohmer 2000).

The auditory anatomy of odontocetes varies widely among species (Kasuya 1973) and it is possible that a species specific pathway of increased sensitivity to BCV may be created. Anatomical features that may be of functional significance regarding these routes of sound conduction include the presence or absence of an osseous connection between the skull and the TPC, the degree of freedom of the *pars cochlearis* from the surrounding periotic bone and the diameter of the cochlear aqueduct (Tonndorf 1966). To date the similarities and differences of these features between *Z. cavirostris* and other odontocetes has not been investigate Alterations in the size of the "third windows" of the cochlea have been shown to affect the sensitivity to both air and bone conducted sounds in terrestrial mammals (Merchant *et al.* 2007). The "third window" refers to all communication between

(Tonndorf 1966). This "third window" is in addition to the oval and round windows and alterations in dimensions will affect the impedance of the cochlea. This has been investigated in humans in regard to large vestibular aqueduct syndrome and dehiscence of the semicircular canals (Merchant & Rosowski 2008) however the dimension of the "third window" of the odontocete cochlea has not been investigated. This thesis will investigate the anatomical similarities and differences between the inner ear and associated structures of *Z. cavirostris* and several other odontocete species. This will be achieved through the use of CT scans and the development of the methodology and implementation of MicroCT techniques to examine the internal osseous structures of the inner ear. The functional implications of any differences will be discussed in regard to the potential for those features to contribute to the creation of an acoustic pathway of increased sensitivity to frequencies used during MFAS in *Z. cavirostris*.

1.7 Thesis Design

In Chapter two of this thesis, the author examines the hypothesis that the auditory anatomy of *Z. cavirostris* may differ from other odontocetes and these features may result in an increased sensitivity to low frequency sounds, such as MFAS. To investigate this hypothesis the morphology of the TPC was assessed using computed tomography for ten species of odontocete, including *Z. cavirostris*. Anatomical parameters that have been previously been assigned functional significance in regard to odontocete sound conduction, or that may be of significance in regard to BCV were examined. These parameters included the tympanic plate, MSMR and the freedom of the *pars cochlearis* from the surrounding periotic bone. The results obtained will be compared between *Z. cavirostris* and other species of odontocete and the potential functional significance of any observed variations discussed.

In this thesis the author examines the hypothesis that the BCV may activate the odontocete cochlea and that the auditory anatomy of *Z. cavirostris* may create a pathway of acoustic sensitivity in this species.

To investigate this hypothesis the structures potentially involved BCV including the course and dimensions of the cochlear aqueduct will be quantified through the use of CT and MicroCT and modelling equations used to estimate the functional significance of this pathway.

The functional significance of an enlarged third acoustic window of the cochlea, such as the cochlear aqueduct, on cochlear dynamics via traditional routes of sound conduction will also be discussed.
Chapter 2: Anatomy of the tympanoperiotic complex in beaked whales and other odontocetes: Potential for cochlear stimulation via bone conducted vibrations

2.1 Abstract

The tympanoperiotic complex of ten species of odontocete was studied using computed tomography to examine anatomical features known to be involved in sound conduction in odontocetes. Anatomical features important for cochlear stimulation via bone conducted vibrations in terrestrial mammals were also examined. The focus of the study was to investigate the potential for a species specific pathway of cochlear stimulation in beaked whales, particularly Ziphius cavirostris, the primary species involved in atypical mass strandings associated with the use of naval sonar. The study also examined anatomical features of Mesoplodon grayi, Mesoplodon layardii, Mesoplodon hectori and Mesoplodon bowdoini, for whom the TPC had not yet been described, and *Physeter macrocephalus*, Kogia sima, Globicephala melas, Peponocephala electra and Tursiops spp. for comparative purposes. Morphological observations of the tympanic bulla in Z. cavirostris included a reduction in the size of the structures involved in traditional routes of sound conduction including the tympanic plate and medial sulcus of the mallear ridge compared with other odontocetes. Noted features of the periotic bone in beaked whales included a pronounced pars cochlearis and an increased size of the aperture of the cochlear aqueduct. The potential acoustic significance of these morphological features is discussed.

2.2 Introduction

Z. cavirostris has received an increased level of interest from the scientific community following several atypical mass stranding events, which appeared to be associated with the use of mid frequency active sonar (MFAS) (Evans and England 2001; Frantzis 2004; Freitas 2004; Martín *et al.* 2004; Servidio and García 2004). A statistically significant correlation between atypical stranding events involving beaked whales and the use of MFAS has been established in some regions (Filadelfo *et al.* 2009) and controlled exposure experiments have demonstrated that beaked whales respond to naval sonar (Tyack *et al.* 2011) and MFAS (DeRuiter *et al.* 2013) with avoidance and altered dive profiles. However, the causal factors required to precipitate a stranding in response to MFAS exposure remain unknown (D'Amico *et al.* 2009; Filadelfo *et al.* 2009).

One reason for the difficulty in defining a causal relationship between MFAS and beaked whale strandings is the limited knowledge of the pathways involved in odontocete audition. The majority of the scientific data supports the "pan bone" theory of sound conduction first proposed over 40 years ago (Norris 1968). This theory describes a series of specialised acoustic fats in and around the thin, posteriorly-flared mandible called the "pan bone". These acoustic fats are responsible for the transmission of acoustic vibrations to the odontocete hearing apparatus, the tympanoperiotic complex (TPC). The TPC is composed of the tympanic bulla and the periotic bone, which act as the middle and inner ears, respectively. The acoustic fats transmit vibrations to the thin ventrolateral wall of the tympanic bulla, which in turn transfers the vibrations through the ossicular chain to the oval window of the periotic bone. Recent advances on this hypothesis have described additional routes of sound transmission including the "gular region"

(Cranford *et al.* 2008), where sound pressure waves enter the area between the mandibles from within the mouth and between the mandibles. A dorsal extension of the mandibular fat body that contacts a specialised area on the dorsolateral edge of the tympanic bulla, called the medial sulcus of the mallear ridge (MSMR) which has also been identified and described as the functional analogue of the tympanic membrane in terrestrial mammals (Cranford *et al.* 2010). These pathways have been demonstrated with a series of modelling papers that examined the mechanics of the TPC (Nummela *et al.*1999; Hemila *et al.*1999) and used morphological parameters to accurately predict audiograms for a number of species (Hemila *et al.*1999; Hemilä *et al.*2001). The same analysis has not been completed for *Z. cavirostris* or any other species of beaked whale.

An alternate mode of cochlear stimulation involving bone-conducted vibrations (BCV) has also been proposed historically (McCormick *et al.* 1970) and has been discussed more recently following the use of finite element modelling and vibrational analysis of the TPC (Cranford *et al.* 2010). This theory originally described the transfer of cranial vibrations to the TPC following sound exposure, resulting in the gross vibration of the TPC and the subsequent creation of movement between the stapedal footplate and the oval window, due to the inertia present within the ossicular chain. This theory was initially refuted due to the inability of the model to explain directional hearing (Ketten 2000). More recently the theory has been used to describe the transfer of low frequency vibrations from the acoustic fats to the TPC in a multimodal model of cochlear stimulation (Cranford *et al.* 2010).

