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BIOLOGY DEPARTMENT
Computerized Scanning and Imaging Facility

HARVARD MEDICAL SCHOOL
DEPARTMENT OF OTOTOLOGY & LARYNGOLOGY



FINAL REPORT

Submitted: 8 March 2013

This revised Report is submitted to assist the reviewing committee. As some members may not be familiar with auditory anatomy, terminology, and the conditions discussed, this revised version contains three parts: 1) an expanded summary of the findings, 2) , an introduction to cetacean ear anatomy with a glossary, and 3) detailed reports for each ear with figures and where appropriate field notes.

Summary Findings:

The scans demonstrate that melon head whales have a typical odontocete auditory anatomy with a two part (tympanic and periotic) temporal bone. As in all other odontocetes, the periotic houses the cochlea, which in this species has a 2.25 to 2.5 turns with a conventional odontocete basal to apical construction; i.e., robust outer osseous lamina throughout the basal turn and a gradual diminution of the cross-section of the cochlear canal diameter towards the apex. These features are best visualized in the three-dimensional reconstructions of the membranous labyrinth (e. g. Figs. 3, 4). The VIIIth nerve and its fibers are well developed and arrayed in segregated channels throughout the modiolar hub, which is a normal condition for a healthy odontocete ear.

There is a well-developed outer osseous lamina in the basal turn running approximately 30% of the cochlear length, consistent with a Type II odontocete cochlea which is consistent with best sensitivities in the mid to high ultrasonic frequencies (approximately 200 Hz to 120 KHz principal hearing range). The outer lamina is absent in the middle and apical turns, which is normal. The vestibular labyrinth is significantly reduced in comparison to the cochlea, which is also a normal odontocete inner ear labyrinth feature.

The significant findings for the 8 melon-headed whale ears (*Peponocephala electra*) examined in these cases are as follows(see also Table 1 and figures for individual ear reports):

- 1) the presence of multiple small mineralized nodules in the peribullar tissues, and in one case a stalked appendage extending from the periotic to a nodule. These small hyperattenuating objects may be consistent with peribullar and middle ear parasites or some as yet undetermined reactive process. They are not the result of fracturing or partitioning of the tympanic and periotic and have not been commonly reported in other odontocetes.

- 2) There are some notable regions of hyper and hypo attenuations of the tympanic and periotic bones, suggesting mild demineralization and adjacent reactive formation, which is not unusual, particularly in mature odontocetes. These are not so severe as to represent definitive otosclerotic cases.
- 3) Some of the ears have roughened margins of the periotic and tympanic that may reflect chronic peribullar infections or repeat acute otitis in the past. This is a common finding across all species, particularly in mature animals.
- 4) Small deposits of bone chips, most of which consistent with chipping from the fragile anterior lip of the tympanic bone. These are likely to be the result of postmortem handling, but should be verified by histologic exam.
- 5) Small pockets of air are seen in several individual cochleae, some in the lower or basal (high frequency hearing region) turn, others in the middle and apical or upper (low frequency region) turns. These are most consistent with postmortem autolysis and may have been introduced in handling or shipping as they are not accompanied by disruption of the inner ear membranes. The air in some cases assists visualization of separate compartments (scalae) of the cochlea. The presence of these isolated air pockets in different divisions of the cochlea of some cases provides further evidence that the cochlear membranes are intact.
- 6) There are no significant findings of hemorrhage, fibrosis or ossification in the inner ear that would indicate injurious exposures or prior or peri-mortem trauma, nor is there evidence of membrane trauma or related abnormalities.

Any significant sensorineural hearing loss typically manifests itself as a reduced auditory nerve diameter, absence of ganglion cells in the ganglionic bulge, and, in advanced cases, the presence of fibrous or fatty replacement tissue in any part of the cochlea and its associated neural tracks. In acute cases, evidence is found of ossicular damage, ruptured membranes and frequently intracochlear hemorrhage. None of the specimens examined exhibit these features.

All findings noted are consistent with chronic or acute infection, parasitic infestation and response, and fibrous or calcified adjunct formations. None of the findings are consistent with acoustically derived auditory damage or recent, acute hearing losses. There is no significant finding consistent with acoustic trauma, blast injury, or impulse noise injury.

It should be noted that many of the tissues examined were of sufficiently poor quality that a definitive determination eliminating any neurosensory loss cannot definitively be stated from the scans and may not be possible even if histologic processing and examination are undertaken since there are extensive postmortem artifacts in most of the ears. However, two specimens as noted below may be worthwhile to pursue histologically as the scans indicate they have intact inner ear structures. Histology may be able to address some questions, such as the nature and origin of small, dense particles adjacent to the tympanics and periotics and would add valuable, unique data better understanding of the comparative anatomy and hearing of this species.

It should also be noted that in at least one animal (please see original field notes and images for MAD 108) that there was a gross necropsy observation of ring lividity in the

blubber which is consistent in cetaceans with a drowning incident. This is also one of the animals which has an adequate ear specimens for histology. A histologic examination of this ear may have an additional advantage in assisting a determination of death by beaching vs. drowning. The cochlea may contain information to clarify the question of drowning, as endolymphatic hydrops (a distension of the middle chamber of the cochlea, scala media) is sometimes found in the inner ear in drowning victims.

Table 1 Key Findings

Specimen	Intracochlear Gas	Peribullar Nodules	Fractures or Fragments	Surface Irregularities (Rugosities)	Demineralized Regions	Parasites
N2008-0623 MAD 308/Pe001	Head only, Code 2					
Right	X apical	XX	X	X		X
Left		X		X	X	
N2008-0624 MAD 308/Pe002	Code 3+ body					
Right	X apical, basal	XX		X	XX	X
Left	X apical, middle, basal	XX	X	X	X	
N2008-0625 MAD 308/Pe003	Code 3+ body					
Right		X	X	X	X	
Left		XX	X	X	X	
N2008-0626 MAD 108/Pe003	Code 2+ body					
Right			X XX (dissection artifact)	X		
Left		X				

Respectfully submitted,



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Introduction

Cetacean Ear Anatomy:

All cetaceans (whale, dolphin, porpoise, etc) have ears encased in two bones external to the main skull: the periotic which houses the inner ear, and the tympanic, which houses the middle ear.

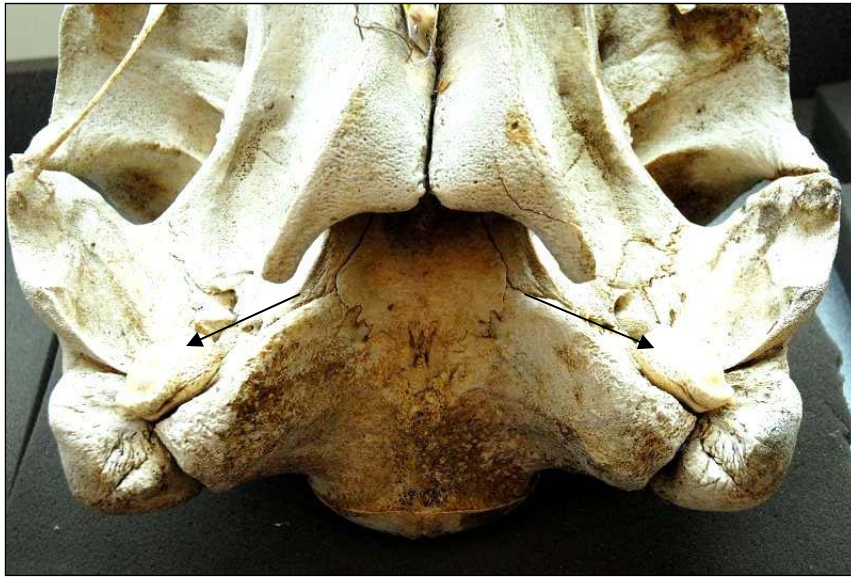


Fig. 1. Ventral view of a Cuvier's Beaked Whale (*Ziphius cavirostris*) skull. The tympanic bones (arrows) are conical and hollow. The denser, ovoid periotics are located dorsal and medial to the tympanic bones and are not visible in this photograph.

The two bones are connected laterally and posteriorly. At the posterior joint, or posterior prominence, there is a spongy (cancellous) bony flange that in some cetaceans stabilizes the ear in its cavity (peribullar fossa) outside the skull.

