Experimental Measures of Blast and Acoustic Trauma in Marine Mammals

Final Report

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ABSTRACT
Blast traumas are essentially mechanical responses, therefore blast effects are inducible and measurable in post-mortem specimens. To determine onset of damage zones for blast trauma in marine mammals, fresh post-mortem specimens were implanted with pressure gages, CT scanned, and exposed to underwater blast pressures of 10-300 psi. Following exposures, specimens were rescanned and necropsied by a team of blast pathology specialists blinded to test pressures. All procedures were documented by UW video and still photography. The results show severity and type of impacts are mass-dependent and correlated with received psi. Classic blast damage was found in all tissues. Some organs unique to cetaceans have distinct damage patterns that may be diagnostic in UW blast cases. For the smallest species, safe margins are in the 10-12 psi received pressure range; for larger species the ranges can be 20-25 psi. Important issues remaining to be tested include near field vs. far field loading effects, exponential vs sinusoidal bursts, and synergistic effects of rate of pressure increase, peak pressure, waveform and duration.

LONG-TERM GOALS

Although marine mammal middle and inner ears are similar to those of land mammals, there are sufficient differences that marine ear damage mechanisms continue to be a hotly debated topic. To date there are surprisingly few direct investigations of marine mammal ear fragility. This project has an immediate, overarching goal of characterizing intense pressure impacts in marine mammal ears. The data provide peak pressure correlates for trauma to assist in determining the distances or zones that provide a functional safety margin for marine mammals in the vicinity of an explosive source. The project also has a second goal which is to provide new, fundamental information about mechanical responses of marine adapted ears that will improve our understanding of mammalian middle and inner ear pressure transduction mechanisms. The study addresses a critical Navy need for explicit data to assign mitigation zones for ship shock and other underwater high velocity explosive tests and procedures.

OBJECTIVES

The explicit objective of this research is to determine the range of impacts toothed whales may sustain in relation to received pressures in when exposed to intense pressure sources. Ears are the bellwethers of pressure induced damage. Equally important, they are a crucial sensory system for marine mammals. Therefore, understanding the nature and parameters of impacts on marine mammal ears as well as traumas to other organs correlated with received peak pressures will provide a marine specific metric for determining blast and impulse noise exposure mitigation zones.

APPROACH

Although responses in live animals would clearly give us the most accurate measures of damage and recoverability from blast exposures, for many reasons, such measures are not feasible. However, post-mortem ears, given proper handling and preservation, have *mechanical* responses that are isomorphic with those of live ears (Fig. 1. Rosowski *et al.,* 1990). Hearing loss and auditory system trauma from blasts and from intense, short-term impulsive sources depend fundamentally upon mechanical responses of the middle and inner ear components. The majority of such effects are inducible, replicable, and measurable post-mortem.

Marine and land mammal ears differ in their robustness, stiffness, mass, vascularization, and pneumatization characteristics. The most extreme of marine mammal ears are those of cetaceans; i.e., whales and dolphins. Because they evolved ears that are fully adapted to the reception and transduction of underwater sounds, it is inappropriate to assume we can extrapolate whale auditory system responses to blasts from data on land mammal ear responses to air-borne blast waves or impulse noise. By applying the techniques developed for cadaveric ears noted above, we can measure blast trauma effects in post-mortem specimens of cetaceans. The experiments underway in this research therefore are designed to provide direct measures of the trauma and pressure responses of cetacean tissues and of the mechanical changes in whale and dolphin auditory systems from received peak pressures from explosive sources.