However, additional routes of BCV have been demonstrated in terrestrial mammals, including the direct compression of the cochlear shell (Tonndorf 1966) and the transmission of audio frequency pressure waves from the intracranial space to the

cochlea via the cochlear aqueduct (Freeman *et al.* 2000). Neither of these pathways has been investigated in odontocetes.

This study aims to compare the auditory anatomy of *Z. cavirostris* and other odontocetes to enhance the anatomical data available regarding the auditory structures of odontocetes and to determine whether species specific differences exist. Anatomical parameters traditionally discussed in regard to sound reception, including the tympanic plate and the MSMR and parameters shown to be significant for BCV in terrestrial mammals including the freedom of the cochlear shell and the cochlear aqueduct will be examined and the possible functional implications following exposure to natural or anthropogenic sound discussed.

2.3 Materials and methods

Samples (n = 34) from odontocetes that stranded and died between 2002 and 2010 along the east coast of Tasmania and the north coast of New South Wales were collected and archived by the Department of Primary Industry, Water and Environment (DPIWE), Tasmania, and the Office of Environment and Heritage (OEH), New South Wales respectively. The time between stranding and collection of each specimen varied, but as the structures to be examined were osseous in nature, their gross morphology was assumed to be unaffected by post mortem degeneration. The specimens examined in this study are listed in Table 1.

The method for extraction of the TPC varied, however for the majority of specimens (n = 31), the entire head was removed and stored frozen at -20° C. The heads were subsequently thawed and each TPC was removed according to the method described by Ketten (2007), and then stored in 10% neutral buffered formalin. For specimens in which the TPCs were removed from fresh carcasses (n = 3), 1 ml of 10% neutral buffered formalin was also injected with a 25 gauge needle into the round window at the time of extraction to preserve the soft tissues within the cochlea, although these features were not examined for this study. In some specimens (n = 6), iatrogenic separation of the tympanic bulla from the periotic bone, and ablation of the posterior process of the tympanic bulla, occurred during extraction. These preparation artefacts are listed in Table 1.

Where available, specimens (n = 5) were identified by genetic analysis conducted at the Cetacean Conservation and Genetics Laboratory, Oregon University. Species were identified from the mtDNA control region sequence using the web-based program DNA Surveillance (http://www.cebl.auckland.ac.nz:9000/) (Ross *et al.* 2003). Species for which genetic confirmation was not available were identified

based on morphological features (Shirihai *et al.* 2006). The method of species identification used for each animal is listed in Table 1. All members of the genus *Tursiops* examined are described as *Tursiops* spp. due to the lack of significant variation in TPC morphology between species (Kasuya 1973) and the current taxonomic confusion related to this genus (Natoli *et al.* 2004; Tezanos-Pinto *et al.* 2009; Charlton-Robb *et al.* 2011). Animals were classed as juveniles if they were equal to, or shorter than, the body length at weaning as stated in the New South Wales (NSW) guidelines

(http://www.environment.gov.au/coasts/publications/cetacean/protocols/pubs/cetace an-protocols -appendix3.pdf).

Computed tomography (CT) (Phillips Brilliance 16 slice CT; 4535 673 86351_E, Phillips Medical Systems, Nederland BV) was used to examine the specimens. Scans were conducted using 120 kV and 200 mAs with a high resolution helical scan, on a thin bone setting, section thickness of 0.8 mm and reconstruction advance of 0.5 mm, following guidelines established by Ketten (2008). The images were stored in Digital Imaging and Communication in Medicine (DICOM) format and the three dimensional reconstructions, volumes and linear measurement of the TPC were obtained using the reconstruction software, Phillips: Brilliance-Extended workspace V3.5.0.2254.

For assessment and acquisition of TPC morphological data the virtual specimen was rotated to provide visualization of standard landmarks described by Kasuya (1973), as illustrated in a CT reconstruction in Figure 5a. These landmarks include the length of the tympanic bulla, as measured from the anterior spine to the posterior margin of the outer posterior prominence; the length of the periotic bone as measured from the anterior to posterior margins and the length of the *pars cochlearis*, as measured from

the anterior to posterior margin of the central globular portion of the periotic bone. All linear measurements were recorded using the listed software and performed in triplicate to minimise operator error. The measurements generated from the software were validated against a single set of manual measurements of the tympanic, periotic, and cochlear lengths using vernier callipers, accurate to 0.05 mm. Volumes of the tympanic and periotic bones were also calculated using an application within the software that selected tissue based on density. Given the density of the TPC a setting of 1000 Hounsfield units was set as a minimum density for tissue to be included into the calculation of bone volume. The validity of the tissue selected for volume calculation was confirmed by visual examination of 2D images, displayed concurrently, which illustrated the tissue included in the calculation.

For TPCs that were articulated at the time of examination, the volume of the tympanic bulla (TV) and periotic bone (PV) were calculated by "virtual" separation of the bulla using the aforementioned software. The two bones were divided between the posterior processes and along the dorsolateral margin of the tympanic bulla including the conical process, sigmoid process and the dorsal ridge of the anterodorsal crest as demonstrated in a CT reconstruction in Figure 5b. Measurements of the area of the aperture of the cochlear aqueduct, the round window and the MSMR were not undertaken due to limitations in the recording software and are therefore presented pictorially. All nomenclature employed is based on Mead and Fordyce (2009).

Where sample size permitted statistical analysis, a Kruskal-Wallis Test (Minitab 15.1.0.0, Minitab Inc.) was used to assess differences within species between sex and age groups. Significance was assumed at p < 0.05.



Figure 5: Computed tomography reconstructions of the *a*) medial and *b*) lateral aspects of the right TPC of *Globicephala melas*. The image is orientated with the anterior end of the specimen facing the bottom of the image. The periotic length (pl), the length of the *pars cochlearis* (pc), the length of the tympanic bulla (tl), the posterior tympanic process (ptp) and sigmoid process (sp) are demonstrated. The dashed line indicates the delineation between tympanic bulla and the periotic bone and the dotted area shows the medial sulcus of the mallear ridge. The scale bar represents 1 cm.

2.4 Results

Morphometric data of the TPC is presented in Table 1.

The tympanic bulla of the four Mesoplodon species had a conspicuous lateral furrow, large posterior prominences with a wide interpromineal notch, and a globular outer posterior prominence. The tympanic plate was relatively small in *Z. cavirostris* compared to the Mesoplodon species, with the involucrum extending beyond the tympanic plate and with minimal development of the structure laterally. A ventral view of the tympanic bulla from CT reconstructions is depicted in Figure 6. The size of the MSMR relative to the TPC varied among species, and was smaller in *Z. cavirostris* compared to those in the other odontocetes examined. The MSMR is depicted in Figure 7. In *Tursiops* spp., *Peponocephala electra* and *Globicephala melas* the accessory ossicle was fused to the tympanic bulla. In *Physeter macrocephalus, Kogia sima* and beaked whale specimens the accessory ossicle was situated more medially and was fused to the ventral surface of the periotic bone. The MSMR of *Mesoplodon grayi* could not be assessed due to iatrogenic separation of the tympanic bulla and the periotic bone during extraction.

