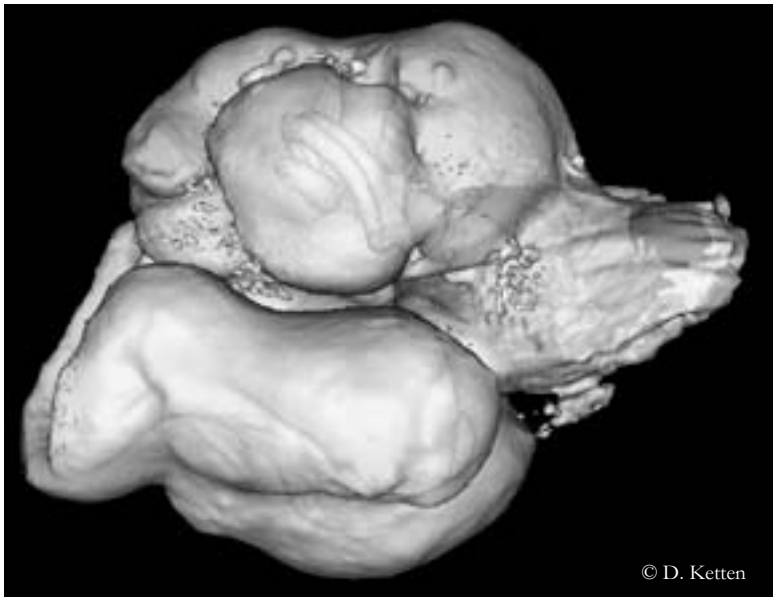
A MANUAL FOR THE REMOVAL, FIXATION AND PRESERVATION OF CETACEAN EARS



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PREFACE

This chapter is intended as an instructional guide for the removal, fixation and preservation of auditory system tissues of marine mammals. Each section describes procedures for a major ear type for marine mammals. The main intention is to provide both inexperienced and seasoned stranding responders with sufficient instructions to locate, document and remove all structures related to the ears and hearing in order to optimize the fixation and preservation of these tissues for later, more extensive examination. It is strongly recommended that examination be performed collaboratively with auditory system experts, but careful documentation and preservation are the critical first steps that will allow accurate diagnoses.

Key Terms: inner ear, cochlea, ossicles, vestibular system, auditory bulla, temporal bones, peribullar tissue, round window, oval window, hearing, auditory system.

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EQUIPMENT

Animal size, condition, and location impact what equipment is available for any necropsy. Items cited in bold and italics are useful in all necropsies. Items cited in plain text are helpful but not critical. **At a minimum, for most ear extractions you will need one small, thin bladed knife; one large heavy bladed knife**.

Surgical Tools
Knives – multiple lengths, serrated and plain edged
Hammer
Scalpels – handles and blades*
Chain Saw
Clamps – forceps and hemostats*
Chisels – narrow to broad blade*
Saws – hand and electric
Rope
Narrow flexible tubing or catheters*
Twine
Probe Sharp*
Plastic Ties
Probe Blunt*
Duct Tape
Metzenbaum Scissors Straight *
Measuring Tape Nylon or Plastic (metric)
Metzenbaum Scissors Curved *
Ruler (metric)
Syringe (1, 5, 10 and 50 cc)*
Thermometer – electronic probe type or conventional
Suture Kits*
Headlamp
Calipers
Flashlight
Femoral Disarticulator*
Screwdriver – Flathead; Long Blade
Ronguers/Bone Shears*
Crow Bar
Meat Hooks (with handles and or hooks with attached chain)
Hack Saw and Blades
Cutting Board or Sheet (plastic)
Sawz-All and Blades
Scalpel Blade Remover*
Cordless Drill
Sharpening Stone

Safety / First Aid
Safety Glasses
Wet Suits
Elastic Bandage
Survival Suits
Ice Packs
Quick Clot *
First Aid Kit - Professional
Sunscreen
Disinfectant Soap/Hand Cleaner

Hand Warmers
Soap/Shampoo
Derma Bond/Super Glue
Ear Plugs
Dry Suits

Bags / Containers / Labels / Pens / Pencils
Whirl Paks *
Permanent Markers
Histology Cassettes*
Sealable Plastic Bags **(e.g. Zip Loc)**
Cooler
Duffel Bag
Plastic Bags
Labels/Tags
Lidded buckets
Garbage Bags
Pencils
Body Bags
Plastic Containers (25 to 500 mls)

Miscellaneous
Necropsy Forms
Cloth Towels
CD's
Formalin*
Microscope Slides*
Expanding Foam
Ethanol

Audio / Video
Digital Video Camera and Tapes
Waterproof Housing for Camera(s)
35mm Digital Camera
Tripod Stand and Case
35mm SLR Camera
Storage media

Clothing
Disposable Latex Gloves
Rain Suit
Sterile Gloves*
Rubber Boots
Nitrile Gloves*
Surgical Gowns*
Dish Gloves
Scrubs*
Plastic/Rubber Aprons

***** Available through medical or veterinary supplies.

Field & Laboratory Necropsy Kit Checklist – SAMPLE

Date_____Location_____Description_____

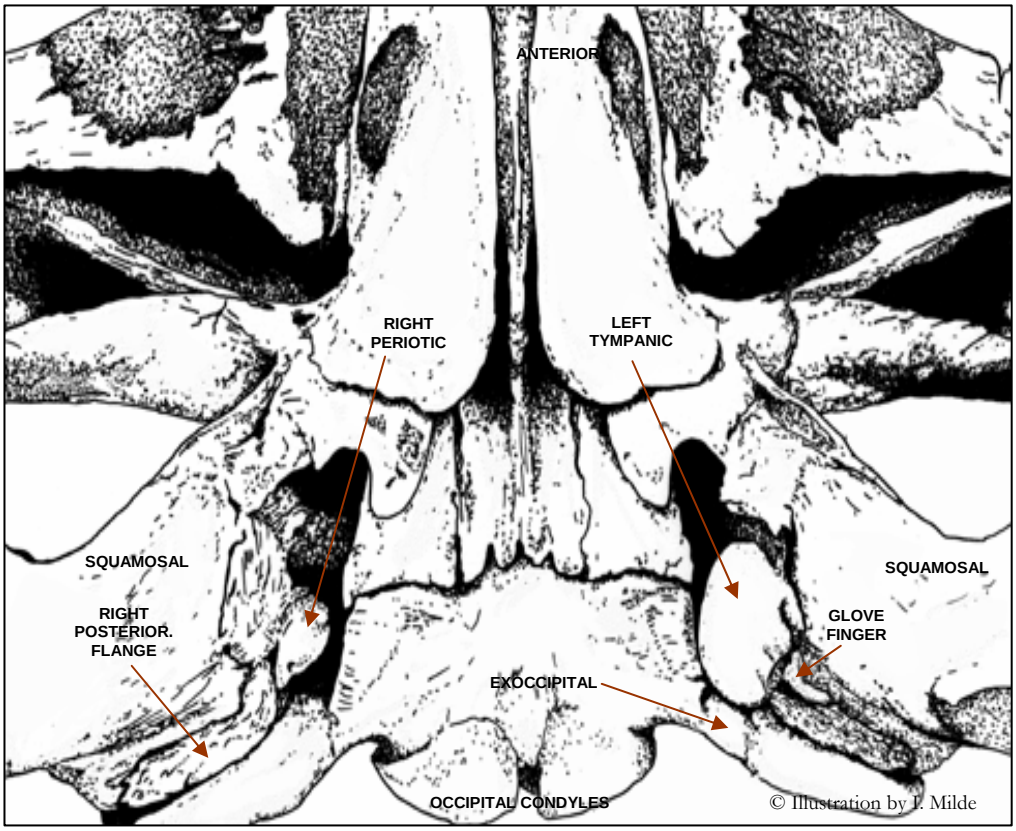
- SURGICAL TOOLS -		
Knives – multiple lengths, serrated and plain edged	***Hammer***	
Scalpels – handles and blades*	Chain Saw	
Clamps – forceps and hemostats*	***Chisels – narrow to broad blade***	
Saws – hand and electric	Rope	
Narrow flexible tubing or catheters*	Twine	
Probe Sharp*	Plastic Ties	
Probe Blunt*	***Duct Tape***	
Metzenbaum Scissors Straight *	***Measuring Tape Nylon or Plastic (metric)***	
Metzenbaum Scissors Curved *	***Ruler (metric)***	
Syringe (1, 5, 10 and 50 cc)*	***Thermometer – electronic or conventional***	
Suture Kits*	***Headlamp***	
Calipers	***Flashlight***	
Femoral Disarticulator*	***Screwdriver – Flathead; Long Blade***	
Ronguers/Bone Shears*	***Crow Bar***	
Meat Hooks (with handles and or hooks with attached chain)	**Hack Saw and Blades**	
Cutting Board or Sheet (plastic)	***Sawz-All and Blades***	
Scalpel Blade Remover*	Cordless Drill	
Sharpening Stone		
- SAFETY/FIRST AID -		
Safety Glasses	Wet Suits	Elastic Bandage
Survival Suits	***Ice Packs***	Quick Clot *
First Aid Kit - Professional	***Sunscreen***	***Disinfectant Soap/Hand Cleaner***
Hand Warmers	***Soap/Shampoo***	***Derma Bond/Super Glue***
Ear Plugs	Dry Suits	
- BAGS/CONTAINERS/LABELS/PENS & PENCILS -		
Whirl Paks*	***Permanent Markers***	Histology Cassettes
***Sealable Plastic Bags* (e.g. Zip Loc)**	Cooler	Duffel Bag
Plastic Bags	***Labels/Tags***	***Lidded buckets***
Garbage Bags	***Pencils***	
Body Bags*	***Plastic Containers (25 to 500 mls)***	
- MISCELLANEOUS -		
Necropsy Forms	Cloth Towels	**CD's**
Formalin*	Microscope Slides*	
Expanding Foam	Ethanol	
- AUDIO/VIDEO EQUIPMENT -		
Digital Video Camera and Tapes	**Waterproof Housing for Camera(s)**	
35mm Digital Camera	**Tripod Stand and Case**	
35mm SLR Camera	**Storage media**	
- CLOTHING/GLOVES -		
Disposable Latex Gloves	Rain Suit	
Sterile Gloves*	***Rubber Boots***	
Nitrile Gloves*	Surgical Gowns*	
Dish Gloves	Scrubs*	
Plastic/Rubber Aprons		