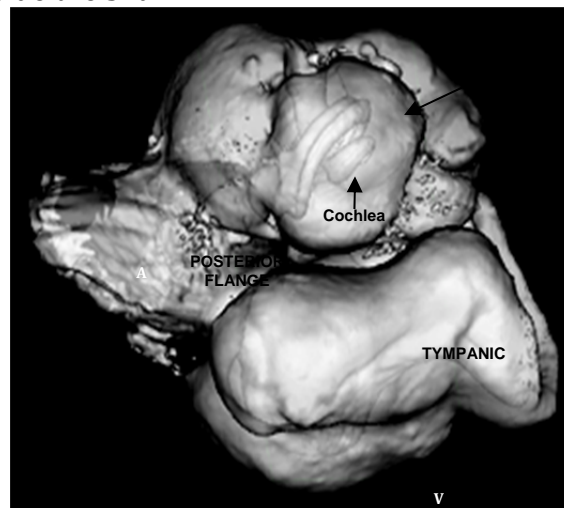
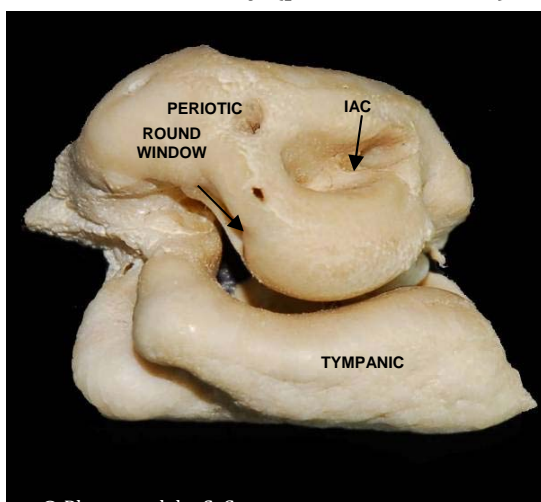


Fig. 2A,B. Photograph (left) and a 3D reconstruction (right) from CT scans of a left ear tympano-periotic from a bottlenose dolphin. medial view. The bone in the 3D is transparent to show the spiral cochlea in the periotic. The vertical shaft above the cochlea is the auditory nerve, which exits the periotic at the opening of the internal auditory canal (IAC on left).

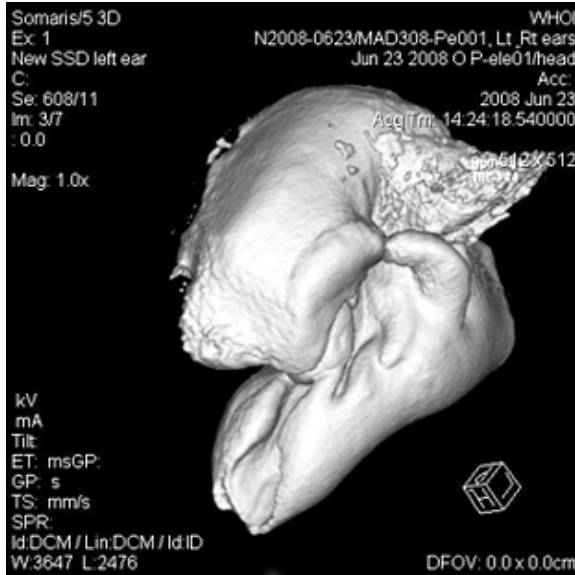


Fig. 2C 3D of left ear from one of the specimens collected and described in this report on melon-headed whale ears (*Peponocephala electra* 0623). The melon head ear bone complex is shown in anterior view in the position it would be *in situ*. The melon ear has a more pronounced posterior flange than most odontocetes and stabilizes the ear in the skull.

The tympanic is a hollow, cone-shaped bone. It forms the middle ear cavity. It is lined with a spongy soft tissue (corpus cavernosum) that may expand to fill the cavity or retract and compress to a thick liner. Within the middle ear cavity are three loosely joined bones (malleus, incus, stapes) that form the ossicular chain. They connect the tympanic membrane (eardrum) to the oval window, one of two membrane sealed openings (fenestrae) to the inner ear. The other membrane sealed opening is the round window, which is in contact with the corpus cavernosum and is a pressure release. The ossicles act as a levered amplification system, transmitting sound vibrations to the cochlea via the oval window. The stapes, the last bone in the chain, acts like a piston on the oval window.

The inner ear consists of two organs, both contained in the periotic. One is the auditory system (cochlea, hearing) and the other is the vestibular system (semi-circular canals, balance and motion). Both are fluid filled labyrinths with sensory cell patches, but they have very different shapes. The cochlea is a single large spiral canal divided with multiple parallel chambers and membranes with several thousand sensory cells that transduce sound energy into neural signals. It is the most pressure sensitive organ in the body.

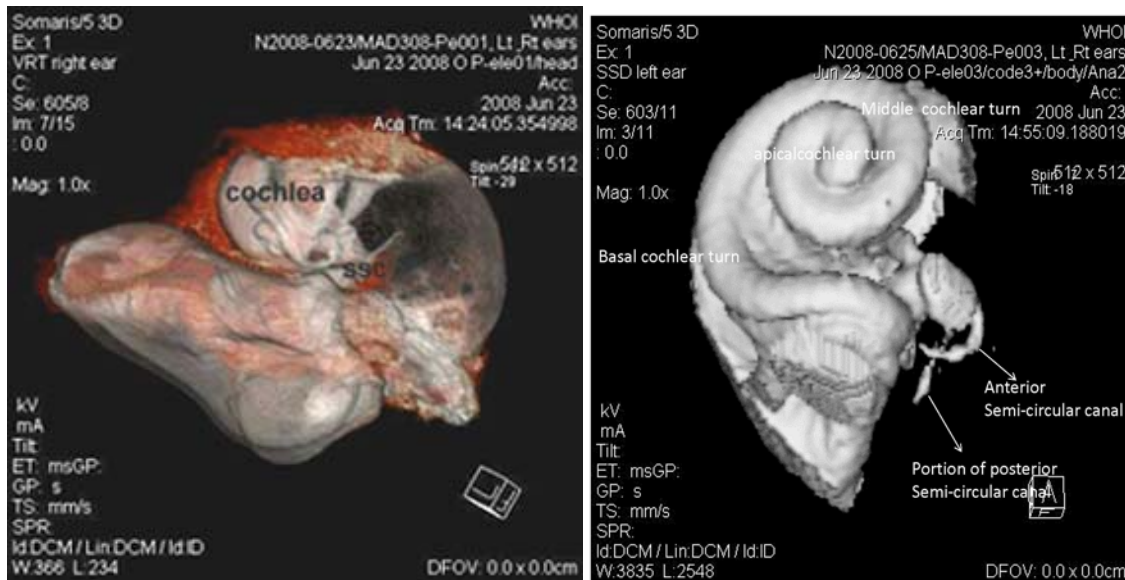


Fig. 3. Left Panel: A melon head whale right ear tympano-periotic 3D reconstruction is shown with the bone transparent to reveal the inner ear. Right panel: 3D reconstruction of the fluid filled canals and divisions of the cochlea and the semi-circular canals of the vestibular system (SSC), digitally “dissected” from the periotic and inverted.

The other organ found in the periotic is the vestibular system. In all mammals the vestibular system consists of three looped canals (the semi-circular canals) that converge at a common base with two major and three minor sensory patches. The fluid in these canals flows in response to motion by the head and neck and gives us our sense of balance and cues about gravity, rotation, and acceleration. In most mammals, the vestibular system canals are nearly as large as the cochlea, but, in all whales and dolphins, the canals and their innervation are drastically reduced. This may be a reflection of reduced vestibular function as a result of the fusion of neck vertebrae in cetaceans.

Glossary:

Cochlea: a spiral organ system devoted to hearing. It processes acoustic energy. It typically has 2-2.5 turns to the spiral and contains fluid filled canals (scalae) with two resonating membranes that support hair cells connected to the auditory nerve. The cochlear spiral is continuous but is referred to in 3 segments or turns: base or basal (high frequency responsive), middle (mid-frequencies), and apical (low frequency). In cross-section it appears like a broad clover shape with central branch (the nerve bundle) with oval leaves (the canals) that are largest at the base and smallest at the apex. The spiral diameter is about 8 mm at the base in melon headed whales, which is similar to bottlenose dolphins.

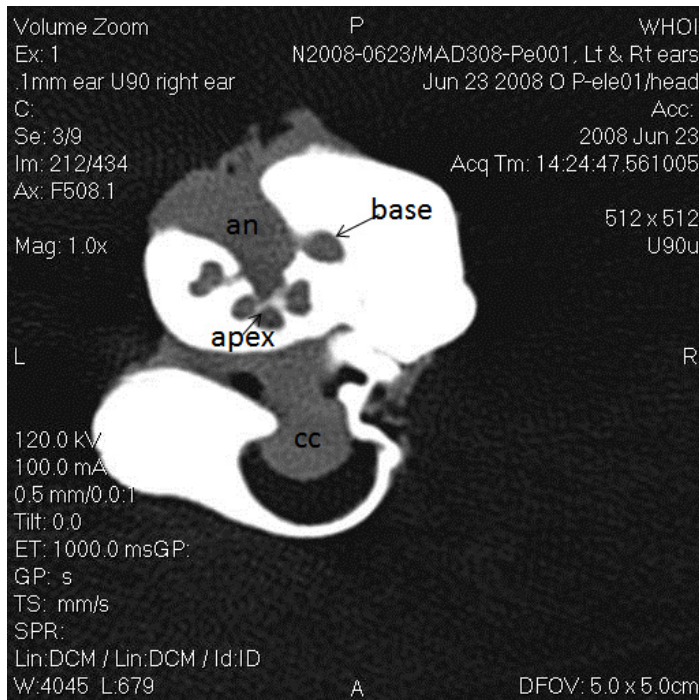


Fig. 4 2D CT scan of right melon head ear at the level of the cochlea. In cross-section the cochlear spiral appears like a broad clover shape with central branch, the auditory nerve bundle (an) with oval leaves (the cochlear canal). The cochlear canal diameter at the base averages 1.7 mm in this species. The smaller vestibular system is not shown because it resides in the periotic posterior and at right angles to the cochlear (see fig. 5).