The morphology of the periotic bone varied among genera. A dorsal view of the periotic bone is shown in a CT reconstruction in Figure 8. In beaked whale specimens examined, the *pars cochlearis* was delineated to a greater extent from the surrounding periotic bone and the ratio of the *pars cochlearis* length to the length of the periotic bone was generally less than in the other odontocetes examined. The aperture of the cochlear aqueduct also appeared larger in beaked whale specimens when compared to the other genera. This observation was most pronounced in *Z. cavirostris*. No variations were noted in the size of the aperture of the vestibular

aqueduct amongst the specimens examined. The *fenestra rotunda* varied in size and shape among species and is demonstrated in a CT reconstruction in Figure 9. The data set enabled statistical investigation into biological trends for *Globicephala melas*. In this species the tympanic length was found to be significantly greater for non-juvenile (n = 10) than juvenile (n = 5) animals (H = 6.08, d.f. = 1, p = 0.014), although there was no significant difference in periotic lengths (H = 0, d.f. = 1, p = 0.947). Given no significant differences were noted for the periotic length, juvenile and non-juvenile age groups were combined to investigate sex differences; periotic length was determined to be significantly shorter in female compared to in male specimens (H = 4.27, d.f. = 1, p = 0.039), despite the mean body length of the non-juvenile females exceeding that of the male *Globicephala melas* examined.

Table 1: Morphometric data obtained from the TPC. The mean and *range* of values are listed for body length (BL), tympanic length (TL), tympanic volume (TV), periotic length (PL), periotic volume (PV), total tympanoperiotic complex length (TPL) and the length of the *pars cochlearis* (CL). Identification of each species was conducted via morphological assessment (M) or via genetic sequencing (DNA). In some species the posterior process of the tympanic bulla was fractured during extraction (*).

Species	ID	Age	Ν	BL (m)	TL (mm)	TV (cm ³)	PL (mm)	PV (cm ³)	TPL (mm)	CL (mm)
Globicephala melas	DNA	NJ	∂ 4	3.3	44.6	` 6.8	39.2	7.4	51.5	17.1
				3.1-4.6	41.9-47.3	6.2-7.8	37.1-40.9	6.6-8.0	50.0-53.9	15.9-18.5
			♀ 6	4.3	44.16	5.5	37.2	69	51.0	16.36
				3.6-4.6	43.4-45.1	4.9-6.2	36.3-37.5	6.3-7.3	49.7-51.6	16.1-16.6
		J	∂1	2.5	43.2	6.5	39.3	7.3	49.9	16.3
			 ⊈ 4	2.5	41.7	5.3	37.7	7.0	49.4	16.6
				2.4-2.6	39.4-43.1	5.3-5.5	36.7-39.5	5.8-8	47.3-50.6	14.8-17.9
Tursiops spp	DNA	NJ	♀ 3	2.8	37.0	4.5	36.6	5.5	43.7	15.52
				2.6-3.0	34.5-38.3	4.4-4.5	35.8-37.7	4.9-6.7	41.3-46.1	15.2-15.7
	М	J	∂ 2	0.9	31.4	3.2	30.8	4.0	38.3	16.08
				0.9-1.0	30.2-32.5	3.2-3.2	30.6-30.9	3.9-4.0	37.7-38.8	15.9-16.2
Peponocephala electra	М	NJ	♀ 1	4.7	36.7	*	33.3	-	-	14.47
Kogia sima	М	NJ	∂ 1	2.7	27.6	3.2	22.3	2.3	-	11.2
Physeter macrocephalus	DNA	NJ	∂ 1	-	58.0	3.1	59.9	31.9	-	25.6
		J	∂ 1	3.7	52.5	2.7	58.2	29.9	-	24.9
Ziphius cavirostris	М	-	3U	-	54.05	*	56.4	18.1	-	20.2
					(51.5-56.3)	*	(55.9-56.7)	(15.2-21.2)	-	19.4-21.1
Mesoplodon hectorii	DNA	J	1U	2.5	48.0	11.0	47.3	10.6	57.4	19.5
Mesoplodon grayii	М	NJ	1U	5.1	42.9	*	44.9	8.7	-	16.3
Mesoplodon layardii	М	NJ	∂ 1	5.9	41.6	7.5	40.3	7.0	-	15.6
		NJ	♀ 1	4.0	40.6	7.6	40.2	7.9	-	17.4
		J	♀ 1	3.6	44.2	8.0	42.3	10.9	-	20.7
Mesoplodon bowdoini	DNA	J	∂ 1	2.6	52.1	14.7	57.4	18.6	-	20.4
			♀ 1	2.4	51.1	12.3	51.1	12.2	-	20.4



Figure 6: Computed tomography reconstructions of the ventral view of the right tympanic bulla of *a*) *Peponocephala electra, b*) *Globicephala melas, c*) *Tursiops truncatus, d*) *Physeter macrocephalus, e*) *Kogia sima, f*) *Ziphius cavirostris, g*) *Mesoplodon grayi, h*) *Mesoplodon bowdoini, i*) *Mesoplodon layardii* and *j*) *Mesoplodon hectori*. Specimens are oriented with the anterior aspect to the bottom of the image and the lateral edge to the right of each image. The inner posterior prominence is illustrated within the dotted circle and the outer posterior prominence is demonstrated with a black arrow and the posterior tympanic prominence with the black diamond. The lack of posterior tympanic processes on specimen f) - i) is due to preparation artefact. The scale bar is 1 cm.



Figure 7: Lateral view of the TPC showing the Medial Sulcus of the Mallear Ridge (MSMR). The MSMR lies ventral to the anterodorsal crest (•) and is outlined with a dotted line in species *a*) *Globicephala melas*, *b*) *Tursiops spp. c*) *Peponocephala electra*, *d*) *Physeter macrocephalus*, *e*) *Kogia sima*, *f*) *Ziphius cavirostris*, *g*) *Mesoplodon layardii* and *h*) *Mesoplodon hectori*. The specimens are oriented with the anterior alignment facing the left hand side of each image and the periotic bone on the top of each image. The scale bar is 1 cm.



Figure 8: Computed reconstruction of the dorsal view of the right periotic bone of *a*) *Globicephala melas, b*) *Tursiops truncatus, c*) *Peponocephala electra, d*) *Physeter macrocephalus, e*) *Kogia sima, f*) *Ziphius cavirostris g*) *Mesoplodon grayi h*) *Mesoplodon bowdoini i*) *Mesoplodon layardii* and *j*) *Mesoplodon hectori*. The specimens are oriented with the anterior process of the periotic bone directed to the bottom of each image. The aperture of the cochlear aqueduct is demonstrated with the solid black triangle and the aperture of the vestibular aqueduct with the triangle outline. Scale bar is 1 cm.