***** Available through medical or veterinary supplies.

Extraction

Documenting the procedures and tissue condition as you proceed with ear extraction is crucial. Photograph the area you are working on before and after each stage of the procedures, being sure to add a scale and a marker indicating any abnormal area. Take wide area and close-up shots of such areas. Label areas on the photograph consistent with the labeling of tissues sub-sampled from each area.
Mysticete ears consist of two joined bullae, or rounded bones, the tympanic and periotic, located just lateral to the occipital condyles in a cavity formed by the squamosal (dorsal and lateral border) and the exoccipital (posterior border) (Figs. 1 and 2a – 2b).

Figure 1. Ventral view of Humpback Whale (*Megaptera novaeanglia*) ears in the skull. All soft tissues, the lower jaw, and the right tympanic are removed.



A dense pad of fibrous and fatty tissue covers the ventral and posterior surface of the tympanic bone (Figs. 2a – 2b). Approaching the ears from the ventral surface, slice through the tissue pad until you reach the smooth ventral surface of the tympanic bone. The pad can be cut away or left intact. If there is any question of explosive or ship strike trauma, leave the pad attached or photograph it carefully and preserve in formalin or by freezing some areas that appear normal as well as abnormal from locations that were photographed.

Figure 2a. Ventral view of a Minke Whale (*Balaenoptera acutorostrata*) head. Lower jaw is removed and left tympanic is exposed

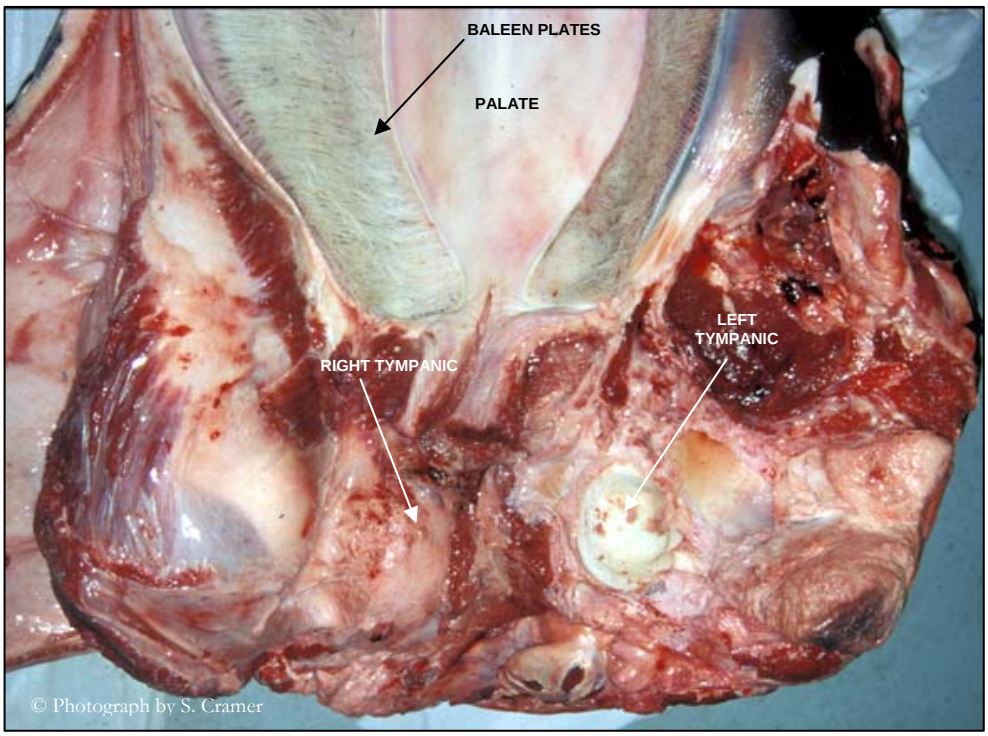
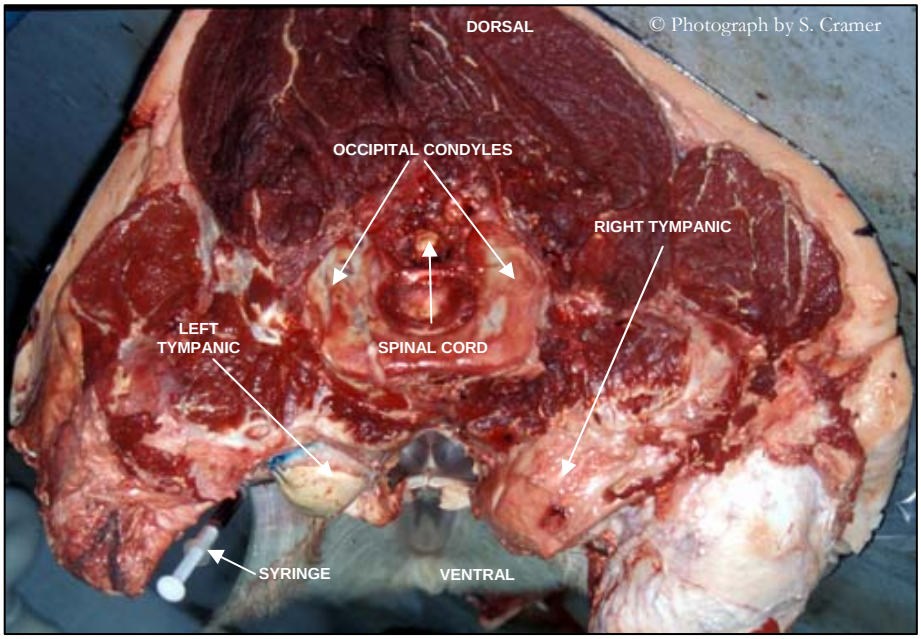


Figure 2b. Posterior view of a Minke Whale (*Balaenoptera acutorostrata*) head. The bulbous white left tympanic bone is visible. The right tympanic bone is still covered by its fibrous and fatty pad. A syringe is inserted into the external auditory canal near the tip of the glove finger.