Corpus cavernosum (cc): Thick layer of soft tissue lining the middle ear cavity (tympanic bone), which is highly vascularized.

VIIIth nerve (an): the acoustic and vestibular nerve bundle. It resides in the center of the cochlear spiral and internal auditory canal (IAC) and exits the periotic at the medial surface. In most mammals, it is divided approximately equally between cochlear and vestibular fibers. In all whales and dolphins, 95% of the VIIIth nerve fibers are exclusively auditory.

Hounsfield Units (HU): Hounsfield units are a measure of X-ray attenuation named after the inventor of CT technology. They are normalized to water (0 HU) and range typically from -1000 HU for air to >4000 HU with extended scales. They relate to density but are not synonymous nor are they a direct measure of density.

Inner Ear: two labyrinths of fluid canals, membranes, and sensory receptors that are divided into two distinct sensory systems: the cochlea, which provides hearing, and the vestibular system, which controls balance. Both systems are found in the temporal bone, which is called the periotic (peri – around; otic – ear).

Modiolus: the central hub of the cochlea through which the auditory nerve fibers branch out to the ganglion cells at the inner edge of the cochlear canals.

Ossicles: The three, connected bones that act as an amplifying lever system for sound, located in the middle ear .

Otitis: an infection of the ear: interna, of the inner ear; media of the middle ear, externa of the outer ear.

Peribullar: The area around the temporal bones

Periotic (peri – around; otic – ear): The bone that houses the cochlea and vestibular system. It also has three neural canals for the auditory, vestibular, and facial nerves, and it has two aqueducts related to endolymph, ending in a sac, and perilymph, connecting to the subarachnoid space of the brain.

Tympanic (tympanum drum head): The conical hollow bone with a central space that forms the middle ear cavity and houses ossicles, eardrum, muscles, and ligaments. The facial nerve fibers enter the periotic at the IAD and exit the periotic near the oval window then cross the middle ear.

Vestibular System: regulates balance and detects gravity and linear and rotational acceleration. It consists of three fluid filled semi-circular canals at right angles to each other with sensory patches (ampullae, saccule, macula) at their base. In cetaceans these canals are extremely small, with about 1/10 the nerve fibers found in land mammals.

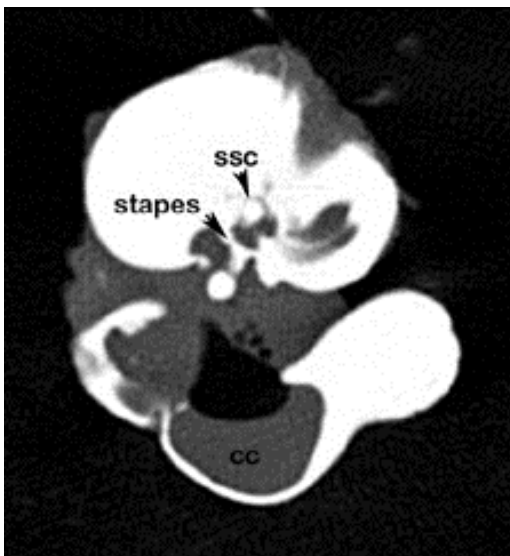


Fig. 5. 2D CT scan of melon head ear at the level of the lateral semi-circular canal (ssc). The stapes is also visible in this cross-section. The arrow is pointing to the “footplate” which is in contact with the membrane of the oval window.

CT Imaging Report

Original Draft Submitted to: Dr. Dee McAloose, DVM, Pathology, Wildlife Conservation Society, 2009

Revised report Submitted to: Dr. Teri Rowles and Dr. Brandon Southall, 2013

Provided by: D.R. Ketten, Ph.D. WHOI and Harvard Medical School

NOTE:

This report is based on images obtained through high resolution CT imaging conducted at the WHOI CT facility. Additional, multi-plane reformats, magnifications of basic scans, and 3D reconstructions were obtained through post-scan processing of the raw data and transaxial image files on the Volume Zoom scanner at WHOI Computerized Scanning and Imaging Center. The 2D images were the source of all critical observations. The 3D images are provided to assist in the visualization of findings from the 2D file examinations.

Copies of images and the reports of results provided here may be distributed only with permission of this office. All data and images from the WHOI/HMS facility are to be treated with a level of confidentiality strictly consistent with the HIPAA guidelines.

These scans and related reports are confidential and should be retained by the requesting agency. Data and findings included in this report are confidential, proprietary and produced only for release to the requesting agency and participants of the official necropsy team. WHOI CSI reformatted images and reconstructions are copyrighted and carry all conventional restrictions for use. Do not distribute this report or related images to third parties without first contacting this office for releases.

Raw scan data were retained by this scan facility; images were distributed on CD formatted for auto-display. Reformatted high-resolution images produced by WHOI OIC were archived on CD and MO disks and are available in DICOM, tiff, or jpg formats but may incur additional costs. If additional images or projections are required for this case, please contact Dr. Ketten at the WHOI address listed at the end of the report.

Specimens received 12:00. 23 June 2008, Fed Ex weigh bill 8634 1495 0562:

ID:	Label	Source Specimen
N2008-0623	MAD 308/Pe001	Head only, Code 2
N2008-0624	MAD 308/Pe002	Code 3+ body/Ana1
N2008-0625	MAD 308/Pe003	Code 3+ body/Ana2
N2008-0626	MAD 108/Pe003	Code 2+ body/Ant1

All scans were performed in a position equivalent to the ear *in situ* in an animal prone, scanned rostral to caudal.

N2008-0623 MAD 308/Pe001

Exam date 23, June 2008

Left and right ears were scanned together, with each re-imaged individually, producing 430 and 431 2D images respectively.

The specimens were scanned with a spiral protocol with 0.5 mm slice acquisition and 0.5 mm/sec table speed. This protocol employed an ultra high resolution 90 UH kernel for the Siemens Somatom Volume Zoom.

Both specimens were imaged in the transaxial plane at 0.1-mm slice thickness and with extended scale due to the extreme density of the tympanic and periotic.

Left ear

The tympanic and periotic are intact. All ossicles are present in the middle ear. There is relatively little soft tissue attached to the surface regions of the specimen. Some residual tissues are fibrous, particularly in the lateral posterior face of the periotic. The periotic has Hounsfield units ranging from 2900 to well over 4000. Some portions of the tympanic show slightly lower density areas with Hounsfield units of 1890 to 1895. An example of this is seen on the spiral images for IMA 280. The demineralized areas also result in surface "bubbling" of the tympanic. (fig. 0623-1).

Middle Ear

There is no evidence of peribullar or internal parasites. The stapes and annular ligament are intact. The round window membrane is also intact as can be seen in image 297. The corpus cavernosum and middle ear components are intact with portions of the malleus and incus still embedded within the corpus cavernosum.

Inner Ear

The cochlea is well imaged with clear evidence of an intact spiral osseous lamina outer spiral osseous lamina. The VIIIth nerve is present in the internal auditory canal (IAC). Intracochlear attenuations are within the normal range. This specimen has a nearly 2.5 turn cochlea, with an 8-mm to 9-mm basal diameter and an axial height of 3.6 mm and a middle turn diameter of 4.6 mm.