Figure 9: Computed tomography reconstruction of the ventromedial view of the *pars cochlearis* of the periotic bone including *a*) *Globicephala melas, b*) *Tursiops truncatus, c*) *Peponocephala electra, d*) *Physeter macrocephalus, e*) *Kogia sima. a*) *Ziphius cavirostris, b*) *Mesoplodon grayi, c*) *Mesoplodon bowdoini, d*) *Mesoplodon layardii* and *e*) *Mesoplodon hectori*. The specimens have been oriented such that the anterior region is directed toward the bottom of each image. The *fenestra rotunda* is denoted with the arrow head, the aperture of the cochlear aqueduct is denoted by the black triangle. The head of the stapes is denoted by the black square in each image. Scale bar is 1 cm.

2.5 Discussion

The aim of this study was to compare the morphology of the TPC of *Z. cavirostris* to that of other species of odontocete and to consider the potential functional implications of any species specific differences noted in regard to both traditional routes of sound conduction and routes of BCV previously undescribed for odontocetes.

The results of this study have increased the biological data available for a number of odontocete species and contributed new data for a number of species for which the TPC had not been described. For the odontocete species that have been described previously, the morphological data from the present study was consistent with past studies (Kasuya 1973; Morell et al. 2007). In regards to biological trends within species, the growth after birth of the tympanic bulla but not the periotic bone was consistent with data recorded by Kasuya (1973) but conflicted with results described by Buffrenil (2004) who stated that the full length of the tympanic bulla and periotic bone were present in new born animals. This inconsistency is most likely reflective of conclusions drawn from analysis of small sample sizes. The growth of the tympanic bulla after birth occurs predominantly in the anterior spine of the bulla (Kasuya 1973) and given this feature has not been discussed in the anatomical papers modelling the conduction of sound (Nummela 1999; Cranford et al. 2010) the acoustic significance of this growth is assumed to be minimal. The decreased periotic length in females relative to male animals in this species is a new finding though the acoustic significance of this is unclear.

The gross morphology of the tympanic bulla varied among species and did not necessarily follow taxonomic or biological relationships. The morphology of the tympanic plate and the MSMR of the Mesoplodon genus were more consistent with

Tursiops spp., than that of *Z. cavirostris*. This was unexpected, as the known auditory and biological capabilities of the Mesoplodon species are closer to *Z. cavirostris* (Zimmer *et al.* 2005; Cook *et al.* 2006) than the shallow diving, social *Tursiops* spp. (Mooney *et al.* 2012).

The finding of a reduced surface area of the tympanic plate and the MSMR observed in *Z. cavirostris* are of interest. The contrast between the thick medial involucrum and the thin dorsolateral tympanic plate of the tympanic bulla has been described as a mechanism required to increase the vibrational amplitude and particle velocity of sound as it travels towards the inner ear (Hemila *et al.*1999). Given the functional role that these features have previously been ascribed, the decreased development of the tympanic plate in *Z. cavirostris* may be expected to decrease the vibrational difference generated between the medial and lateral components of the tympanic bulla, potentially resulting in a more uniform vibration of the tympanic bulla in response to sound. The gross vibration of the tympanic bulla in this case would result in cochlear stimulation due to the inertia present within the ossicular chain as described by Cranford's multimodal model of cochlear stimulation for low frequencies (Cranford *et al.* 2010).

In terrestrial mammals, the surface area of the tympanic membrane relative to the stapes footplate has been shown to be linearly related to its sound collecting ability (Nummela 1995) and this ratio primarily determines the middle-ear transfer gain. The MSMR has been described as the functional analogue to the tympanic membrane in odontocetes (Cranford *et al.* 2010), and hence a decrease in the surface area or volume of this space that is not matched by a relatively equal decrease in the surface area of the footplate of the stapes, may decrease the sound receiving

potential and subsequent middle ear gain, thereby decreasing the efficiency of this structure to transfer vibrations through the ossicular chain.

The prominent pars cochlearis and the enlarged aperture of the cochlear aqueduct present in beaked whales may have functional significance with respect to cochlear stimulation via BCV. The prominence of the pars cochlearis relative to the cochlea may facilitate compressional distortion of the cochlear shell when the TPC is exposed to vibrations within the peribulary sinus or from surrounding cranial tissues. The role of an enlarged cochlear aqueduct, as observed in Z. cavirostris, may also impact upon the dynamics of the cochlea. Previous studies have shown that this is a potential pathway of acoustic transmission (Freeman et al. 2000; Sohmer and Freeman 2004) and may act as a third acoustic window (Tonndorf 1966). Future study modelling the impact of MFAS upon these structures would be of interest. The functional hypotheses that can be drawn from the observed anatomy of the TPC of Z. cavirostris are two-fold. Firstly, there is a potential reduction in the ability to transfer vibrations through the ossicular chain via traditional routes of sound conduction, given the decreased surface area of both the tympanic plate and the MSMR. Secondly, there is an increased potential for cochlear stimulation via pathways of BCV given the prominence of the pars cochlearis and relative size of the aperture of the cochlear aqueduct. Further work is required to assess the validity of this hypothesis including the acquisition of accurate measurements of the cochlear aqueduct and the frequencies that may be capable of stimulating the cochlea via this route. All routes of sound conduction and the interaction between these routes should be evaluated when assessing the impacts of anthropogenic sound.

Acknowledgements

Specimens for the study were provided by Department of Primary Industries and Water, Tasmania, and the National Parks and Wildlife Service, New South Wales. Special thanks to Dr Rosemary Gales, Dr Isabel Beasley, Drew Lee and Andrew Irvine for the collection of samples from the field We thank The University of Sydney Veterinary Teaching Hospital for access to their imaging facilities and, in particular, Helen Lauendet for her imaging expertise, The University of Auckland for assistance in identification, The Australian Registry of Wildlife Health for sample collection and Dolphin Marine Magic, Coffs Harbour for the provision of in kind support.

Chapter 3: The cochlear aqueduct of Cuvier's beaked whale (*Ziphius cavirostris*): a potential route of sound conduction at low frequencies?

3.1 Abstract

Microcomputed tomography was used to examine the dimensions of the acoustic windows of the cochlea in odontocete species, Tursiops truncatus, Orcella heinshoni, Mesoplodon layardii, Mesoplodon bowdoini and Ziphius cavirostris in order to investigate the presence of a third acoustic window. In addition, computed tomography was employed to examine the cranial anatomy surrounding the tympanoperiotic complex of Tursiops truncatus, Mesoplodon layardii, Mesoplodon bowdoini and Ziphius cavirostris to examine and measure the route of the cochlear aqueduct as it traverses between the intracranial space and the periotic bone. The main findings included an absolute and relatively greater diameter of the aperture of the cochlear aqueduct in Z. cavirostris when compared to the other species examined, as well as a greater degree of osseous support for the course of the cochlear aqueduct in all beaked whales examined. Where both length and diameter of the cochlear aqueduct were measured, the impedance of the aqueduct was calculated for a range of frequencies with the lowest impedance determined in Z. *cavirostris* at all frequencies. The potential significance of this finding in regard to the potential for the cochlear aqueduct to activate the cochlea following exposure to low frequency sounds is discussed.