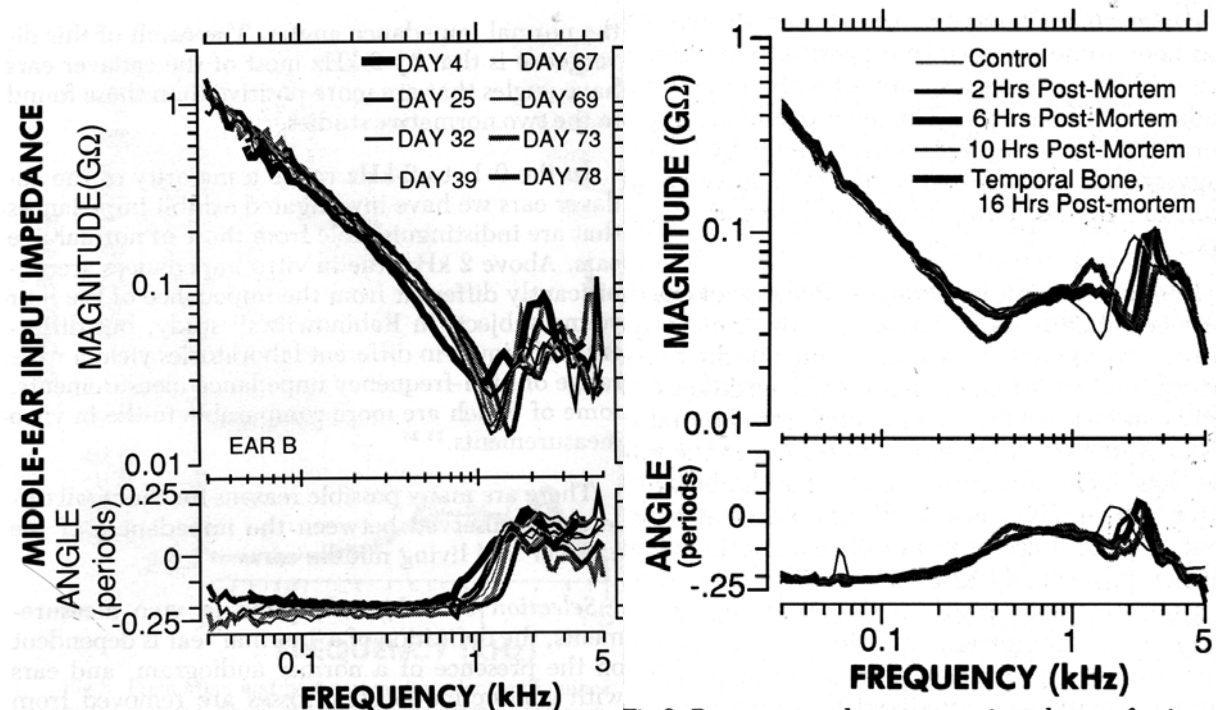

Fig 4. Eight in vitro input impedance measurements made in same cadaver ear over period of 74 days. Key notes days postmortem of each measurement. Largest changes occur near impedance minimum at time of 32-day measurement.

Fig 8. Premortem and postmortem impedances of guinea pig middle ear. Small changes are apparent in some frequency ranges. Largest changes occur immediately after death in 1- to 2-kHz range and after removing temporal bone at 16 hours postmortem.

Fig. 1. Immitances and impedances measured in live and postmortem guinea pig and human ears. (Rosowski et al., 1990)

There are three principal steps that are involved in determining how peak pressures impact cetacean ears: selection and preparation of fresh post-mortem specimens, blast exposure of the prepared specimen, and comprehensive necropsy and measurement of the blast effects.

For the first component, carcasses of stranded marine mammals that are euthanized for medical reasons are obtained within four hours post mortem. They are then examined by computerized tomographic scanning (CT) to assure normal ear structures and intact head and body cavity organs. Scans are conducted at Mass. Eye and Ear Infirmary/Mass. General Hospital Dept. of Radiology or Woods Hole Oceanographic Institution's Ocean Imaging facility, under Dr. Ketten's supervision. The CT units employed are both Siemens Volume Zoom High Resolution Spiral scanners funded through ONR and DURIP programs.

The ears and post-cranial organs that are susceptible to pressure damage are then implanted with pressure gauges. The implanted, intact specimen is transported to the Carderock Undersea Warfare Center and immersed in the blast test pond for exposure to a single blast designed to deliver received pressures ranging from 300 to 0 psi. Blast tests are designed and conducted by a team under the direction of James Craig. The animal is then re-examined post blast by CT at the Smithsonian Institution or at Walter Reed Army Hospital's Radiology Dept. in order to document gross tissue changes in situ and transported to the Walter Reed Army Institute for Research/Naval Medical Research Center (WRAIR/NMRC) surgical suite for a complete necropsy to visually assess gross structural damage and for sampling of tissues to be further assessed at the cellular and infrastructural level. Necropsies are conducted by a team comprising Dr. Ketten; Mr. Scott Cramer, a marine mammal specialist; Dr. Joy Reidenberg, a marine mammal laryngeal specialist; Dr. Sherman McCall, an AFIP blast and clinical pathologist; Dr. Virginia Naples, a comparative anatomist specializing in abdominal anatomy; and Dr. Dale Dunn, an AFIP veterinary pathologist. The necropsy team, with the exception of Dr. Ketten and Mr. Cramer, do not know the received or test pressures on any animal they examine to avoid bias in their assessments.