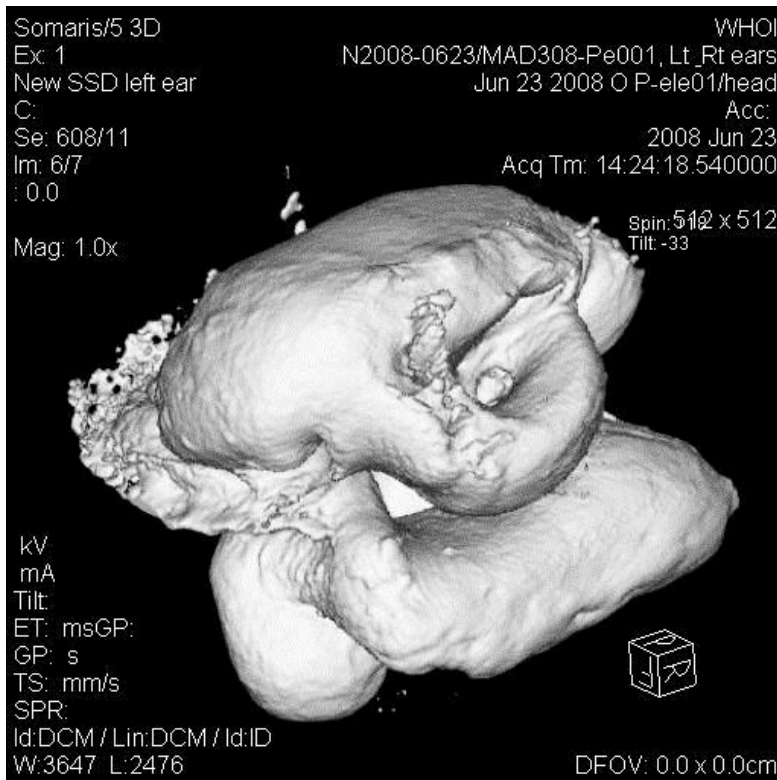


Fig. 0623-1. Medial view, Left ear.
Peribullar particles (upper left, lateral to periotic flange and near IAC) and bubbled, mildly lytic areas of tympanic (lower right of image)

Impression:

Periotic and cochlear anatomy consistent with a normal ear with minor evidence of past disease. The tympanic and periotic are on average well mineralized throughout. Small regions of demineralization and mildly lytic areas in the tympanic bone that suggest past, probably chronic disease. No evident broad spongiotic or sclerotic processes. Cochlea and vestibular systems are normal with typical odontocete characteristics.

Right Ear:

486 images were obtained.

The right ear has an intact tympano-periotic complex.

There are small high attenuation (250 to 400 HU) spheroids within the corpus cavernosum, primarily located in the anterior tympanic conus.

There are multiple, low attenuation fragments on the medial surface of the periotic (fig. 0623-1). The 3D reconstructions also show some pitting of the tympano periotic with some hypoattenuation, possible thinning near the posterior juncture of the tympanic and periotic. This joint is typically calcified, although it may be more flexible or hypo-mineralized in younger animals.

There is a very mild pitting of the ventral surface of the tympanic with accompanying fragments in and adjacent to the anterior aperture of the tympanic (fig. 0623-2).

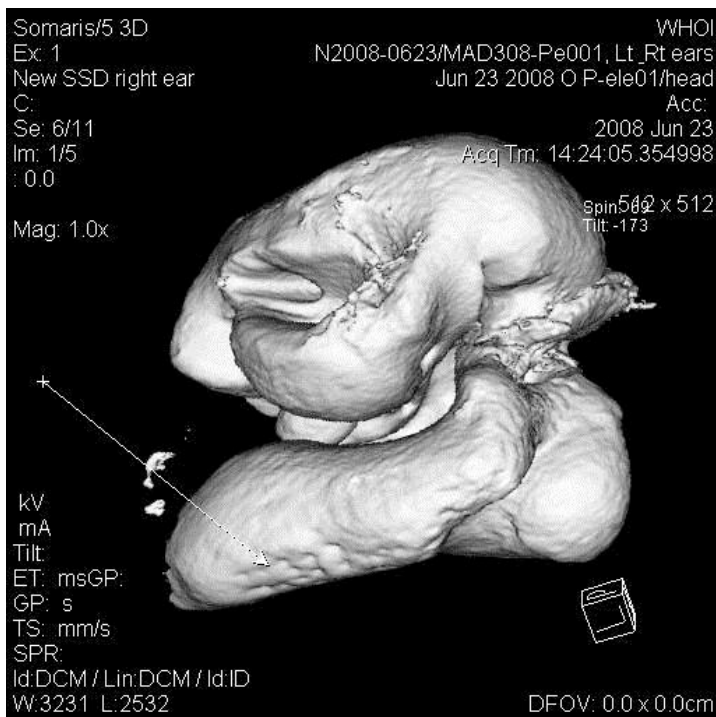


Fig. 0623-2. Right ear, medial view. 3D with arrow indicating tympanic anterior particles and surface pitting.

Middle Ear

The 3D CT scans show all ossicles are present, articulated, and normal in position and appearance. The corpus cavernosum is well developed but contracted in some areas, adhering closely to the wall of the middle ear. A pendular extension of the corpus cavernosum is remarkable (fig. 0623-3) but has been observed in other odontocetes. There is no evidence of congestion.

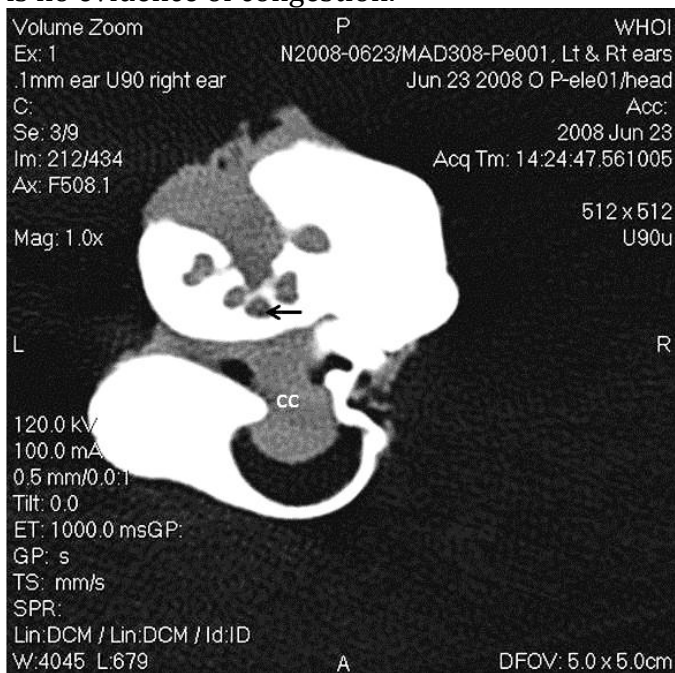


Fig. 0623-3. The image shows a mid-modiolar section of the right cochlea with an arrow indicating a small apical gas inclusion (black bubble). The corpus cavernosum (cc) has a normal extended tongue of tissue occupying part of the middle ear cavity.

Inner Ear

The cochlear anatomy is normal. At the apex there are minor inclusions with low attenuations (-300 or less) consistent with intracochlear gas pockets (fig. 0623-3).

Impression:

Normal inner ear and middle ear anatomy for this species. There is no indication of chronic or acute inner ear disease. Intracochlear gas is most likely attributable to autolytic processes as the cochlear membranes are intact. The IAC is a common postmortem entry route for air into the cochlea as well.

Small hyper-attenuating spheroids present in the middle ear and retro bullar near the IAC are consistent with calcified parasitic cysts commonly found in some species of odontocetes. Incidence in this species is not known. These may also be reactive tissues associated with the presence of nematode peribullar parasites documented in the field dissection notes and Fig. 0623-4, below

Rugosities and pitting of the ventral surface of the tympanic are consistent with chronic infection or inflammatory processes and may indicate past otitis media events. Peri-tympanic fragments are likely derived from breakage of the fragile tip of the conus, which is a common postmortem phenomenon from handling.

Field Dissection Notes

10-June-2008//308-pe001

Chalalavana

1 head, chilled

2 whole animals/ Hauled via boat from Antsuis. Released from boat, towed near shore via Zodiac, then brought by local porters to beach. These two decapitated by Marcie and Dee based on my markings. Single head brought in cooler to beachhead. All photoed.

No sex on single head. Chilled head removed from plastic container (chest) & from plastic bag on beach. All external surfaces photographed on board in chest. 4 cuts done to determine planes of lividity. External lividity diffuse, uniform-suggesting in H2O death and long term post rotation. Right eye partially compromised. Palpated narial dorsal sacs & descending nares. Sloughing epithelium, blackened but not bloated

Rt side:

Exposed serially all jaw fats & lower ridge of mandible. Disarticulated hyoid at cartilaginous cap.

No evidence of hemorrhage or clots/no contusions.

Fats clean-creamy with collagen fibers distinct. Minor region of serosanguinous staining consistent with post-mortem migration. Autolysis moderate. Fats well preserved. Small regions of effusion surrounding capillaries. Right eye erythematous, superficial scrapes anterior to posterior. Peribullar sinus normal, vascularized mucosa collapsed. Periotic ligaments intact. No evidence of parasites.

Terminating due to darkness.

Animals all male. Question of earlier all female as reported or just not extruded.

11-June-2008

Head moved: in cooler.

06:00-09:00

Return to Auschett (?) via Zodiac 06:00.

Recommence dissection/ 11-Jun-08

11:00: Head from cooler, poor condition/insufficient chilling.

Oral cavity filled with mud.

Head tissue-Notes by Marcie:

Lt globe removed into formalin. Rt globe removed intact. Increased dissolution of fat bilateral, but color remains unremarkable/ normal.