3.2 Introduction

Cuvier's beaked whale (*Ziphius cavirostris*) is the odontocete species most commonly involved in atypical stranding events associated with naval exercises using mid frequency active sonar (MFAS) (Evans and England 2001; Frantzis 2004; Freitas 2004; Martín *et al.* 2004; D'Amico *et al.* 2009). Several theories have been proposed to explain this association, including their unique dive physiology (Zimmer and Tyack 2007), anatomical variations (Rommel *et al.* 2006), acoustic resonance in the lungs (Evans *et al.* 2002) and the formation of gas emboli in blood vessels and internal organs (Jepson *et al.* 2003). Despite these proposed associations, a causal relationship between exposure to MFAS and atypical stranding events remains undefined. This study examines the anatomical structures involved in odontocete audition and explores the hypothesis that the auditory anatomy of *Z. cavirostris* may create a species specific pathway that is sensitive to low frequency sounds, such as those frequencies used in MFAS.

The cochlea can be described schematically as a fluid filled spiral, encased in a bony shell and divided into three portions, called scala vestibuli, scala media and scala tympani. Scala vestibuli communicates with the stapedal footplate at the oval window and scala tympani communicates with the middle ear at the round window (Evans 2013). Scala vestibuli is separated from scala media by a compliant membrane, and scala media is separated from scala tympani by an epithelial lamina that houses the sensory organ and hair cells required to transform mechanical inputs into neural impulses. This occurs when an input into the cochlea creates a pressure differential between scala media and scala tympani, and functionally the sensory organ acts as a pressure divider. This pressure differential displaces cochlear hair cells, resulting in stimulation of the afferent neurons which the brain is able to interpret as sound (Fay

1994). A pressure difference between scala media and scala tympani only occurs due to the presence of the round window and/or other pressure shunts in scala tympani, and without these pressure shunts the cochlea's sensitivity to sound is greatly diminished.

However, the cochlea also has a "third acoustic window", which refers to the sum of communications, excluding the round and oval windows, between the cochlea and the endocranial spaces (Tonndorf 1966). This includes the apertures of the cochlear and vestibular aqueducts and a schematic representation of these anatomical structures is shown in Figure 10.

In humans, the cochlear aqueduct is diminutive (Su *et al.* 1982; Gopen *et al.* 1996) and study on the functional impact of an enlarged third acoustic window have thus far focused on mobile windows on the scala vestibuli side of the cochlear partition. This is seen in such conditions as semicircular canal dehiscence or large vestibular aqueduct syndrome (Merchant and Rosowski 2008). The presence of this third window has been shown to result in a simultaneous decrease in sensitivity to air conducted sounds and an increase in cochlear sensitivity to bone conducted vibrations (BCV) (Merchant and Rosowski 2008). However the relative size of these structures is reversed in odontocetes, where an enlarged aperture of the cochlear aqueduct and a relatively small aperture of the vestibular aqueduct is seen (March *et al.* 'in press'). The acoustic significance of this arrangement has not been investigated.



Figure 10: Schematic view of the tympanoperiotic complex and the cochlea demonstrating placement of the acoustic windows. The oval and round window account for the first two acoustic windows and the "third window" is the sum of all other entry points into the cochlear including the cochlear and vestibular aqueducts.

Bone conducted vibrations (BCV) can activate the cochlea in terrestrial mammals via a number of routes including; the pressure induced in the external ear canal, inertial forces acting on the ossicular chain and cochlear fluids, alteration of the cochlear space and pressure transmission through the third acoustic window (Tonndorf 1966; Stenfelt 2011). Vibrations can also travel from the intracranial space to the cochlea and enter via the third acoustic window via non osseous routes including the cochlear aqueduct (Freeman *et al.* 2000; Sohmer and Freeman 2004). The impact of these routes of sound conduction upon odontocete audition has not been investigated and the functional significance of these routes may vary between species depending upon the cranial anatomy surrounding the tympanoperiotic complex (TPC).

Beaked whales have several anatomical features that are of interest in this regard including an osseous connection between the skull and the TPC that many other odontocetes do not share (Kasuya 1973), relatively larger pterygoid sinuses (Cranford *et al.* 2008) and a unique bony strut present at the opening of the Eustachian tube into the tympanic cavity (Ketten 2000; Rommel *et al.* 2006). The net input of acoustic energy into the cochlea is the vectorial summation of all routes of sound conduction (taking into account impedance) and the impact upon audition of these anatomical features, following exposure to sound, will vary with both pressure and frequency.

Micro-computed tomography (Micro-CT) and traditional computed tomography (CT) were used to examine the communication between the intracranial space and the TPC and to obtain dimensions of the acoustic windows of the cochlea including the aperture of the cochlear aqueduct and the round and oval windows. These results were used to create a quantitative model to assess the acoustic impedance of

cochlear aqueduct. The results obtained are compared among *Z. cavirostris* and other odontocetes to examine the potential for a species specific pathway of acoustic vulnerability to MFAS in *Z. cavirostris*.

3.3 Materials and Methods

For standardization, all nomenclature is based on "The Therian Skull" (Mead and Fordyce 2009).

The specimens examined in this study were collected and archived by the Department of Primary Industry, Water and Environment (DPIWE), Tasmania, the Office of Environment and Heritage (OEH), New South Wales and the Queensland Museum from odontocetes that stranded and died between 2002 and 2013. The specimens used in this study are listed in Table 2. The majority of specimens were identified by genetic analysis conducted at the Cetacean Conservation and Genetics Laboratory, Oregon University. Species were identified from the mtDNA control region sequence using the web-based program DNA Surveillance (http://www.cebl.auckland.ac.nz:9000/) (Ross *et al.* 2003). For those species where genetic confirmation was not available, species identification was based on morphology (Shirihai *et al.* 2006).

Archived cranial CT scans, collected using established protocols (Ketten and Montie 2008) were obtained from the DPIWE for *Tursiops truncatus*, *Mesoplodon layardii* and *Mesoplodon bowdoini*. Images from cranial CT scans for *Z. cavirostris* were made available courtesy of Dr. Ted Cranford, San Diego State University. For morphological assessment, the path of the cochlear aqueduct was assumed to be the same as that of the internal auditory meatus. This assumption was based on the close proximity of the two structures at the point of entry into the basicranium and periotic bone (Mead and Fordyce 2009) and the common identification of these structures in a previous publication (Evans and England 2001). The length of the cochlear aqueduct was taken from the internal acoustic meatus of the periotic bone to the posterior lacerate foramen of the basicranium as demonstrated in Figure 11.