The tympanic bone contains the middle ear bones (ossicles), a spongy soft tissue (corpus cavernosum), and the tympanic membrane (eardrum; glove finger). The glove finger looks exactly like

its name: a pink or tan hollow, fibrous tube closed at the lateral edge. If you do not see it, do not be concerned. It protrudes from the lateral side of the tympanic (Fig. 3) but may be deteriorated or lost in the extraction. It will vary by species from approximately 5-15 cm in length and 2-5 cm in diameter.

Figure 3. Lateral view of a Northern Right Whale (*Eubalaena glacialis*) ear with the glove finger exposed.

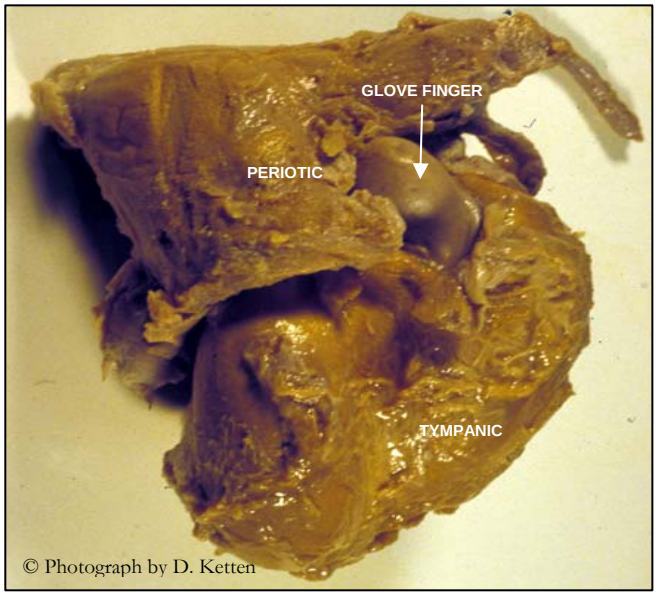


Figure 4a. Lateral view of a Northern Right Whale (*Eubalaena glacialis*) ear bone.

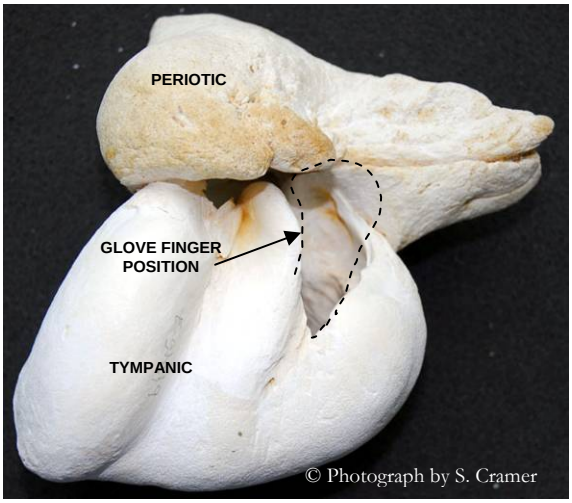
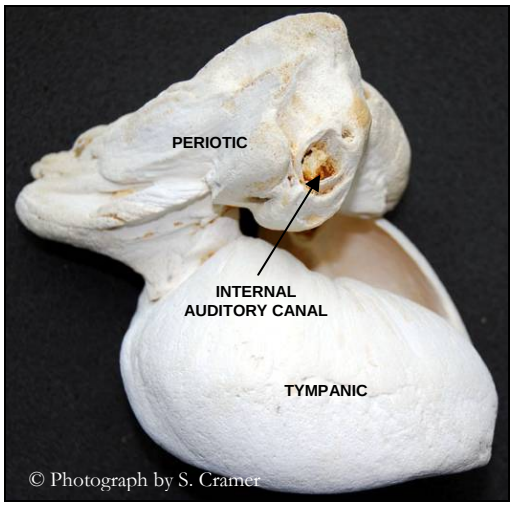


Figure 4b. Medial view of a Northern Right Whale (*Eubalaena glacialis*) ear bone.



In some animals, tissue attached to the lateral tympanic wall obscures the glove finger. Leave as much of this tissue intact as possible to protect the glove finger and its wax plug if you locate it. If there is a question of blast injury in particular, leave as much soft tissue around the tympanic membrane and ear bones as possible.

Just dorsal to the tympanic is the periotic which contains the inner ear. In Figure 1, the right tympanic has been removed to show the periotic's location and relative size. Ideally, remove the periotic and

tympanic bones together as a unit. If the tympanic separates from the periotic during removal or is loose, please be sure to preserve the ossicles and do not forget to remove the periotic, as it is crucial for hearing and auditory trauma analyses.

The periotic is attached to the skull by anterior (short) and posterior (long) flanges (Fig. 1). The flanges are blunt spikes of spongy bone that are wedged into bony channels in the exo-basioccipital and squamosal. The dense, spherical bone between the two spongy flanges is the actual periotic bulla which contains the inner ear.

To remove the ear, try first to free the posterior flange by prying it out with a chisel. Use a screwdriver to lever the flanges from their troughs. Pulling upward gently simultaneously on the tympanic may help. Several nerves and vessels exit the periotic on its medial side. The largest of these is the auditory nerve (Fig. 4 - 4b). Cut these rather than pulling on them if possible. If the anterior flange appears fused to the skull, lever it out with a chisel or screw driver. If the flanges cannot be freed, cut them with a saw or heavy serrated knife, or crack them off with a chisel placed on the neck of the flange approximately 2 cm behind the tympanic, then try to cut the retrobullar nerves and soft tissue to free the periotic.

Fixation/Preservation

After removal, place the ears in 10% buffered formalin for at least one week before shipping or further processing. They may remain in formalin for up to several months but the formalin should be changed after the first week and at least twice during the first month. The best fixative is 10% buffered formalin available commercially through scientific chemical suppliers. If formalin is not available, use 70% ethanol. Freezing is not optimal but is acceptable. If the ears are frozen, do not thaw before fixation or shipping. Ship them frozen or if fixative becomes available later, place the frozen specimens in the fixative to thaw. Do not thaw in water or in air.

If concentrated formaldehyde liquid or formaldehyde powder is used, mixing it with sea water in lieu of fresh water provides an adequate temporary buffered solution. Perfusion of the inner ear by injection is not necessary, but if the specimen is very fresh and if you are comfortable with the anatomy, inject formalin through the round window with a 22-25 gauge needle. Do not inject if only large bore needles are available. DO NOT INJECT IF TRAUMA IS SUSPECTED.

Place the specimen in the field in sufficient formalin to cover. As soon as possible, increase the volume to five times that of the specimen. During the first week, check the formalin daily or every other day to determine if it is saturated (dark reddish brown). If so, replace the saturated formalin with fresh solution. Continue this process, gradually decreasing the volume of formalin until no further coloration is evident and soft tissues show some hardening. The formalin at this point may be clear or have a slight yellowish color. The volume should be kept at one and one half to two times the specimen minimum.

Extraction

Documenting the procedures and tissue condition as you proceed with ear extraction is crucial. Photograph the area you are working on before and after each stage of the procedures, being sure to add a scale and a marker indicating any abnormal area. Take wide area and close-up shots of such areas. Label areas on the photograph consistent with the labeling of tissues sub-sampled from each area.

Approach odontocete ears from the side of the animal unless the lower jaw has been removed. Each ear consists of two joined spheres of dense bone, one hollow (tympanic) which forms the middle ear cavity and one nearly solid (periotic) which contains the inner ear. These paired bones sit in a cavity (peribullar sinus) below the brain case, bounded by the squamosal (lateral and dorsal) and the exoccipital (posterior and medial).

Figure 1a. Bottlenose Dolphin (*Tursiops truncates*) with a marker for lateral ear extraction incisions.

Figure 1b. Lateral view of a Harbor Porpoise (*Phocoena phocoena*) head heavily flensed to show the right tympanic ear bone and anterior region of the peribullar sinus.