Throat structures that remain are coated with mud/anatomy normal. No overt lesions.

Bilateral parasites, possibly *Stenurus*. Retrobullar bilaterally, only in sinus. None evident at Eustachian tube level. (Check post fixation for epitympanic parasites). Also seen in NADZ08 Pe003. Bilateral in peribullar sinus retro and medial- numerous extracted-middle ear-expect corpus cavernosum infested.

Lt tympano-periotic complex: lateral surface tympanic remarkably clean. Healed fracture comminuted-lateral tympanic below PG hemi circle + 2 longitudinal 1 transverse -Photo

Both ears cleaned, extracted. Surfaces are normal, minor rugaosity on inferior surface, left ear.

Examined the museau de singe, no abnormalty, no foreign body.

Measurements:

	Left ear	Right ear
TP length	34	36
P length	33	34
Height	35	30

Inter bullar:165

Mouth:

No teeth on the middle line

Right side

Rt maxillary: 16 teeth + 4 broken or worn

Mandible right: 18 teeth + 4 broken or worn

Left maxilla: 16 teeth + 3 broken or worn

Mandible left: 18 teeth + 2 broken or worn

Mandibles bilaterally are free of lesion and fractures.

Brain in 001 removed/breadloafed through major horns lateral ventricles. No hemorrhage/ Severe autolysis in some regions (poor temporal, dorsal, cerebellum)

but basic structure intact/ Normal capillary/ white matter/ventricles intact but green in color with exception of left lateral/ choroid plexus intact.

5 animals/ 3 heads completed at 18:00/ Ice brought in (frozen buckets/sacks)

Animals on plastic/ covered/ ice on top 19:00.

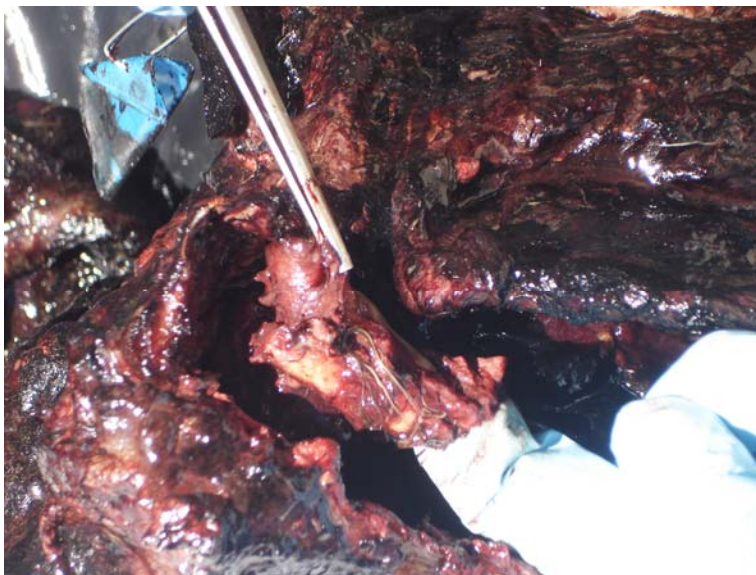


Fig. 0623-4. Nematodes (thread like white bodies, center of photo, extending diagonally from the opening to the middle ear cavity) infesting right peribullar sinus and associated soft tissues.

N2008-0624 MAD 308/Pe002 P-ele 02

23, June 2008

The specimen was scanned on the WHOI Siemens Volume Zoom spiral CT scanner using a ultra high resolution protocol with a 90 UH acquisition kernel, kV 120, effective mAS 200, acquisition table speed 0.5-mm at 0.5 seconds. 419 images were obtained in the transaxial plane at 0.1 mm slice thickness.

Left ear

Specimen presented is a left ear in with an intact tympano-periotic and abundant, attached soft tissue. There are multiple hyperattenuating spheroids (600 to 1200 HU) embedded in the soft tissues on the medial and lateral surfaces of the tympanic and periotic.

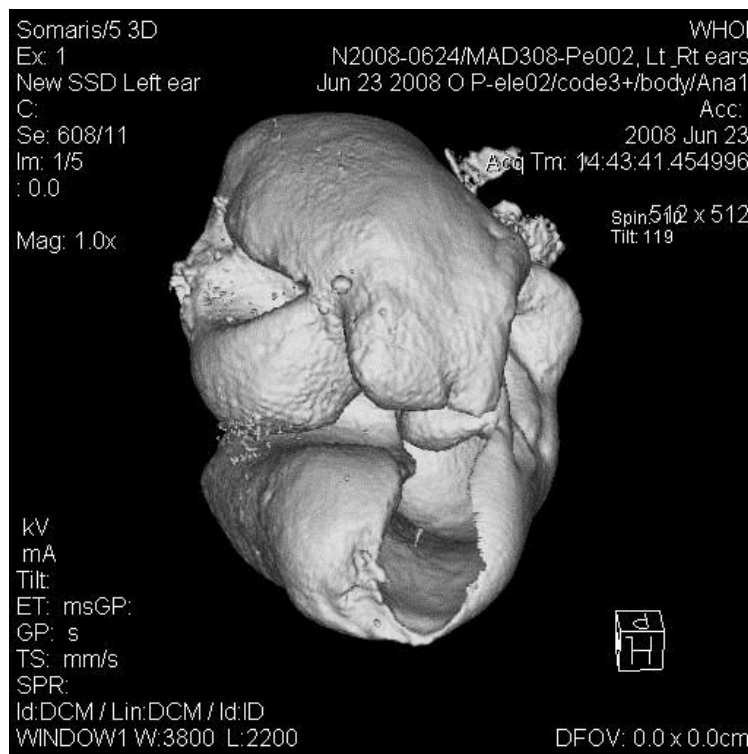


Fig. 0624-1. Lt. ear, anterior view. Peribullar nodules and notched anterior lip of tympanic bone.

Middle Ear

All ossicles are present and in their normal orientation. The annular ligament is intact.

Inner Ear

There is a well-developed cochlea which is generally normal with the exception of a small gas inclusion in the apical turn, a larger gas deposit in the upper middle to apical turns (fig.0624-2), and a small pocket of intracochlear gas in the basal turn adjacent to the round window. There is no evidence of intracochlear blood or of clotting associated with entrapped air.

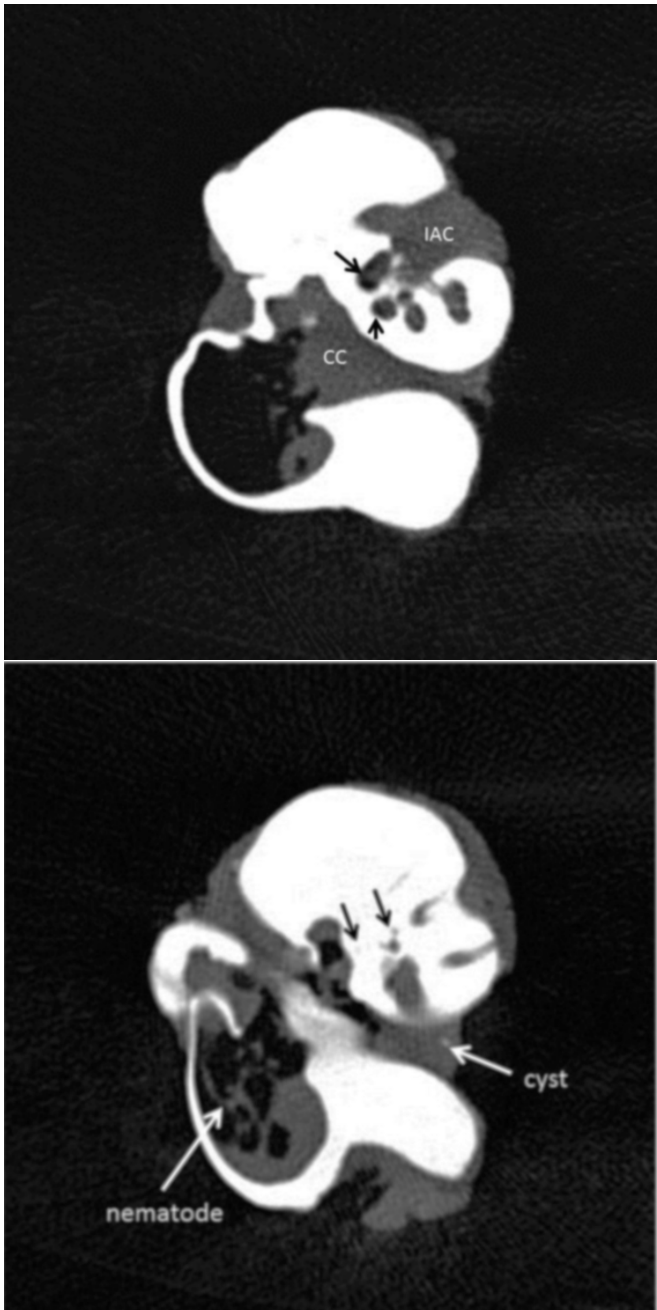


Fig. 0624-2. Left ear.
Upper panel: at the mid level of the cochlear spiral showing all turns and a well formed auditory nerve in the IAC. Gas is evident in the middle (angled arrow) and apical (vertical arrow) turns in this image.