Table 2: Morphometric data of the inner ear of individual odontocetes for body length (BL) and periotic length (PL) the areas of the acoustic windows of the cochlea including the cochlear aqueduct (Aca), the oval window (Aow) and the round window (Arw). The length of the cochlear aqueduct (Lca) and the method of species identification; morphological assessment (M) or via genetic sequencing (DNA) and the age of the animals; juveniles (J) or non-juveniles (NJ) is also shown

Species	ID	Age	BL (m)	PL (mm)	Aca (mm²)	Aca:PL	Aow (mm²)	Aow:PL	Arw (mm²)	aRW:PL	Lca (mm)
Tursiops truncatus	DNA	NJ	2.8	33.7	2.26	0.07	2.59	0.08	2.44	0.07	21
Kogia sima	М	NJ	2.7	22.3	0.59	0.03	1.36	0.06	1.85	0.08	-
Ziphius cavirostris	М	NJ	-	56.4	9.07	0.16	7.08	0.13	5.72	0.10	90
Orcella heinshoni	М	NJ	-	-	1.00	-	1.17	-	1.85	-	-
Mesoplodon bowdoini	DNA	J	2.5	54.3	1.65	0.03	4.62	0.09	5.06	0.09	46
Mesoplodon layardii	М	NJ	5.9	40.9	3.94	0.10	4.65	0.10	3.48	0.09	67



Figure 11: Magnified section of the cochlear aqueduct (\nearrow) running from the periotic bone (•) to the intracranial space at the posterior lacerate foramen (\blacktriangleright) for *a*) *Ziphius cavirostris, b*) *Mesolpodon layardii, c*) *Mesoplodon bowdoini* and *d*) *Tursiops truncatus*. Image a) was provided by Adjunct Assistant Professor Ted Cranford. Scale bar is 1 cm.

Micro-CT scans were performed on the Xradia MicroXCT -400 (Xradia, California, USA), with scanning parameters optimized on an individual basis to obtain maximum X-ray dynamic energy. The tomographical datasets were imported into a hardwarebased back-projection reconstruction software supplied by Xradia, outputting serial images of 10242 pixels in 16-bit, with a voxel resolution of 17 µm. Measurements from the Micro-CT scans were obtained using VG-Studio Max 2 software, which allowed image stacks to be rotated, permitting a 2D plane to be orientated to achieve ideal visualisation of the parameter to be measured. Measurements collected and shown in Table 2 included the diameter of the oval window at the footplate of the stapes, the diameter of the round window at the termination of the scala tympani and the diameter of the aperture of the cochlear aqueduct at the point of insertion into the basal turn of the cochlea, based on landmarks described by previous studies (Su *et al.* 1982; Gopen *et al.* 1997). To account for allometric scaling between species, the areas of the acoustic window were also expressed as a ratio of the length of the periotic bone.

This study performed MicroCT scans of the *pars cochlearis* and opportunistically used archived cranial CT scans and hence measurement of the length of the cochlear aqueduct was not possible for all species. MicroCT and CT data was available for *Z. cavirostris, M. layardii, M. bowdoini* and *T. tursiops* and the length and the diameter of the cochlear aqueduct was recorded. This data was used to model the impedance of this structure, based on previous studies (Gopen *et al.* 1997) calculating the impedance of the human cochlear aqueduct using the following equations:

1. $Z_{ca} = R_A + j\omega M_A$

Where Z_{ca} is the impedance of the cochlear aqueduct.

2.
$$R_A = 8\eta I / \pi a^4$$

Where R_A is the resistance of the cochlear aqueduct.

3. $M_A = 4p_0 I / (3\pi a^2)$

Where M_A is the acoustic mass of the fluid within the aqueduct.

The additional parameters are:

 η -co-efficient of the shear viscosity of the fluid within the cochlear aqueduct

(assumed to be equivalent to water, 1×10^{-3} Pa-s);

I-length of the cochlear aqueduct (m);

a - radius of the cochlear aqueduct (m);

j - the imaginary number $\sqrt{-1}$;

 ω - the radian frequency, or 2π times the frequency in Hz;

 p_0 - density of the perilymph (equal to 1000kg/m³).

3.4 Results

Based on examination of CT scans, the cochlear aqueduct in beaked whales traversed a greater distance of bone between the subarachnoid space and entry into the peribullary sinus compared to *T. truncatus*. The TPC also demonstrated an osseous attachment with the bones of the cranium in beaked whales compared to the suspension of the TPC in soft tissue within the peribullary sinus in *T. truncatus* as shown in Figure 11.

The length of the cochlear aqueduct, the areas of the acoustic windows and the ratio of these areas relative to the length of the periotic bone are displayed in Table 2. The diameter of the cochlear aqueduct was both absolutely and relatively larger in *Z. cavirostris* when compared to other species and the size of the other acoustic windows respectively. The point of insertion of the aqueduct into the basal turn of the cochlea is displayed in Figure 12. The calculated impedance of the cochlear aqueduct was lower in *Z. cavirostris* than *T. truncatus, M. bowdoini* and *M. layardii* at all frequencies and the impedance of the structure increased exponentially with frequency in all species. The results of these calculations are displayed in Figure 13.



Figure 12: Microcomputed tomography scans of *a*) *Z. cavirostris, b*) *M. layardii, c*) *M. bowdoini* and *d*) *T. truncatus* demonstrating the cochlear aqueduct (\blacktriangleright) at the point of entry into the basal turn of the cochlea and the round window (Δ). The scale bar is 1mm.



Figure 13: Theoretical acoustic impedance (Pa-s/m; Pascal per second per meter) of the cochlear aqueduct calculated from measurements obtained in the current study for *Z. cavirostris, M. layardii* and *T. truncatus* at a range of frequencies.

3.5 Discussion

This is the first study to examine the dimensions and impedance of the cochlear aqueduct in odontocetes. Due to an absence of some fundamental data of the inner ear properties in each species, such as the impedance of the oval and round windows, we are limited here to discussing each of the anatomical features and the functional impacts that these features may have, in isolation.

The most widely accepted route of sound conduction in odontocetes includes the transfer of vibrational energy from the mandibular fats to the TPC and then through the ossicular chain to the oval window (Ketten 2000; Mooney *et al.* 2012). The presence of a patent cochlear aqueduct will decrease the impedance on the scala tympani side of the cochlear partition, which may in turn increase the sensitivity of the cochlea to these acoustic inputs, given the cochlear partition acts as a pressure divider. However, the impedance of the cochlear aqueduct has been shown to increase with frequency (Gopen *et al.* 1996), hence this increased sensitivity would be most pronounced at low frequencies.

Vibrational input into the oval window via the ossicular chain may potentially be delivered via two routes and is schematically represented in Figure 14a. These routes include the transfer of energy through the chain from the dorsolateral margin of the tympanic plate (Nummela *et al.*1999; Hemila *et al.* 1999) or gross movement of the TPC, and creation of a relative motion between the cochlea and stapes given the inertia present within the ossicular chain. The latter route may result from the transfer of cranial vibrations to the TPC (McCormick *et al.* 1970) or via sound waves delivered via the mandibular fats (Cranford *et al.* 2012). Given the decreased impedance of the cochlear aqueduct in *Z. cavirostris*, the impact of low frequency

sounds delivered via this route may be more significant than for other odontocetes and could result in a supranormal sensitivity to low frequency sounds.