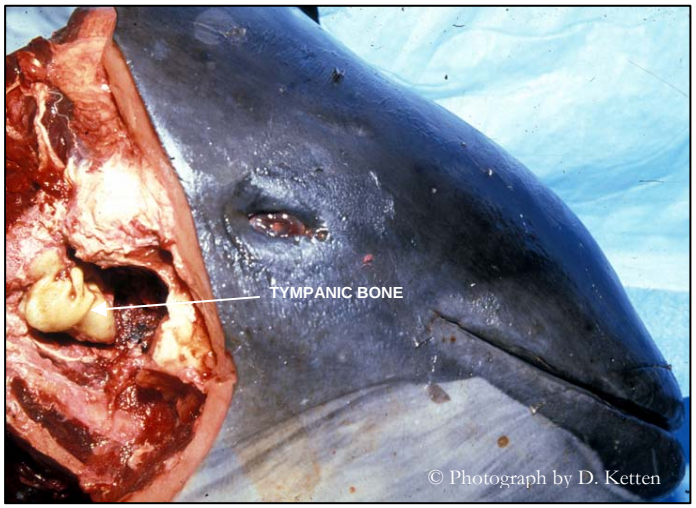


Figure 1c. Harbor Porpoise (*Phocoena phocoena*) head showing the ear position in relation to the lower jaw (mandible). Periotic (p); Tympanic (t); Mandible (m); Exocciptial (e).

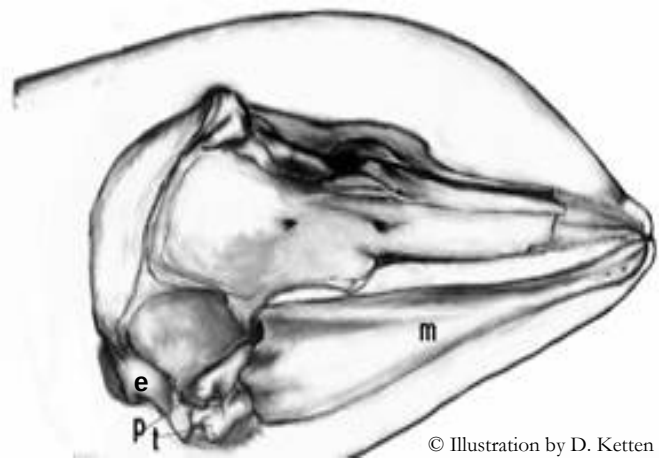


Figure 1d. Ventral view of a Harbor Porpoise (*Phocoena phocoena*) skull. Ear bones have been removed.

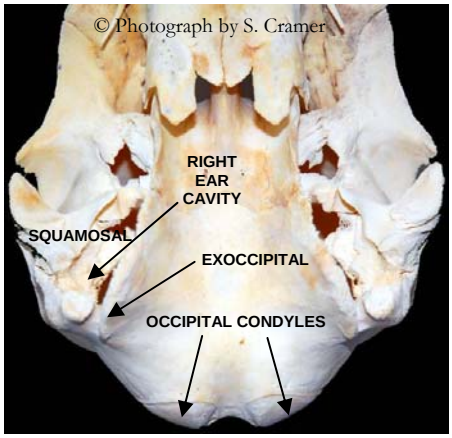


Figure 2a. Medial view of a delphinid left ear; tympanic, periotic and neural canals. This ear is slightly rotated downward from the image at the right.

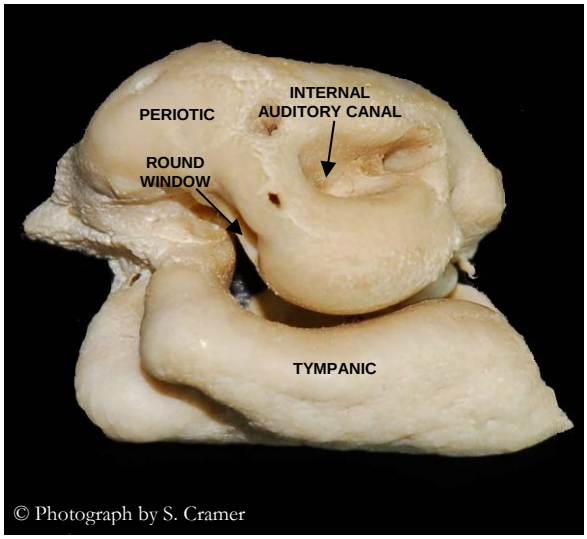
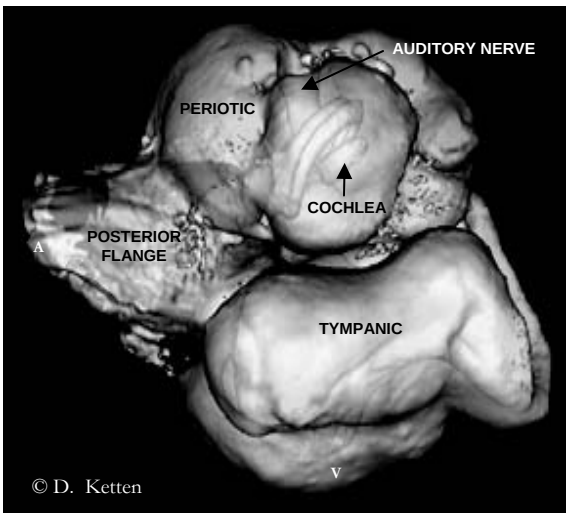


Figure 2b. Medial view of a 3D reconstruction of a Pygmy Sperm Whale (*Kogia breviceps*) right ear from CT scans. The periotic is rendered transparent to show the actual position of the cochlea and auditory nerve (VIII) in the periotic.



The ears are located just behind and deep to the lower jaw, on a line about mid-way between the eye and the insertion of the pectoral fin (Figs. 1 – 1d). To extract the ears from a lateral approach, first make an X-shaped incision about mid-way between the eye and the pectoral fin, with the midpoint of the X in line with the lower jaw. Pull the flaps back and down, cutting through the blubber and muscle. There is considerable soft tissue filling the cavity around the ear bones, and you will probably not see either the tympanic or periotic at this point. Pushing a probe straight in, you will feel a hard surface which is the posterior section of the tympanic bone. If possible, photograph the area to document its appearance

before cutting further. Then, gently cut away the tissue with a scalpel or knife until you find the tympanic bone. In a typical delphinid, the tympanic will be about 40 mm in length and 25 mm wide. It resembles a conch shell, with a hollow interior that contains the middle ear bones (ossicles), a spongy soft tissue (corpus cavernosum), and the eardrum. The periotic bone contains the inner ear. It is slightly smaller and is located just dorsal and medial to the tympanic (Fig. 2a – 2b). The hyoid bones are generally attached to the posterior/lateral edge of the tympanic by a cartilaginous cap. Cut this juncture with bone shears or a scalpel.

The tympanic and periotic are partly fused to each other at the rear edge by a semi-fused (synosteotic) joint and at the lateral edge by a curved or sigmoid process. These joints are relatively weak. Try to keep the two halves together and remove them as a unit. If the tympanic separates from the periotic during removal or is loose, be sure to extract both and check for ossicles that may have fallen from the middle ear.

Having cleared enough tissue to identify the two bones, you will now need to cut a set of five to eight suspensory ligaments and the facial and auditory nerves located on the medial surface of the ear (Fig. 2a). Gently rock the ear while cutting the attached soft tissue on the medial, anterior, and posterior surfaces with a narrow, sharp knife or scalpel. If the ear is difficult to move, the periotic may be attached to the skull by a short bony process or the ligaments may be calcified. This is particularly common in older animals. (Note: Some groups such as Ziphiids (Beaked Whales) and Physeterids (Sperm Whales) have substantial bony connections. Separate protocols are given for these ears). Any bony attachments that are resisting removal should be cut with bone shears or pried loose with a small chisel, screwdriver or flat bladed instrument. Scrape the posterior area where the periotic joins the exoccipital and try to locate suture margins. Insert your screwdriver or chisel into these lines and gently tap it into the bone, periodically wiggling the blade to see if the flange can be levered free of the skull. Do not use a scalpel blade for this procedure. It will snap. Some soft tissue will be attached to the ear. Simply leave that in place.