Lower panel: in a more posterior section, thread like nematode bodies are found in the middle ear cavity as is a high density sphere consistent with a calcified parasitic cyst. The black arrows point to two semi-circular canals.

Impression:

The origin of the intracochlear gas deposits is uncertain. The intracochlear gas in the vicinity of the round window may be the result of a small perforation of the round window, which may be answered through histology or microdissection, or may be a postmortem artifact, particularly in light of the additional gas in the apex and middle turns. The origin may be decomposition or potential entry points via the IAC or the cochlear aqueduct. The absence of associated hemorrhage or clotting suggest the latter.

The significance of the peribullar nodules is unclear. It cannot be determined from these scans whether these represent anomalous calcifications or parasitic cysts.

Right ear.

The specimen was scanned using parameters noted above. Scans were obtained in the transaxial, paramodiolar plane, and images formatted using a 90 UH kernel for bone windows with extended scale. There are 493 slices at 0.1 mm thickness in the transaxial plane.

There are diffuse and extensive hypoattenuating regions in the tympanic and periotic bone. This is most notable on the medial edge of the ventral tympanic bone and in the posterior periotic. .

On the anterior medial edge of the tympanic there is a high attenuation, 3 mm long pedunculated projection; on the medial periotic anterior to the IAC there is a shallow mineralized ovoid, 2 mm in diameter and 2.4 mm in length (see scan 104 and fig. 0624-3).

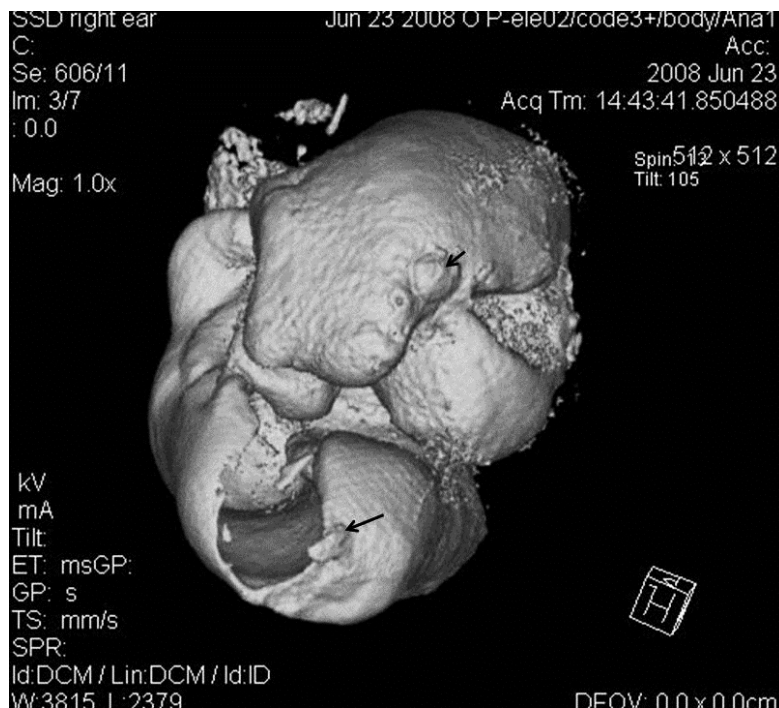


Fig. 0624-3. Rt.ear, anterior view. This 3D image shows the abundant, mineralized particles surrounding the ear bones. The arrows indicate the projections attached to the periotic (upper) and tympanic (lower).

Middle Ear

The middle ear ossicles are present and normal. The oval and round windows are intact.

Inner Ear

There are multiple regions of entrapped intracochlear gas in the basal, middle and apical turns. Some of the gaseous inclusions are restricted to a single scala, usually scala tympani, and thus demonstrate that the basilar membrane is intact (position -511.1 and fig. 0624-4)

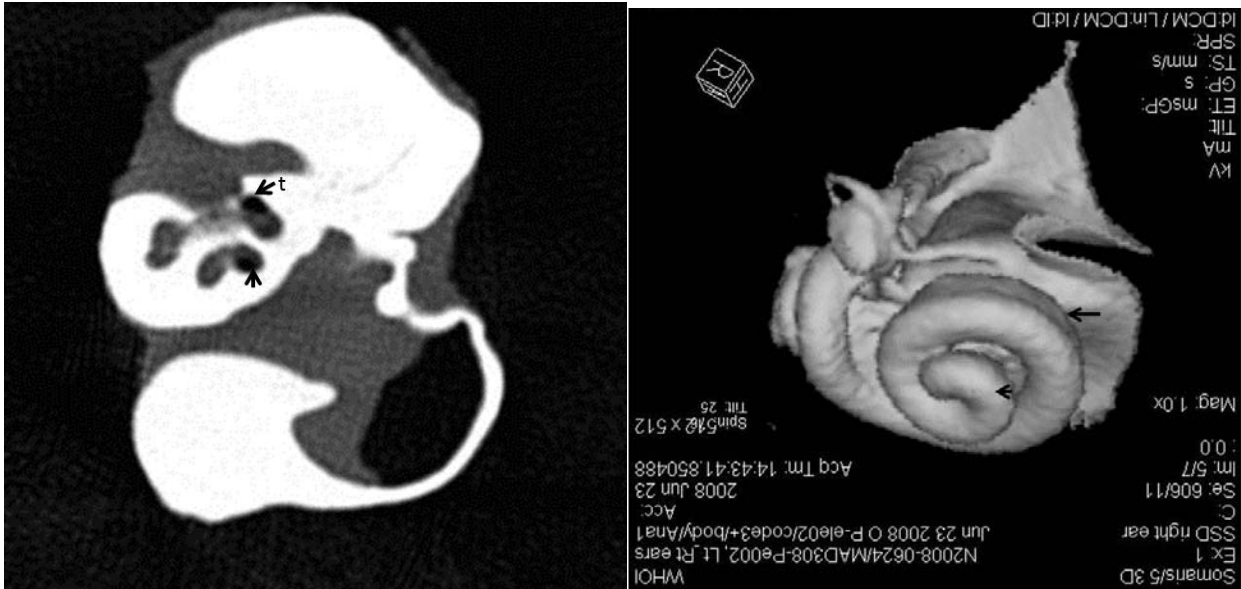


Fig. 0624-4 Rt ear. Left panel: Gas confined to one chamber (scala tympani, t) in the upper basal turn of the cochlea outlines the intact basilar membrane. A second area of gas is found in the apical turn. Right panel: A 3 D reconstruction of the cochlear scalae walls. The two arrows indicate the equivalent positions in the 2D

Impressions:

The extensive low attenuation sites in the tympanic and periotic are non-specific and may be the result of a number of demineralization processes; e.g., otosclerosis, arthropathy, chronic or acute osteolytic disease. The most extensive sites (ventral tympanic) are consistent with rugosities seen in other specimens that are likely to be associated with otitis media, however, this may be coincidental.

There is notable intracochlear gas of unknown origin. The fact that gas is present with intact cochlear windows weighs in favor of postmortem autolysis in a longer term or poorly preserved case that was designated as a code 3. This impression is reinforced by the fact that there are gas deposits isolated within the scalae.

N2008-0625 MAD 308/Pe003

Scan date 23 June 2008

Left ear

The specimen presented in formalin, and is an intact odontocete tympano-periotic complex with attached soft tissue. Gross examination shows no overt evidence of fracture or disease.

The specimen was scanned using an ultra high resolution spiral protocol on the Siemens Volume Zoom at the WHOI CT Facility. Scan parameters employed were the same as for preceding cases, kV120, effective MAS 200, acquisition table speed 0.5-mm at 0.5 seconds. Scans were obtained in the transaxial, paramodiolar plane, and images formatted using a 90 UH kernel for bone windows. 442 images were obtained at 0.1-mm slice thickness, formatted from the raw data in the transaxial plane.

Tympanic and periotic are attached at the posterior margin. There is no fluid and soft tissue evident in the middle ear. There are abundant hyperattenuating particles floating in the middle ear with equally abundant external particles haloing the periotic and tympanic, some of which are rounded, others forming long (3-5 mm) spicules. The anterior medial margin of the periotic and the outer wall of the tympanic have extensive low attenuation regions, and the anterior lip of the tympanic is compromised (fig. 0625-1).

Middle ear

The middle ear structures are normal. All ossicles are present and articulated with normal orientations. The oval and round windows are intact and the annular ligament is evident in the scans. The round window is clearly evident at spiral position -502.4

Inner Ear

All cochlear labyrinth and neural tissues are normal; with normal fluid and soft tissue attenuations. The scans demonstrate a 2.5 turn cochlea with no intracochlear evidence of disease or disruption.