All stages of the experiments were video or still photo documented and blast sequences were filmed using ultra-high speed video to confirm the position and overt reaction of the specimen to the pressure wave as well as to graphically document pre and post-exposure external condition of the specimen. All necropsies were documented on film and by digital camera. Although auditory system effects are the focus of the exam, all major organs were examined and documented.

WORK COMPLETED

The following experiments were completed during the funded years:

1) Four preliminary test pond mapping shots were performed to test the resilience of the specimen suspension system and to confirm received pressure model simulations for expected specimen placements within the test pond.
2) 2) Two specimen simulation tests utilizing gages implanted in hams and two simulated cetacean ear tests were performed to confirm *in situ* gage integrity when implanted in soft tissues, in flexible air cavities, and at bone-soft tissue interfaces. For the latter tests, four pseudo-cetacean ears were constructed of acrylic shells equivalent to the volumes of small delphinid and larger baleen ears. Each shell was filled with varying combinations of flexible or semi-rigid walled air-cavities (balloon catheters or acrylic chambers), hydrated soft tissues (calf livers), and air only chambers.

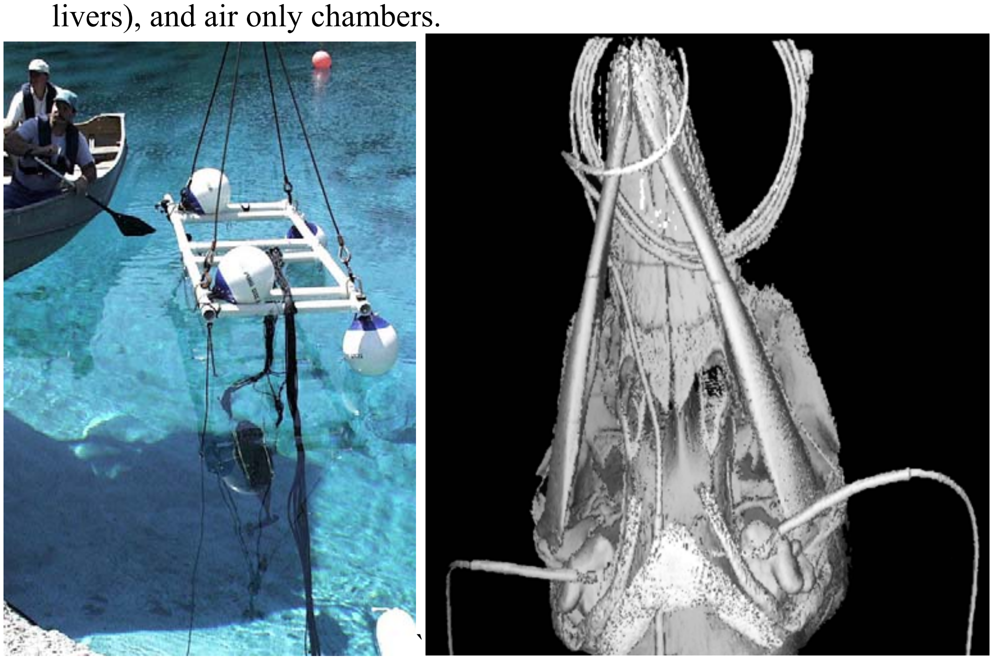

Fig. 2. Left: Instrumented specimen suspended in test pond. Right: 3D reconstruction of post-exposure CT scan documenting peribullar (middle ear) gage positions.