Fixation/Preservation

After removal, place the ears in fixative immediately. The best fixative is 10% buffered formalin. If concentrated formaldehyde liquid or formaldehyde powder is used, mixing it with sea water in lieu of distilled water or a chemical buffer provides sufficient buffering. If formalin is not available, 70% ethanol may be used. If the specimen is very fresh, and if you are comfortable with the anatomy, it is best to inject formalin through the round window with a 22-25 gauge needle. The round window is located at the posterior/medial edge of the periotic, ventral to the stapes (Fig. 2a). If you are not familiar with the anatomy, please do not attempt this injection. Also, please do not inject if only large bore needles are available. IF TRAUMA IS SUSPECTED, DO NOT INJECT at the round window, instead, insert the needle into the center of the VIIIth nerve and slowly inject formalin. If you are injecting the round window, insert the needle in the middle of the membrane approximately 3 mm and SLOWLY inject a small quantity of formalin. Be certain to record the location, needle size and fluid quantity injected in all cases and send with the other data for the animal's ears to the examiner.

In the field, getting the tissues into any quantity of formalin that surrounds them is acceptable, but, ideally, place the ears in a fixative volume five times that of the specimen as soon as possible. After one week, move them to half that volume changing to fresh formalin once or twice weekly until they are well fixed. Once fixed, the soft tissues will be moderately stiff and brown and the formalin clear or light tan in color. The ears can be held for several months as they are, moved to another preservative, or shipped at this point.

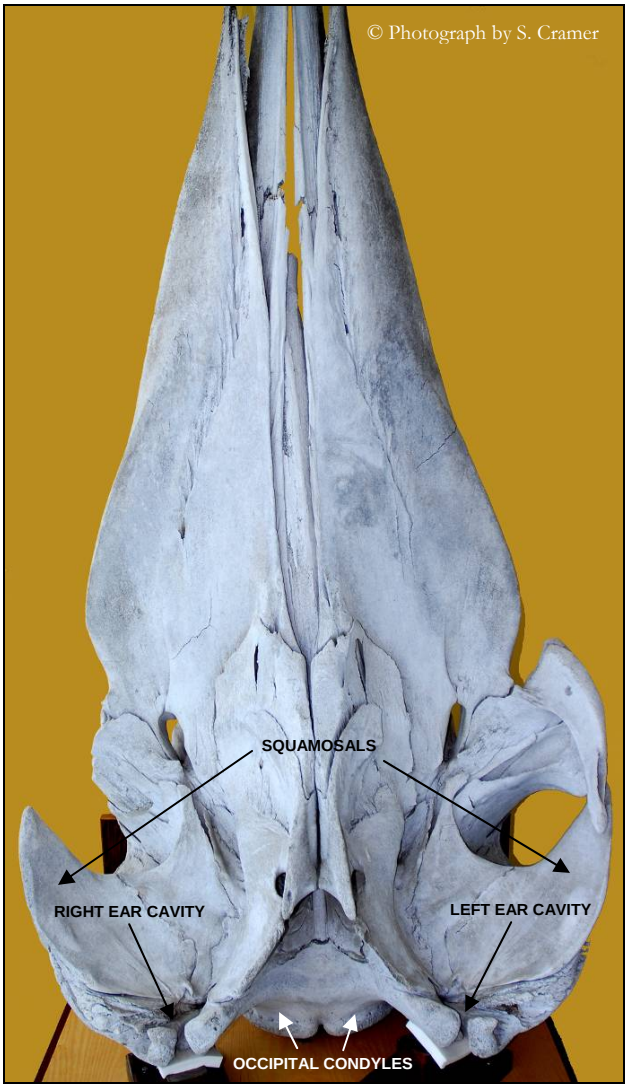
Freezing is acceptable only if fluid fixation is not possible. If possible, try to obtain fixative later, and place the frozen specimens in the fixative to thaw. Do not thaw in water or in air. If the ears are frozen, do not thaw before shipping. Hold them without thawing and contact the receiving lab to discuss shipping methods.

Extraction

Most important in any necropsy procedure, document photographically the tissue condition at each major step, external to final removal. Label all images consistent with any cassette labels of tissues sampled from the region photographed. Include in the picture a label with the animal ID, indication for dorsal, ventral, anterior and posterior directions, and a metric or other scale. Take both broad and close up views of suspected abnormalities.

Sperm whale ears can be approached from the ventral or lateral side. Ventral approaches require the removal of the lower jaw. The ears sit in cavities below the brain case, located either side of the occipital condyles and behind a large squamosal shield (Fig. 1). If you are taking a ventral approach, part of the ears will be visible as two large white, egg-shaped bones (Fig. 2).

Figure 1. Ventral aspect of an adult Sperm Whale (*Physeter macrocephalus*) skull.



From the side, the ears are located just behind and deep to the lower jaw, about mid-way between the eye and the posterior insertion of the pectoral fin (Fig. 3). On a newborn or very young sperm whale, they are located approximately 17 cm behind the rear edge of the lower jaw on a head that is 120 cm long. The distances should be proportional on an adult.

For a lateral approach, make an X-shaped incision about mid-way between the eye and the pectoral fin, with the midpoint of the X in line with the lower jaw. Pull the flaps back and down, cutting through the blubber and muscle. Your incision should be just posterior to the jaw. As you probe towards the center of the head, the next bone you will come to is the squamosal, which in this species is a large, lateral wing or shelf extending from the skull. Because of this "squamosal shield", it is easier in this species to approach the ears ventrally or to remove the head and work from the posterior face than to attempt a lateral approach.

Figure 2. Posterior view of the right ear of a young Sperm Whale (*Physeter macrocephalus*) head**.**

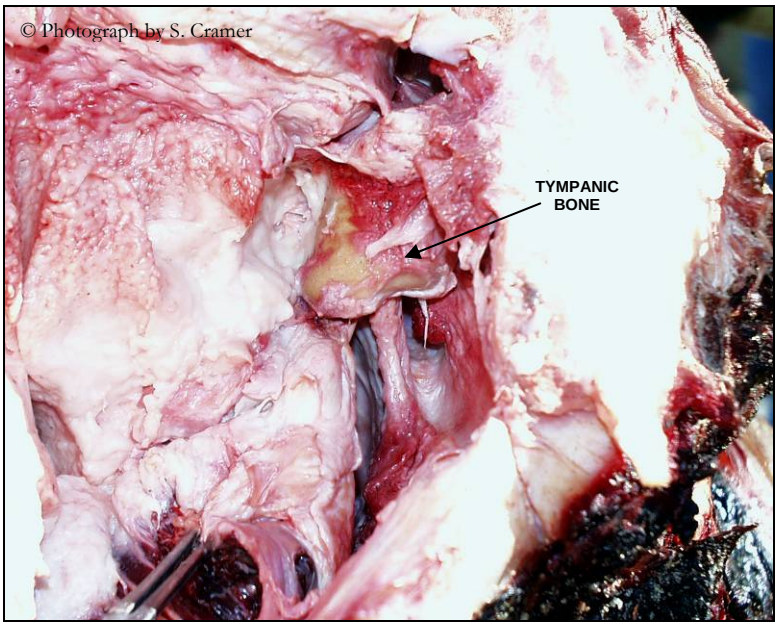


Figure 3. Incisions for a lateral approach to remove a Sperm Whale (*Physeter macrocephalus*) ear. © Photographs by D. Ketten and S. Cramer