Impression:

All major features of the ear are normal with abnormalities restricted to the bony and peribullar elements. The significance of the abundant and disseminated intrabullar and peribullar particles is unclear. This is an unusual finding worth further investigation and characterization and is not currently attributable to any definitive process.

The low attenuation foci are consistent with diffuse demineralization most commonly associated with aging or active osteolytic disease.

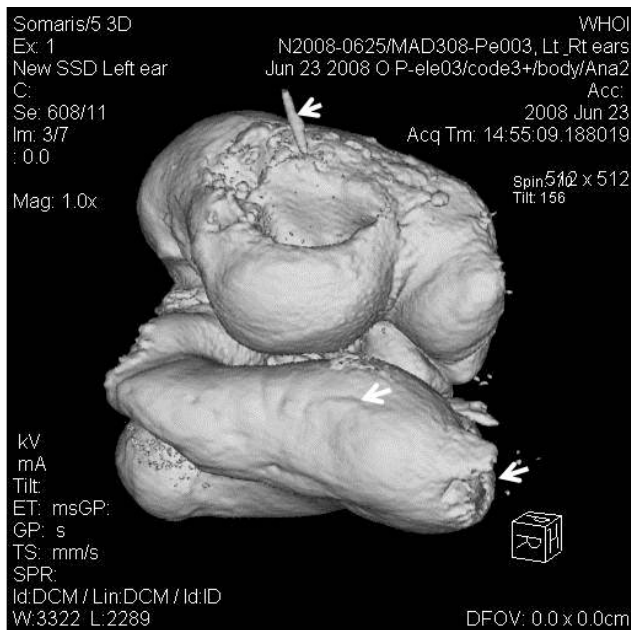


Fig. 0625-1. Upper panel: Lt ear, medial view. Arrows indicate peribullar particles, lytic region on the tympanic, and anterior lip degradation.

Left lower panel: Lt ear 2 D at level of hypomineralized, lytic tympanic area.

Right lower panel: Rt ear, medial view. Internal auditory canal lip degradation



Right Ear

439 images were produced with 0.1 mm slice thickness.

The specimen consists of a right intact tympano-periotic complex. . Both the tympanic and periotic have lower average attenuations than seen in the other specimens in this series. The lowest attenuations are found at the the medial face of the promontorium, the anterior processs of the periotic, and the anterior lip of the tympanic, which is rugose and serrated.

There are hyperattenuating inclusions in the peribullar soft tissues, with greatest deposits along the lateral periotic. The IAC has roughened margins that overlies the low mineralization areas (fig. 0625-1).

Middle Ear

The middle ear is intact with all ossicles present and in their normal anatomic relation. Little soft tissue is evident in the middle ear.

Inner Ear

The cochlear labyrinth is intact with a patent canal and no evidence of chronic or acute abnormalities. The cochlea is slightly under 2.5 turns and consistent in size with that found in other specimens.

Impressions:

With the exception of peribullar hyperattenuating particulates and low attenuation foci, of the periotic and tympanic margins, the ear is unremarkable. The extent and nature of these abnormalities suggest they are the result of chronic processes, likely response to infections, and possibly consistent with an older ear.

Specimen N 2008 -- 0626/MAD 108 -- Pe003

Exam date 23, June 2008

Left Ear

The specimen was scanned using an ultra high resolution spiral protocol on a Siemens Volume Zoom at the WHOI CT Facility. Scan parameters employed were kV120, effective MAS 200, acquisition table speed 0.5-mm at 0.5 seconds. Scans were obtained in the transaxial, paramodiolar plane, and images formatted using a 90 UH kernel for bone windows. 368 sections at 100 micron slice thickness were imaged with extended scale.

The right and left ears were in a common container. The ears were scanned separately. Tags with wires attached to the specimens were removed from the specimens to avoid metal artifacts in the scans and reattached following scanning.

The specimen was presented in formalin and consists of an intact left odontocete tympano-periotic complex with attached soft tissue comprising the suspensory ligaments and remnants of the peribullar plexus as well as peripheral fats. Gross examination shows no overt evidence of fracture or disease.

Both the tympanic and periotic have exceptionally high Hounsfield readings, even for odontocete tympano-periotic bones, ranging from 2000 to > 4000 HU in the cochlear capsule. The attenuations are evenly distributed with no evidence of spongiotic or sclerotic foci and therefore, no hyper/hypomineralized areas as noted in noted for some preceding cases.

There are 5 small, distinct hypoattenuating nodules (1-3 mm diameter) embedded in the peribullar soft tissues distributed along the medial surface of the periotic and tympanic. Three are ovoid with a reading of >3000 HU and 2 mm x 1 mm, long and short axes. The remainder are located on the posterior margin of the periotic.

Middle Ear:

The middle ear ossicles are intact with normal positions. There is no evidence of disarticulation or subluxation. The stapes is in its normal orientation, and the annular ligament is intact. The round window membrane is also evident in the scans.

Inner Ear

The scans demonstrate a 2.5 turn cochlea with good preservation. The auditory nerve and residual fibers are well preserved and evident throughout the IAC.

The cochlea is patent with no evidence of early or late labyrinthitis ossificans or other occlusive processes. The intracochlear fluids have normal attenuations with no evidence of hemorrhage.

Impression:

Normal tympano-periotic complex, middle and inner ear for an adult odontocete. This specimen may provide valuable material for this species as ears for this species have not previously been described.

The cochlea, vestibular, middle ear and bony elements of the periotic are normal with no indication of disease, chronic or acute, and no indication of trauma. The VIIIth neural fibers are well preserved with no indication of punctate, regional. or broad loss. This specimen has the best inner ear preservation of the available cases.

There are small peribullar, free-standing nodules that are not conventionally found as part of the odontocete tympanic periotic complex but have been found in several of these cases.

Right Ear

The specimen was presented and scanned in formalin using a spiral protocol of KV 120, MAS 205 at 0.5 mm table speed, 0.5 mm acquisitions and ultra high resolution kernels and extended scale. 360 images were obtained in the transaxial plane with 0.1-mm slice thickness.

The right ear was received with separated tympanic and periotic components, which based on appended field notes was done to examine the middle ear. The two components were placed to approximate normal anatomical positions for scanning but could not be stabilized (fig. 0626-1).

Abundant soft tissue remnants of the peribullar plexus are attached to the individual components of tympanic and periotic. Because of the disarticulation of the tympanic and periotic and the distribution of parts of the tympanic within the soft tissues, the axial images of this specimen do not represent a completely normal positioning, and therefore the transaxial images should not be directly compared with intact complexes.

The periotic bone is intact, but the tympanic has multiple longitudinal and comminuted fractures. Approximately 5 major and multiple smaller tympanic fragments are distributed throughout the surrounding soft tissues. The middle ear ossicles are present but disarticulated. The stapes is seen in a conventional position in the oval window, consistent with an intact annular ligament. The round window membrane is visible in the scans and is intact.

There are minor rugose areas regions on the ventral tympanic and an irregular tympanic lip.

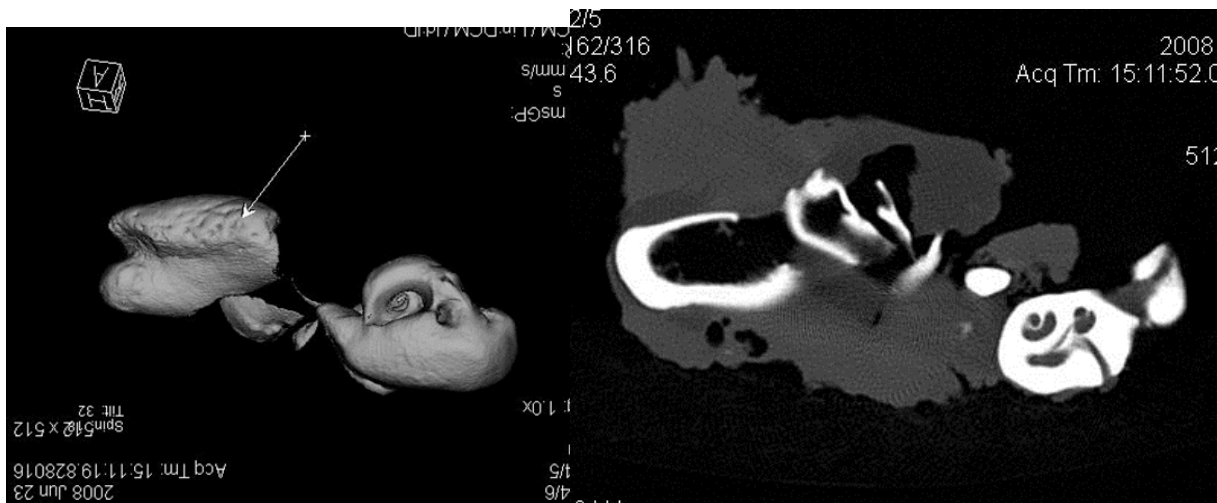


Fig. 0626-1. Rt. Ear:

Left panel: 3D of separated periotic (intact) and tympanic with fractures. The arrow indicates pitting.