The direct transfer of acoustic energy from the intracranial space to the cochlea via the cochlear aqueduct has been demonstrated in terrestrial mammals (Freeman et al. 2000; Sohmer and Freeman 2004) but not considered for odontocetes. This route of BCV is schematically represented in Figure 14b. The cochlear aqueduct in beaked whales had a greater degree of osseous support compared with the other odontocetes examined and the reduced exposure to soft tissue will decrease the dissipation of acoustic energy (Freeman, Sichel & Sohmer 2000) and may create pressure fluctuations in the cochlea originating from the cerebrospinal fluid. The opposite of this may be true in regard to *T. truncatus*, where the cochlear aqueduct has less osseous support and travels through a greater amount of soft tissue within the peribullary sinus prior to reaching the TPC. Hence though the impedance of the cochlear aqueduct was less than that of *M. layardii* and *M. bowdoini*, the exposure of the aqueduct to the soft tissue in the peribullary cavity may decrease the efficiency at which vibrations are transmitted to the cochlea. The increased impedance of the cochlear aqueduct in *M. layardii* and *M. bowdoini* compared with *Z. cavirostris* is a result of a narrowing of the aqueduct within the periotic bone at the point of entry into the basal turn of the cochlea, despite a well-developed aperture on the surface of the periotic bone. Increases in sample size to assess the variation of this structure between individuals and age groups would be of interest. The increased size and decreased impedance of the cochlear aqueduct observed in Z. cavirostris increase the likelihood that the cochlear aqueduct may act as a conduit for pressure fluctuations in this species, particularly at low-frequencies. The frequencies that may be capable of traversing this route and stimulating the cochlea have not been

calculated as they are dependent upon the knowledge of additional parameters, such as the impedance of the cochlea, which are currently unknown for *Z. cavirostris*, however future work modelling these frequencies to assess the impact of MFAS would be of interest.

Compression of the cochlear shell and subsequent displacement of the intracochlear fluid has been described in terrestrial mammals (Tonndorf 1966; Stenfelt 2011) and is schematically represented in Figure 14c. Beaked whales have several anatomical features that may facilitate this form of cochlear stimulation including a bony strut at the opening of the Eustachian tube (Ketten 2000; Rommel 2006) presumably to maintain patency at depth, well developed pterygoid sinuses, presumably to provide the peribullary sinus with air during deep dives (Fraser and Purves 1960) and a bulbous pars cochlearis (Kasuya 1973), resulting in relative freedom from the surrounding periotic bone compared to other odontocetes. If resonance of the Eustachian tube was to occur at depth, as has previously been discussed (Evans et al. 2002) the prominent pars cochlearis would be exposed to vibrations under great pressure with enough force to displace the fluid within and stimulate the cochlea. The addition of the cochlear aqueduct as a third acoustic window on the scala tympani side of the cochlea, which already has a markedly decreased impedance relative to the scala vestibuli (Merchant et al. 1996), may increase the cochlear sensitivity to this pathway.

The delivery of vibrations into the peribullary sinus via this method may also impact directly upon the round window, as schematically represented in Figure 14d. If the round window was directly exposed to vibrations within the peribullary sinus, a patent cochlear aqueduct may facilitate a release of this pressure and effectively buffer the cochlea against these inputs. The increasing pressure levels within the presumably
air filled peribullary sinus may increase the impedance of the round window, whereas the cochlear aqueduct and the spaces that it communicates with are fluid-filled and therefore incompressible. This means that although the impedance of the cochlear aqueduct will change with frequency, it should not change with depth. This could result in a point during the dive profile where the impedance of the cochlear aqueduct becomes less than that of the round window, and acts as the primary membrane being displaced on the scala tympani side of the cochlear partition following input from the ossicular chain.



Figure 14: Schematic of the odontocete tympanoperiotic complex demonstrating the proposed movement of the cochlear fluid, as shown by the thin black arrows, following the transfer of acoustic vibrations, as shown with the speaker icon. Demonstrated routes sound conduction include *a*) the ossicular chain, *b*) the cochlear aqueduct *c*) compression of the cochlear shell and *d*) the round window.

Theory on Low-Frequency Sound Localization Underwater

BCV has been previously problematic as a mode of hearing in odontocetes due to the inability of this pathway to explain directional hearing (Ketten 2000). However, it may be possible that the interaction between the multiple routes of BCV discussed above result in the creation of an interaural intensity difference (IID) which would enable the animal to determine the direction of the sound. The hypothesis is represented schematically in Figure 15. The creation of this IID is explained in the following hypothetical scenario. Exposure to an underwater soundwave originating from the left of the odontocete will stimulate the whole skull, moving it to the right, and due to an osseous attachment between the skull and the TPC, the stimulus is effectively transferred to the cochlea via whole-head BCV. Given the medial location of the oval window relative to the ossicular chain, this will result in the stapes of the left cochlea moving into the oval window and the right stapes moving away (i.e. the pressure waves in the left and right cochleae will be 180 degrees 'out-of-phase'). However, the stapedal movement would be similar in magnitude and there would be little intensity difference to enable the animal to ascertain the direction of the sound. Similarly, if the cochlear aqueduct is capable of transmitting audio-frequency pressure waves to the cochlea via a fluid-conduction pathway, following exposure to an underwater soundwave the magnitude of input would be identical in each cochlea and no IID would be present. However, sound entering the cochleae via fluidconduction pathways would produce stapes motions that were 'in-phase' between the two cochlea. Therefore, when both bone-conducted and fluid-conducted stimulation pathways are present, the sound induced pressure fluctuations in the cochlea will partially cancel in the right cochlea but sum in the left, creating a strong IID. The relationship between bone- and fluid-conducted stimulation pathways and

sound localization have not yet been explored, but are worthy of further research on theoretical grounds.



Figure 15: Schematic representation demonstrating the creation of an interaural intensity difference (IID) via the interaction of bone conducted vibration (BCV) and fluid conducted pressures transmitted to the cochlea from cranial tissues via the cochlear aqueduct. Increased fluid pressure in the cochlea would displace the oval window (OW) in both cochlea outwards. The bulk movement of the head following exposure to sound would accelerate the periotic bones relative to the tympanic bulla (TB) resulting in movement of the stapes and OW due to the inertia of the ossicular chain, but this movement would be of equal magnitude but in opposite direction between the two ears. The fluid and BCV effects add in the left ear, but cancel in the right, which generates a strong IID.

3.6 Acknowledgements

Specimens for the study were provided by Department of Primary Industries and Water, Tasmania and the National Parks and Wildlife Service, New South Wales. Special thanks to Dr Rosemary Gales, Dr Isabel Beasley, Drew Lee and Andrew Irvine. Special thanks also to Adjunct Assistant Professor Ted Cranford, San Diego State University, who supplied the computed tomography scan of the head of *Z. cavirostris*. We thank The Australian Centre for Microscopy and Microanalysis for access and assistance to their imaging facilities and, in particular, Dennis Dwarte, Dr. Matthew Foley and Dr. Chris Wong. The Faculty of Veterinary Science at The University of Sydney financially contributed and Dolphin Marine Magic, Coffs Harbour.