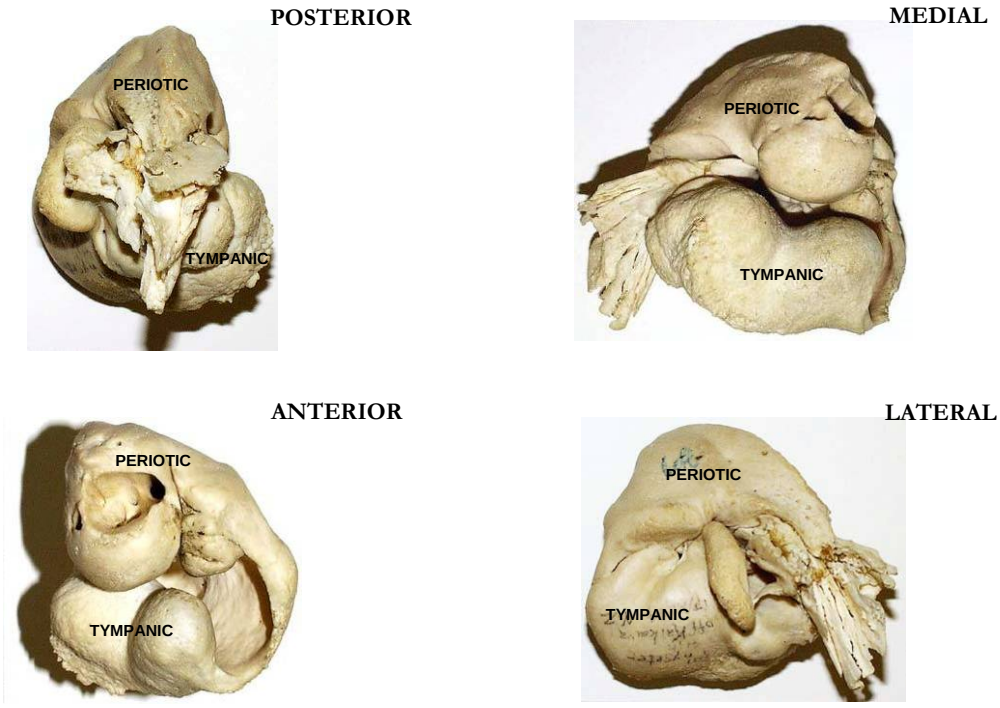
Depending upon the animal's position and the need to preserve the skull parts, you can either reach under the squamosal flange or cut through its narrow neck, which is just above the two bones (tympanic

and periotic) that make up the ear (Fig. 4). You may also cut away a block of tissue using a Sawz-all or chain saw. The block should be approximately 20 cm on a side to include both ear bones, but please be certain that you have included both parts of the ear, described below, in the block.

Each ear consists of two dense joined bones that sit in the cavity below the brain case adjacent to the squamosal(s) and the exoccipital(s). Each of the bones is about the size of a tightly closed fist (Fig. 4).

There is considerable soft tissue surrounding the ear bones. Remove this tissue with a scalpel or knife until you find the tympanic, the lower and more ventral and lateral of the two bones.

Figure 4. Left ear of a Sperm Whale (*Physeter macrocephalus*)**.** © Photographs by S. Cramer



The tympanic resembles a dense conch shell, with a hollow interior that contains the middle ear bones (ossicles), a spongy soft tissue (corpus cavernosum), and the eardrum. With the jaw removed, the ventral tympanic is readily visible. The periotic contains the inner ear and is just above and medial to the tympanic. The tympanic and periotic are fused but the joint may be weak. Remove the ears as a unit if at all possible. If the tympanic separates from the periotic during removal or is loose, be sure to preserve the ossicles and any soft tissue from the middle ear.

Once you locate both bones you will need to locate five to eight ligaments as well as the auditory nerve on the medial and posterior faces of the periotic. In the sperm whale there are also substantial flanges protruding from the posterior edge of the periotic. The ear will likely be difficult to move, and the periotic flanges will need to be cut or levered out of the skull. Chisel any bony attachments that are resisting removal using a screwdriver, narrow chisel or other stiff, flat bladed instrument. Do not use a scalpel blade to pry the ear. The blade will snap and is difficult to remove from the ear cavity. Do not attempt to chisel into the dense periotic. Instead, probe until you find softer, spongy bone on its posterior margin. Wedge your chisel or screwdriver into this flange or into the skull at its juncture. Pry gently until the suture separates or the flange breaks. If this specimen is to be used for osteologic studies, try to maintain the flange.

Once the ear bones can be moved, try to locate the ligaments and nerves retrobullar (behind and medial to the ear bones). Cut these with a sharp knife or scalpel. Grasping the two ear parts, rock the ears gently until they can be cut. Do not pull soft tissue to free them or they may evulse the nerve; i.e., rip the nerve out of the ear.

Fixation/Preservation

After removal, place the ears in fixative immediately. The best fixative is 10% buffered formalin. If concentrated formaldehyde liquid or formaldehyde powder is used, mixing it with sea water in lieu of distilled water or a chemical buffer provides sufficient buffering. If formalin is not available, 70% ethanol may be used. If the specimen is very fresh, and if you are comfortable with the anatomy, it is best to inject formalin through the round window with a 22-25 gauge needle. The round window is located at the posterior/medial edge of the periotic, ventral to the stapes. If you are not familiar with the anatomy, please do not attempt this injection. Also, please do not inject if only large bore needles are available. IF TRAUMA IS SUSPECTED, DO NOT INJECT at the round window, instead, insert the needle into the center of the VIIIth nerve and slowly inject formalin. If you are injecting the round window, insert the needle in the middle of the membrane approximately 3 mm and SLOWLY inject a small quantity of formalin. Be certain to record the location, needle size and fluid quantity injected in all cases and send with the other data for the animal's ears to the examiner.

In the field, getting the tissues into any quantity of formalin that surrounds them is acceptable, but, ideally, place the ears in a fixative volume five times that of the specimen as soon as possible. After one week, move them to half that volume changing to fresh formalin once or twice weekly until they are well fixed. Once fixed, the soft tissues will be moderately stiff and brown and the formalin clear or light tan in color. The ears can be held for several months as they are, moved to another preservative, or shipped at this point.

Freezing is acceptable only if fluid fixation is not possible. If possible, try to obtain fixative later, and place the frozen specimens in the fixative to thaw. Do not thaw in water or in air. If the ears are frozen, do not thaw before shipping. Hold them without thawing and contact the receiving lab to discuss shipping methods.

Extraction

Documenting the procedures and tissue condition as you proceed with ear extraction is crucial. Photograph the area you are working on before and after each stage of the procedures, being sure to add a scale and a marker indicating any abnormal area. Take wide area and close-up shots of such areas. Label areas on the photograph consistent with the labeling of tissues sub-sampled from each area.

Approach Beaked Whale ears from the side of the animal, or ventrally, if the lower jaw has been removed. Each ear consists of two joined dense bones, one hollow (tympanic) and one spherical (periotic), that sit in the cavity lateral to the brain case and are bordered by the squamosal laterally and the exoccipital posteriorly (Figs. 1 and 2).

Figure 1. Ventral aspect of a Cuvier's Beaked Whale (*Ziphius cavirostris*) skull. The tympanic bones are ovoid. The periotics are not visible in this photograph and are located dorsal and medial to the tympanic bones.