Right panel: 2D cross-section at the level of the cochlea (right of image) illustrating multiple fragments. The separation and resulting fractured tympanic occurred during dissection in order to determine the middle ear condition and to inject the cochlea to preserve it (see field note documentation below).

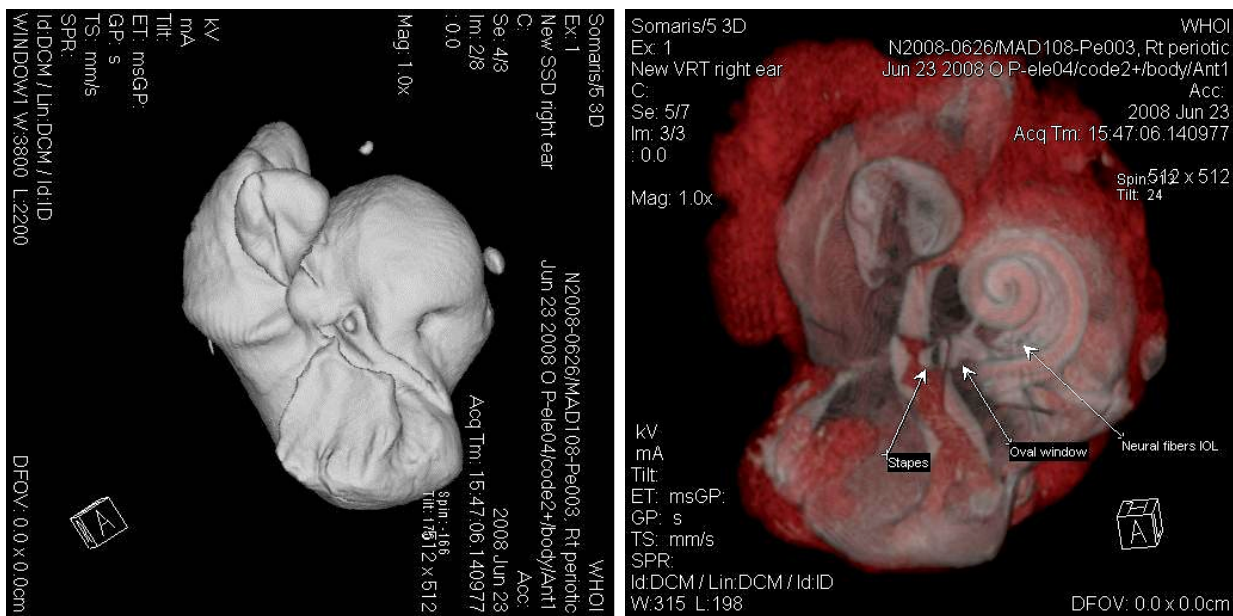


Fig. 0626-2 Rt. Ear. Left panel: 3D of the periotic bone, rotated for consistent position with right panel image. The large pointed ovoid is the head of the malleus in the epitympanic recess.

Right panel: The periotic bone has been made transparent to allow visualization of the intact cochlea. The stapes is clearly intact and attached to the oval window. The right hand arrow indicates a healthy array of auditory nerve fibers innervating the basal turn of the cochlea/

Middle Ear

The head of the malleus is visible in the epitympanic recess of the periotic (fig. 0626-2). The incus is not in evidence and is presumed lost when the tympanic was fractured and separated from the periotic. There is no evidence of any clotting or hemorrhagic tissues in the tympanic cavity nor in the remaining peribullar plexus.

Inner Ear

The cochlea was scanned in an oblique plane due to the difficulty of positioning the periotic. The scans demonstrate a 2.5 turn cochlear labyrinth with normal fluid compartments (-16 to 160 HU), consistent with normal intracochlear fluid, laminae, and membranes. There is no evidence of labyrinthitis (fig. 0626-2).

Impression:

The specimen most likely is from a mature adult based on the presence of minor bony changes on the ventral surface and tympanic lip. These are most consistent with prior and/or long term inflammation and chronic infection. This is a common finding in odontocetes, particularly in older animals.

Despite the appearance of possibly chronic external and middle ear disease, there is no evidence of inflammatory response nor obliterative disease in the inner ear.

The separation of the tympanic and periotic complex resulted in several significant abnormalities, all of which occurred postmortem as noted in the field dissection notes appended below for this case. Separation requires considerable focused force. The tympanic sustained multiple fractures, the number likely exacerbated by the poor postmortem condition of this head.

Due to the absence of staining, absence of hemorrhage, absence of clotting, it is further affirmed that these fractures occurred postmortem through handling. However, it requires histologic examination to confirm definitively. No other evidence of trauma was found. The disarticulation of the ossicles is consistent with the separation of the tympanic and periotic. The ossicles have simple articulations with only a thin mucosal sheath. It is common for their linkage to be disrupted and the incus to be absent if the two bullae are separated.

Field Dissection Notes

12-Jun-08// Male MAD108-PE003

06:00-07:00 on site

Probable juvenile to young adult.

Tooth marks Lt dorsolateral. Abdominal incision- Blubber thin but creamy.

Head: Rt side normal/ moderate to good condition. Left side, scrapes running diagonally - probable conspecific encounter.,

Teeth: good condition/ no evident loss

Mandible Left-23, Right-22

Maxilla Left-21, Right-20 + 2 spaces

Largest posterior to anterior. Note that teeth do not interlace but overlap due to ~ 3.5mm maxillary displacement

Thorax & Abdomen:

Lungs normal, no markings

Photo on gut/GI/air / peri-testicular parasites (encysted-5)/ cranial margin genital slit.

Rt ear: Peribullar region with grey to green necrotic tissue retrobullar. Ligaments discolored. Tympanic separated from the periotic to examine middle ear: symphysis devoid of normal fascia. Necrotic/consistent with retrobullar appearance.

Body of tympanic friable, corpus cavernosum absent in middle ear, small. Parasites intrabullar: Few compared to prior ears. Periotic in-situ/ retro region degraded, ossicles intact. Injecting with formalin.

Decapitated/ Left & Right parotids removed/ normal.

Nares: mucoid foam

Trachea/ bronchi: clear

Blubber overlying melon with vessel distention. Mild congestion.

Larynx: laryngeal congestion. Ring/stain near sphincter

No clear evidence CoD in head or majority of body, but ring and lividity in tail region. Consistent with found floating head up in H₂O.

Lt ear: Normal with no evidence of fracture or disease/ mild peribullar & middle ear infestation. (10's) of nematodes (probable *Stenurus major dujardin*)

Brain: Normal surface appearance. Cross section shows good differentiation of grey & white matter/ no congestion, no hemorrhages. No abnormal regions.

Conformation: typical odontocetes anatomy with hypertrophied temporal lobes.

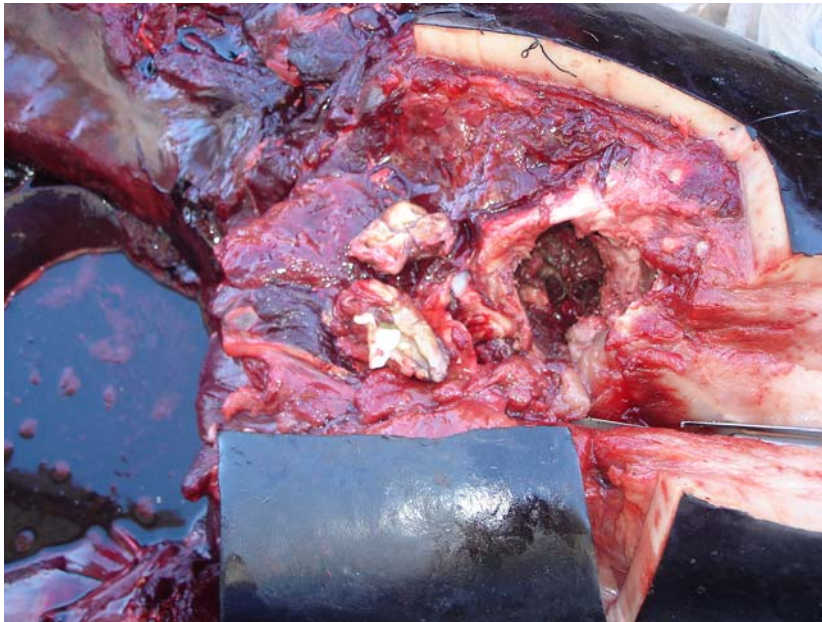


Fig. 0626-3. Rt. Ear. Tympanic and periotic opened for middle ear examination.