3) Twenty actual tests of porpoise and dolphin post-mortem specimens were conducted with gages implanted in the ear, nares, pharyngeal, esophagus, lung, and hypaxial musculature as well as a full suite of external gages placed alongside the ear region and ventrally (Fig. 2).
4) Following implantation, the test animals were suspended in the test pond and subjected to a pre-determined blast pressure or, as controls, to no blast exposure, removed from the pond, transported for CT examination (30 -50 mins. post blast), and then necropsied at the Walter Reed Army Institute for Research (onset of exam 1-1.5 hours post-blast, completed 6-10 hours post-blast). The specimens tested were all odontocetes from five common Atlantic odontocete species (delphinids and phocoenids) and ranged from 15.5 kg to 87.3 kg (Table 1). They were tested at 300 psi (2 animals), 200 psi (2), 100 psi (2), 50 psi (4), 25 psi (3), and 10 psi (4) received pressure at the animal's surface (Fig. 3). Where possible, one specimen was tested at each received psi that was chosen to be 50 % the weight of the maximum weight test animal at that pressure. In addition, three control animals were processed through all stages to provide controls for handling artifacts. For the controls, the animal is handled identically as a test specimen through all stages and with consistent timing of scanning, thawing, rigging, immersion, removal, transport, post scanning, and necropsy with the exception that no explosion is employed. High received pressures were purposely chosen for initial tests to confirm that conventional blast effects could be found postmortem and to determine how well the gages would respond in actual tissues

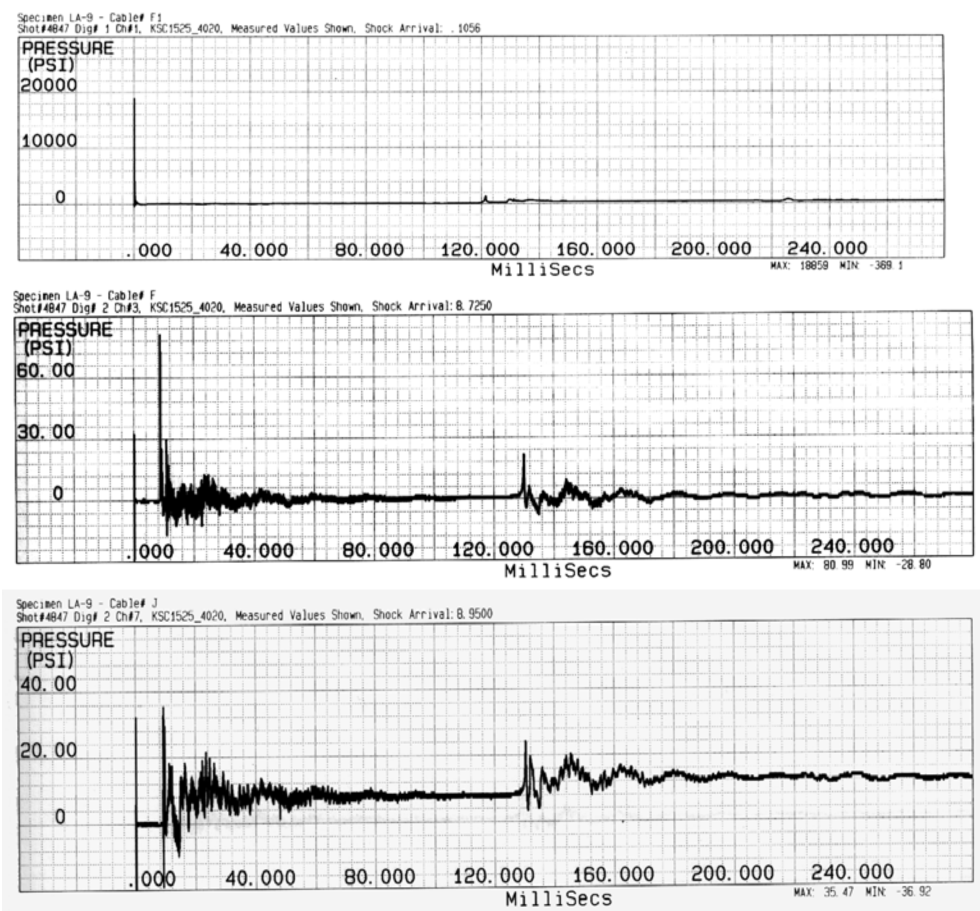

Fig. 3. Sample records from 3 gages.
Top: at explosive source

Middle: implanted peribullar (at middle ear level)