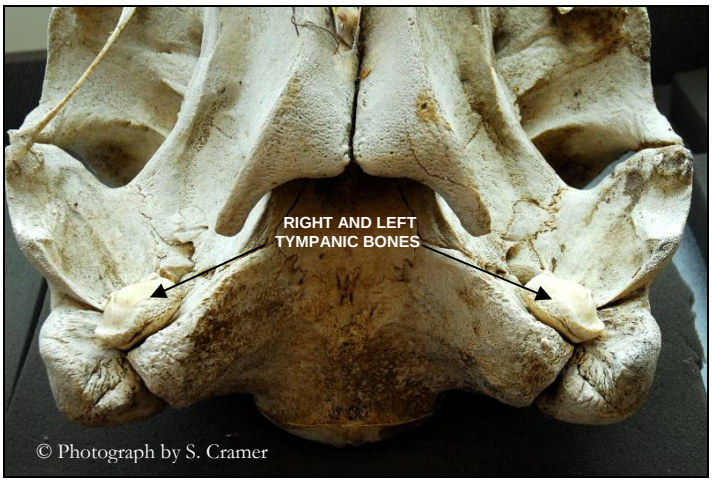
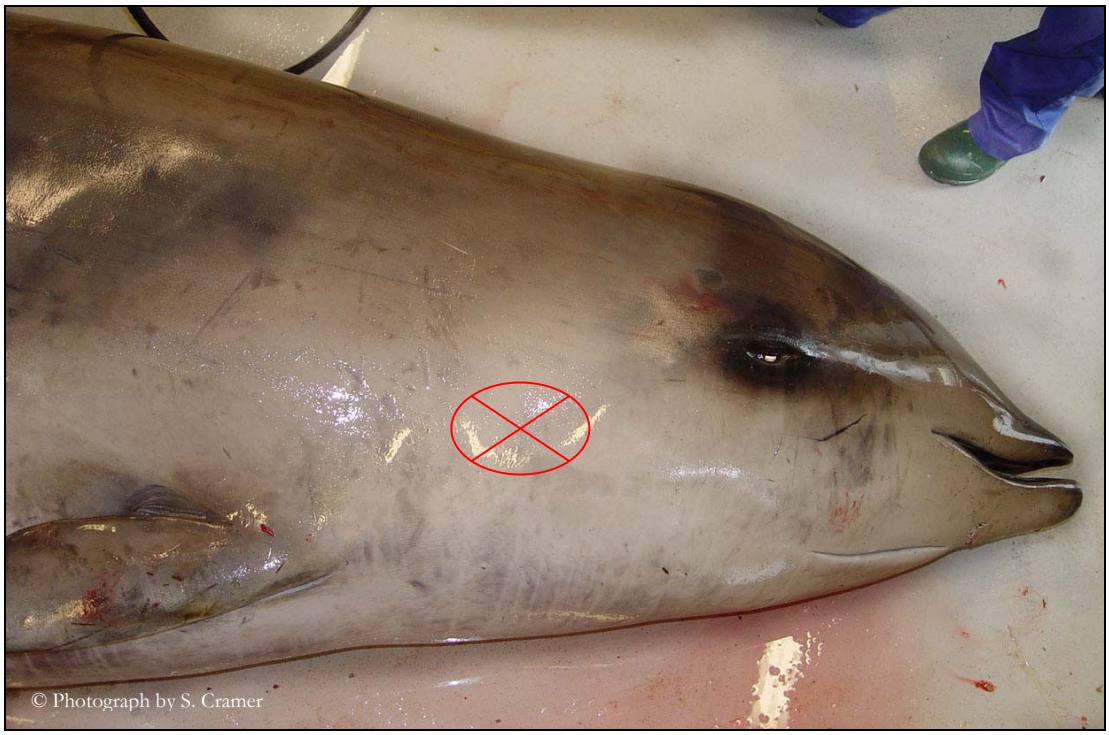


Figure 2. Left ear of a Cuvier's Beaked Whale (*Ziphius cavirostris*). © Photographs by S. Cramer.

The ears are located just behind and deep to the posterior edge of the lower jaw. To extract the ears using a lateral approach, locate by palpation the posterior edge of the jaw. Make an X-shaped incision about mid-way between the eye and the pectoral fin, with the midpoint of the X in line with the jaw (Fig. 3).

Figure 3. Incision location for a lateral ear extraction.



Bend the tissue flaps back and down. Probe straight in from the center of your incision until you feel a hard surface. That is the tympanic bone of the ear.

There is considerable soft tissue filling the cavity around the ear bones. Gently cut away the tissue with a scalpel or knife until you expose the tympanic. In a typical ziphiid, the tympanic will be ~ 40 - 50 mm in length and 30 mm wide. It resembles a conch shell, with a hollow interior that contains the middle ear bones (ossicles), a spongy soft tissue (corpus cavernosum), and the eardrum. The periotic bone, which contains the inner ear, is slightly smaller and is dorsal and medial to the tympanic. One of the hyoid bones generally attaches to the posterior/lateral edge of the tympanic by a cartilaginous cap. Cut this juncture with bone shears or a scalpel. On beaked whales there is also a thick sliver of bone loosely attached to the anterior margin of the tympanic.

The tympanic and periotic are partly fused to each other at the rear edge by a semi-fused (synosteotic) joint and at the lateral edge by a curved or sigmoid process, but the joints may be weak. Try to extract tympanic and periotic as a unit. If the tympanic separates from the periotic during removal or is loose, please be sure to extract both bones and be certain to get any ossicles that may have fallen from the middle ear.

Having cleared enough tissue to identify the two bones, you will need to free them by cutting or levering a posterior flange attached to the skull. Try to move the ear bones, looking for motion in the sutures of the skull posterior to the ear. It may help to scrape the soft tissue from the skull in this area. Place a chisel or screwdriver in these sutures and gently pound the wedge in with a hammer or mallet until you can lever the periotic and tympanic out of their cavity with an approximately 2 cm chunk of softer skull material attached at the posterior edge. You will now cut five to eight suspensory ligaments and the facial and auditory nerves located on the medial surface of the ear. Gently, rock the ear while cutting the attached soft tissue on the medial, anterior, and posterior surfaces with a narrow, sharp knife or scalpel. This is the most difficult part in that it is difficult to cut the tissues blindly. A narrow or curved

scalpel helps. Any attachments that are resisting removal should be cut with bone shears or pried loose with a small chisel, screwdriver or flat bladed instrument. Do not use a scalpel blade for this procedure. It will snap. Leave any soft tissue attached to the ear.

Fixation/Preservation

After removal, place the ears in fixative immediately. The best fixative is 10% buffered formalin. If concentrated formaldehyde liquid or formaldehyde powder is used, mixing it with sea water in lieu of distilled water or a chemical buffer provides sufficient buffering. If formalin is not available, 70% ethanol may be used. If the specimen is very fresh, and if you are comfortable with the anatomy, it is best to inject formalin through the round window with a 22-25 gauge needle. The round window is located at the posterior/medial edge of the periotic, ventral to the stapes (Fig. 2a). If you are not familiar with the anatomy, please do not attempt this injection. Also, please do not inject if only large bore needles are available. IF TRAUMA IS SUSPECTED, DO NOT INJECT at the round window, instead, insert the needle into the center of the VIIIth nerve and slowly inject formalin. If you are injecting the round window, insert the needle in the middle of the membrane approximately 3 mm and SLOWLY inject a small quantity of formalin. Be certain to record the location, needle size and fluid quantity injected in all cases and send with the other data for the animal's ears to the examiner.

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SHIPPING

Once the specimen appears well fixed, call the lab receiving the specimen to confirm that you are ready to ship and the day for shipment. If shipping to this lab, contact us by phone first at the numbers listed below.

On the day of shipping, wrap the specimen in several layers of formalin soaked gauze and place in three or more sealed plastic bags with an absorbent material such as diapers inside each bag to prevent leakage. If you use a jar, seal the jar with wax or waterproof tape and place it in a sealed plastic bag. Do not use glass containers. The important point is to preserve moisture around the ears without a large fluid volume and to have several leak proof seals. Place the packaged samples inside a cooler or reinforced box. The shipping container should be capable of withstanding a drop of at least three feet without damage.

Within the USA, ship by Federal Express or other expedited service for one or two day delivery. If sending from over seas, please use a method that will deliver within 7 days. You will also need to confirm with us any permit numbers that are required for domestic or international shipping for some species. Check with your shipper that formalin fixed, non-liquid samples are allowable and considered non-hazardous. If so, mark the container: Scientific Specimen – No Medical Hazard - Deliver Immediately.

Comments or Questions on Extraction Procedures:

We welcome your comments on this manual and will be happy to answer additional questions.

Contacts – For further questions please contact:

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Mobile: 774-836-5012
Fax: 508-457-2041
Email: dketten@whoi.edu or scramer@whoi.edu