Chapter 4: General Discussion

4.1 Introduction

The causal factors required to precipitate an atypical stranding event following exposure to MFAS, and why *Z. cavirostris* appears to be particularly vulnerable to these frequencies, are unknown. Advances in our understanding of the auditory anatomy and physiology of *Z. cavirostris* since these events have included the documentation of new pathways of sound reception (Cranford, Krysl & Hildebrand 2008) and modes of cochlear stimulation (Cranford, Krysl & Amundin 2010). Biological studies have investigated behavioural attributes such as dive profiles (Tyack *et al.* 2006), echolocation frequencies (Zimmer *et al.* 2005) and behavioural responses following controlled exposure to MFAS (DeRuiter *et al.* 2013). However, there is still no conclusive explanation for the atypical stranding and unusual pathology observed in *Z. cavirostris* following these events.

This thesis was designed to assess the morphology of the *Z. cavirostris* TPC from a functional perspective to investigate the presence of an acoustic pathway of supernormal sensitivity to frequencies used during MFAS. This assessment included a morphological comparison the TPC of *Z. cavirostris* and other odontocetes in regard to features that have previously been credited with having a functional significance and the examination of anatomical features that are involved with cochlear stimulation via BCV in terrestrial mammals. The potential routes of BCV considered included the previously described creation of a relative motion between the stapes and the oval window following the transfer of cranial vibrations to the TPC and the inertia inherent within the ossicular chain (McCormick *et al.* 1970). Two routes of BCV not previously discussed before in regard to odontocete audition were

also discussed including the compression of the cochlear shell following exposure of the TPC to vibrations and the subsequent disturbance of the intracochlear fluid (Tonndorf 1966) and the transmission of audio-frequency pressure waves from the cerebrospinal fluid (CSF) to the cochlea via the cochlear aqueduct (Freeman, Sichel & Sohmer 2000).

The morphology of the Z. cavirostris TPC may decrease the efficiency at which vibrations from the mandibular fats are transferred through the traditionally described route of sound conduction in odontocetes. The tympanic plate and the MSMR of Z. cavirostris are reduced relative to other odontocetes, as described in Chapter 2 of this thesis. The development of the thin ventrolateral tympanic plate, contrasted by the thicker medially located involucrum is believed necessary to achieve the required amplification of particle velocity as vibrations are transferred from the middle to the inner ear (Hemila 1999). Without the development of the tympanic plate to receive the vibrations, there will be less differential motion created between the medial and lateral edges, resulting in an increased likelihood that the entirety of the tympanic bulla may vibrate in response to the vibrations received from the mandibular fats. This would decrease the sensitivity of the TPC to high frequency sounds and may explain the peak ossicular movement generated at lower frequencies in Cranford's modelling experiments (2010). The decreased size of the MSMR may also impact upon the efficiency of vibrational transfer via this route. The MSMR has been described as the functional analogue of the tympanic membrane (Cranford, Krysl &Amundin 2010) and given the size of the surface area of the tympanic membrane has been shown to be functionally significant in the middle ear of terrestrial mammals (Nummela & Sirpa 1995) it stands to reason that a similar relationship will be present in odontocetes. Hence the reduced surface area of the MSMR in Z.

cavirostris may not receive or transfer the vibrations from the dorsal branch of the mandibular fat body through to the ossicular chain as efficiently as other odontocetes.

The auditory anatomy of Z. cavirostris may increase the sensitivity of the cochlea to stimulation via BCV. The osseous attachment between the squamous and bassioccipital bones of the skull and the TPC may also support the transmission of cranial vibrations to the TPC accelerating the complex and activating the cochlea via ossicular inertia, as has previously been described (McCormick et al. 1970). The transfer of these vibrations to the periotic bone may also directly compress the cochlear shell and given the freedom of the pars cochlearis from the surrounding periotic bone observed in Z. cavirostris may enable these vibrations to more efficiently disturb the intra-cochlear fluid and activate the cochlea. The increased diameter of the cochlear aqueduct and the application of measurements from the present study to a simple formula indicated that impedance of this structure was lower in Z. cavirostris than other odontocetes. This will increase the potential impacts of this structure upon the stimulation of the cochlea through both an increased ability to transfer audio-frequency pressure waves from the cerebrospinal fluid and the potential shunting of pressure away from the round window. The simultaneous presence and phase differences of these routes of BCV may provide the basis for an exciting novel hypothesis for the creation of an IID and directional hearing. The stimulation of the cochlea is the vectorial summation of all pathways conduction sound (Freeman, Sichel & Sohmer 2000) and the presence of these routes of sound conduction, how they interact with each other, and how they may interact with vibrations transmitted to the TPC via the mandibular and gular pathways is an interesting concept worthy of further investigation.

4.2 Future directions

This thesis has opened up several novel areas for future investigation. Further modelling studies investigating the resonant frequencies of the cochlear aqueduct in *Z. cavirostris*, relative to MFAS would be of merit. The impedance of this structure will be significantly reduced at the frequencies at which resonance occurs and hence able to conduct audio-frequency pressure waves from the CSF to the cochlea at much lower SPL. To calculate these values the impedance of the cochlea is required which has been estimated for odontocetes (Hemila 1999) and could be calculated using methodologies previously conducted in terrestrial mammals (Merchant, Saumil N, Ravicz & Rosowski 1996). The identification of the cochlear impedance would also enable a more informed hypothesis to be created regarding the role the cochlear aqueduct will play affecting the pressure dynamics within the cochlea for both BCV and vibrations delivered via the ossicular chain. This is because the functional significance of the impedance of the cochlear aqueduct is relative to the impedance of the cochlea and round window, and how these values change with frequency (Gopen, Rosowski & Merchant 1997).

Direct experimental confirmation of these pathways and the frequencies capable of traversing would also be of interest to assess to investigate the sensitivity of these pathways in *Z. cavirostris* and other odontocetes at frequencies involved with MFAS exercises. This could be achieved with an experimental design similar to the paradigm employed by Sohmer (2000) where BCV were applied to sections of the skull and the cochlear stimulation measured via the reception of auditory evoked potentials. Direct demonstration of the pathway underwater and the creation of an IID to determine directionality may also be possible in cadaver specimens. Intracochlear pressure gauges could be inserted into each cochlea and the cadaver

specimen submerged and exposure to a range of frequencies including those used during MFAS. The experiment could be repeated following removal of the lower jaw to assess the impact of the mandibular fats upon cochlear stimulation. The presence of intracochlear pressure fluctuations, in the absence of the lower jaw would confirm the presence of these pathways of BCV. Variation in the pressure levels recorded between cochleae would also allow the assessment of the creation of an IID and the subsequent ability of the animal to determine the direction of the sound source through BCV.

4.3 Conclusions

This thesis has identified aspects of the auditory anatomy of *Z.cavirostris* that may simultaneously decrease the efficiency of sound conduction through the ossicular chain and increase the efficiency of cochlear stimulation via BCV. This includes two routes of BCV not previously considered in odontocetes and the potential for these routes to contribute to the animal's ability to localize sound underwater. These ideas are new to the field and this thesis has created the opportunity for the future investigation of the role that these pathways may play in normal audition or may have played following exposure to MFAS to be explored with experimental work.

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