Bottom: implanted gage at hypaxial muscle near dorsal fin

After the demonstration of substantial trauma in high pressure exposures, pressures were randomized for subsequent trials to avoid any obvious sequencing in the necropsy observations. More replicates were required at 50, 25, 10, and 0 psi to refine judgments and observations of trauma correlates at these more critical (for mitigation) pressures and to clarify transition points amongst species for lethality, recoverable injury, permanent, temporary, and no significant functional auditory damage. These lower pressure groups probably represent non-lethal injury impact zones for most marine mammals, but it is necessary to investigate them with additional variations in species and mass because of the critical nature of the decisions for which these data may be used. Four Kemp's Ridley turtles were tested also as a proof of concept under joint funding with NMFS to determine applicability for future, similar tests on sea turtles.

TABLE 1*

(Weight kg) 15.5 - 87.3

Specimens n=20	**m=9**	**f=11**	**psi received 0-300**
Delphinus delphis		**72**	**0**
Phocoena phocoena		**26.9**	**0**
Phocoena phocoena		**22.2**	**0**
Lagenorhynchus acutus	**85.4**		**10**
Lagenorhynchus acutus		**73.2**	**10**
Lagenorhynchus acutus	**51.5**		**10**
Phocoena phocoena		**25.2**	**10**
Phocoena phocoena		**25.3**	**25**
Phocoena phocoena		**21.6**	**25**
Phocoena phocoena		**15.5**	**25**
Stenella coeruleoalba	**69.1**		**50**
Phocoena phocoena		**60.2**	**50**
Lagenorhynchus acutus		**48.5**	**50**
Lagenorhynchus acutus	**38**		**50**
Phocoena phocoena	**39.6**		**100**
Phocoena phocoena	**24.9**		**100**
Lagenorhynchus acutus	**57.5**		**200**
Phocoena phocoena		**29.3**	**200**
Lagenorhynchus acutus	**87.3**		**300**
Phocoena phocoena	**24.7**		**300**

*In addition to these specimens, 6 sea turtle were tested using the same procedures. Those results are not included in this report.

RESULTS

The results of the blast tests to date are as follows:

(1) Tests without target specimens confirmed pressure simulations for the blast test pond are correct for pressures down to 25 psi and showed that the suspension rigging would withstand the test pressures anticipated without compromising received psi measurements.

(2) Simulated ear and tissue tests provided data for improvements in gage design and acted as trials for video equipment placement. These preliminary tests were required because the rarity and delicacy of appropriate postmortem specimens requires optimizing all recording equipment parameters prior to actual tests.

(3) In order to maintain the same source charge type and weight require, a greater standoff distance than the NSWC test pond can accommodate is required for the 25 and 10 psi tests. Consequently, those tests were conducted at a DoD contracted quarry in Lynchburg Virginia. An underwater rover equipped with video is used to confirm specimen position for the quarry tests. The major concern with

employment of the quarry is that a longer post-test transport is required than for the NSWC tests. This issue was addressed by comparing necropsy results for animals processed both through quarry based control tests and through lower charge weight tests at NSWC.
(4) Actual specimen tests were completed for two or more animals for all basic pressures originally proposed. All specimen necropsies were performed and documented by cetacean anatomists and AFIP-trained forensic and blast pathologists who were not privy to the received levels. Based on retrospective analyses of their "blind" observations, there are clear gradations of damage to multiple tissue suites that are related to both animal mass and received pressures. Necropsy findings for the specimens examined to date show distinct and unequivocal damage consistent with - and only with - blast effects. These results are summarized in Table 2.