Order Cetacea - Whales and Dolphins	
Suborder Odontoceti - Toothed Whales (Odontocetes)	
Superfamily Delphinoidea - Dolphins & small toothed whales	
Family Delphinidae - Dolphins (Delphinids)	
Subfamily Cephalorhynchinae	
Commerson's Dolphin	*Cephalorhynchus commersonii*
Black Dolphin	*Cephalorhynchus eutropia*
Heaviside's Dolphin	*Cephalorhynchus heavisidii*
Hector's Dolphin	*Cephalorhynchus hectori*
Subfamily Delphininae	
Longbeaked Common Dolphin	*Delphinus capensis*
Common Dolphin (Shortbeaked)	*Delphinus delphis*
Risso's Dolphin (Grampus)	*Grampus griseus*
Fraser's Dolphin	*Lagenodelphis hosei*
Atlantic Whitesided Dolphin	*Lagenorhynchus acutus*
Whitebeaked Dolphin	*Lagenorhynchus albirostris*
Peale's Dolphin	*Lagenorhynchus australis*
Hourglass dolphin	*Lagenorhynchus cruciger*
Pacific Whitesided Dolphin	*Lagenorhynchus obliquidens*
Dusky Dolphin	*Lagenorhynchus obscurus*
Pantropical Spotted Dolphin	*Stenella attenuata*
Clymene (ShortsnoutedSpinner) Dolphin	*Stenella clymene*
Striped Dolphin	*Stenella coeruleoalba*
Atlantic Spotted Dolphin	*Stenella frontalis*
Spinner Dolphin (Longsnouted)	*Stenella longirostris*
Bottlenose Dolphin	*Tursiops truncatus*
Subfamily Globicephalinae	
Pygmy Killer Whale	*Feresa attenuata*
Shortfinned Pilot Whale	*Globicephala macrorhynchus*
Longfinned Pilot Whale	*Globicephala melas (= melaena)*
KilIer Whale	*Orcinus orca*
Melonheaded Whale	*Peponocephala electra*
False Killer Whale	*Pseudorca crassidens*
Subfamily Lissodelphinae	
Northern Right Whale Dolphin	*Lissodelphis borealis*
Southern Right Whale Dolphin	*Lissodelphis peronii*
Subfamily Orcaellinae	
Irrawaddy Dolphin	*Orcaella brevirostris*
Subfamily Steninae	
Tucuxi	*Sotalia fluviatilis*
IndoPacific Humpbacked Dolphin	*Sousa chinensis*
Atlantic Humpbacked Dolphin	*Sousa teuszii*
Roughtoothed Dolphin	*Steno bredanensis*
Family Monodontidae - Narwhal and Beluga	
Beluga (White) Whale	*Delphinapterus leucas*
Narwhal	*Monodon monoceros*
Family Phocoenidae - Porpoises	
Spectacled Porpoise	*Australophocoena dioptrica*
Finless Porpoise	*Neophocaena phocaenoides*
Harbor Porpoise	*Phocoena phocoena*
Vaquita (Gulf of Calif. Harbor Porp.)	*Phocoena sinus*

Burmeister's Porpoise	*Phocoena spinipinnis*
Dall's Porpoise	*Phocoenoides dalli*
Superfamily Physeteroidea - Sperm Whales	
Family Physeteridae	
Pygmy Sperm Whale	*Kogia breviceps*
Dwarf Sperm Whale	*Kogia simus*
Sperm Whale	*P. catadon (= P. macrocephalus)*
Superfamily Platanistoidea - River Dolphins and Franciscana	
Family Iniidae	
Boutu (Boto, Amazon River Dolphin)	*Inia geoffrensis*
Family Lipotidae	
Baiji (Chinese River Dolphin)	*Lipotes vexillifer*
Family Platanistidae	
Ganges Susu (Ganges River Dolphin)	*Platanista gangetica*
Indus Susu (Indus River Dolphin)	*Platanista minor*
Family Pontoporiidae	
Franciscana (La Plata Dolphin)	*Pontoporia blainvillei*
Superfamily Ziphioidea - Beaked Whales	
Family Ziphiidae	
Arnoux's Beaked Whale	*Berardius arnuxii*
Baird's BeakedWhale	*Berardius bairdii*
Northern Bottlenose Whale	*Hyperoodon ampullatus*
Southern Bottlenose Whale	*Hyperoodon planifrons*
Sowerby's Beaked Whale	*Mesoplodon bidens*
Andrews' Beaked Whale	*Mesoplodon bowdoini*
Hubbs' Beaked Whale	*Mesoplodon carlhubbsi*
Blainville's Beaked Whale	*Mesoplodon densirostris*
Gervais' Beaked Whale	*Mesoplodon europaeus*
Ginkgotoothed Beaked Whale	*Mesoplodon ginkgodens*
Gray's Beaked Whale	*Mesoplodon grayi*
Hector's Beaked Whale	*Mesoplodon hectori*
Straptoothed [Beaked] Whale	*Mesoplodon layardii*
True's Beaked Whale	*Mesoplodon mirus*
Longman's Beaked Whale	*Mesoplodon pacificus*
Pygmy Beaked Whale	*Mesoplodon peruvianus*
Stejneger's Beaked Whale	*Mesoplodon stejnegeri*
Tasman (Shepherd's) Beaked Whale	*Tasmacetus shepherdi*
Cuvier's Beaked(Goosebeaked) Whale	*Ziphius cavirostris*
Suborder Mysticeti – Baleen Whales (Mysticetes)	
Family Balaenidae - Right Whales	
Bowhead Whale	*Balaena mysticetus*
Southern Right Whale	*Eubalaena australis*
Northern Right Whale	*Eubalaena glacialis*
Family Balaenopteridae – Rorquals	
Minke Whale	*Balaenoptera acutorostrata,*
Sei Whale	*Balaenoptera borealis*
Bryde's Whale	*Balaenoptera edeni*
Blue Whale	*Balaenoptera musculus*
Fin Whale	*Balaenoptera physalus*
Humpback Whale	*Megaptera novaeanglia*
Family Eschrictiidae - Gray Whale	
Gray Whale	*Eschrichtius robustus*
Family Neobalaenidae - Pygmy Right Whale	
Pygmy Right Whale	*Caperea marginata*
Order Carnivora - Carnivores (in part)	
Family Mustelidae - Otters (Mustelids, in part)	

Sea Otter	*Enhydra lutris*
Marine Otter	*Lutra felina*
Family Odobenidae - Odobenids	
Walrus	*Odobenus rosmarus*
Family Otariidae - Eared Seals (Otariids)	
Subfamily Arctocephalinae - Fur Seals	
South American Fur Seal	*Arctocephalus australis*
New Zealand (W. Australian) Fur Seal	*Arctocephalus forsteri*
Galapagos Fur Seal	*Arctocephalus galapagoensis*
Antarctic (Kerguelen) Fur Seal	*Arctocephalus gazella*
Juan Fernandez Fur Seal	*Arctocephalus philippii*
South African & Australian Fur Seal	*Arctocephalus pusillus*
Guadalupe Fur Seal	*Arctocephalus townsendi*
Subantarctic (Amsterdarn I.) Fur Seal	*Arctocephalus tropicalis*
Northern Fur Seal	*Callorhinus ursinus*
Family Phocidae - True = Earless = Hair Seals (Phocids)	
Subfamily Monachinae - Monachids	
Leopard Seal	*Hydrurga leptonyx*
Weddell Seal	*Leptonychotes weddellii*
Crabeater Seal	*Lobodon carcinophagus*
Northern Elephant Seal	*Mirounga angustirostris*
Southern Elephant Seal	*Mirounga leonina*
Mediterranean Monk Seal	*Monachus monachus*
Hawaiian Monk Seal	*Monachus schauinslandi*
Caribbean (W. Indian) Monk Seal	*Monachus tropicalis (Extinct?)*
Ross Seal	*Ommatophoca rossii*
Subfamily Otariinae - Sea Lions	
Northern (Steller) Sea lion	*Eumetopias jubatus*
Australian Sea lion	*Neophoca cinerea*
South American (Southern) Sea Lion	*Otaria byronia (= O. flavescens)*
Now Zealand (Hooker's) Sea Lion	*Phocarctos hookeri*
California, Galapagos, and Japanese (extinct) Sea Lion	*Zalophus californianus*
Subfamily Phocidae - Phocinids	
Hooded Seal	*Cystophora cristata*
Bearded Seal	*Erignathus barbatus*
Gray Seal	*Halichoerus grypus*
Harp Seal	*Phoca (Pagophilus) groenlandica*
Caspian Seal	*Phoca caspica*
Ribbon Seal	*Phoca fasciata*
Ringed Seal	*Phoca hispida*
Spotted (Largha) Seal	*Phoca largha*
Baikal Seal	*Phoca sibirica*
Harbor (Common) Seal	*Phoca vitulina*
Family Ursidae - Bears (in part)	
Polar Bear	*Ursus maritimus*
Manatees & Dugongs (Sea Cows) - Order Sirenia	
Family Dugongidae - Dugongs	
Dugong	*Dugong dugon*
Steller's Sea Cow	*Hydrodamalis gigas*
Family Trichechidae - Manatees	
Amazonian Manatee	*Trichechus inunguis*
West Indian (Florida, Carib.) Manatee	*Trichechus manatus*
West African Manatee	*Trichechus senegalensis*