Table 2 - TRAUMA MATRIX

S = Severe M = Moderate mi = mild - = absent

Organ	300		200		100		50				25			10				C
psi/animal weight (kg)	25	87	29	58	25	40	38	49	60	69	16	22	25	25	52	73	85	all
Bone	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Blubber	S	-	mi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Intestine	S	mi	mi	mi	mi	mi	mi	mi	mi	mi	mi	mi	mi	mi	mi	mi	mi	mi
Liver	S	mi	mi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Kidney	S	-	M	mi	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Brain	S	-	M	-	mi	-	-	-	-	-	-	-	-	-	-	-	-	-
Melon	S	-	M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lung	S	M	M	mi	mi/M	mi	mi	mi	mi	mi	mi	-	-	mi	-	-	-	-
Larynx	S	mi	M	M/mi	mi		mi	mi	mi	mi	mi	mi						-
Jaw Fat	S	M	S	M	M	mi	M	M	mi	mi	-	-	-	-	-	-	-	-
Mid-Ear	S	M	S	S	S	M	M	M	M	M	mi	-	-	mi	-	-	-	-
Inner Ear	S	S	S	S	S	S	S	S	S	S	M	M	M	mi	-	mi	-	-

It should be noted that the presence of dark, intraluminal material throughout the large intestine and portions of the small intestine was a finding common to all specimens (Intestine "mi" in Table 3). It was only in the smaller specimen challenged with a 300 psi received exposure that a clearly demarcated segmental gut hemorrhage like that reported in humans was documented. The mild notation reflects observations of confounding material in the gut which was found on histology to be digested material with pigmentation affected by bacterial degradation post-mortem as well as the presence in many specimens of squid tissues and ink. These observations were deemed unrelated or at least unreliable signs for blast injury.

Serious injuries sustained by all specimens at high psi (100-300 psi received), included liver disruption and hemorrhage, classic blast lung, laryngeal hemorrhage, segmental gut hemorrhages, cerebral ventricular inflation, intraorbital hemorrhages, middle ear ossicular fractures, and inner and middle ear hemorrhage. The majority of these traumas were profound and would likely be mortal for exposures over 100 psi received (Fig.4, Tables 2, 3). Most are consistent with classic blast damage reported from land mammal experiments and human combat or civilian explosions (Fig. 4).

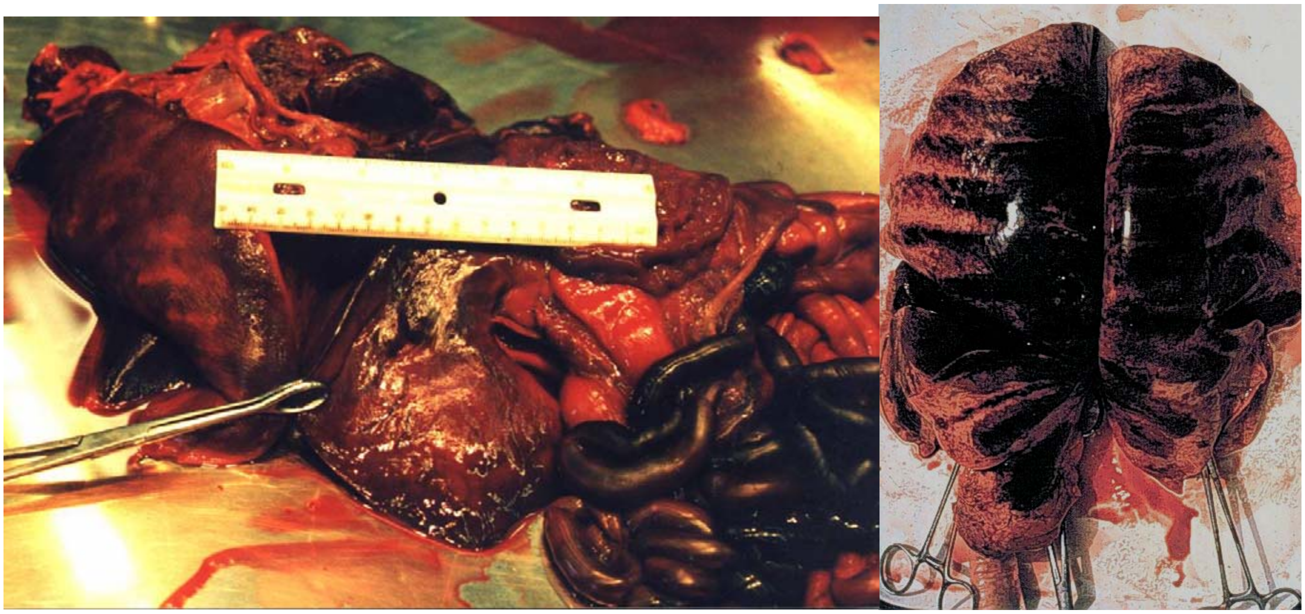

Fig. 4A. Blast Lung
Left: Hemorrhages on the lateral lung surface (top left of left image) and segmental gut hemorrhage (blackened intestine loops, lower right of left image) in a harbor porpoise *(P. phocoena)* that received > 100 psi internal peak pressure based on an intrarectal gage. Right: Lungs taken from a sheep exposed to an air blast (Sharpnack et al. 2006). Both specimens exhibit "rib markings" that characterize blast lung and must be differentially diagnosed to avoid erroneous reports in strandings (see discussion below).

It is important to note that hemorrhagic rib markings from blast have a distinct pattern that differentiates them from striped lividity marks in lungs that appear commonly in animals that strand and die in a prone position. In stranded animals, the darkened regions coincide with the ribs and result from the compression of the tissues medial to the ribs as the weight of the body presses against them and the subsequent pooling of blood in these tissues. In blast lung, the "rib markings" is a terminology that is conventional but somewhat misleading. The darkened areas in the case of blast are actually found in the intercostal tissue spaces and result from greater compression of the intercostal tissues which are less protected than tissues shadowed by the ribs. Thus, blast darkened stripes are found intercostal, NOT subcostal. To differentiate and diagnose blast lung, it is thus imperative to document and verify in any stranded animal the relationship of ribs to striping and not simply the presence of lung striping alone. An important correlative finding also present in the test specimens was the presence of
periodic, parenchymal subpleural pulmonary hemorrhage, which are believed to be the result of differential compressions coincident with the foci from the shock wave reflections.

Other airway tissues were also compromised in the higher blast pressure cases, including laryngeal (Fig. 4B) ligature and in a few cases, pharyngeal contusions.

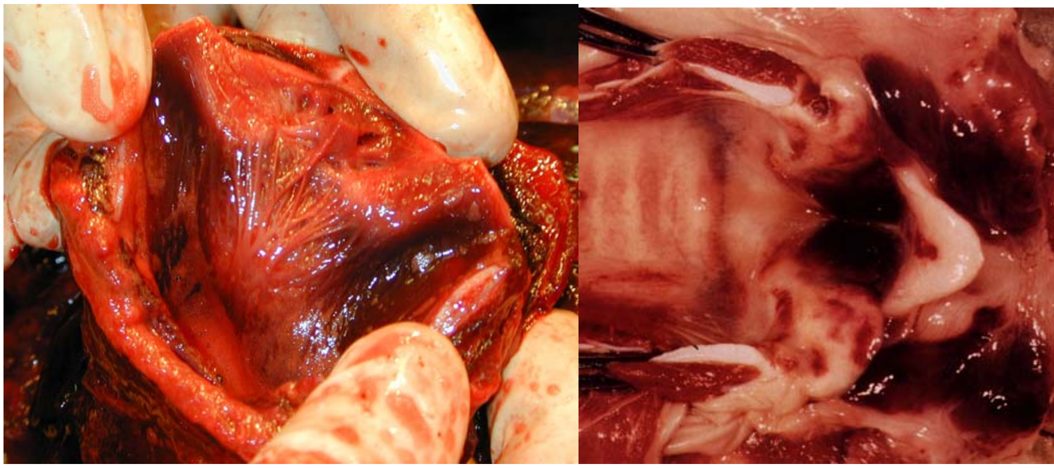

Fig. 4B. Left: Larynx of a porpoise that was exposed to 170 psi internal. Right: Larynx of a sheep that received 200-300 surface psi (Sharpnack et al., 2006).

Cetacean specific traumas were also found, most notably a distinctive ring hemorrhage found at the interface of the two blubber layers that may be diagnostic for the direction of the received blast wave, hemorrhages of the acoustic jaw fats, and melon hemorrhages. The ring hemorrhage phenomenon is particularly interesting since it demonstrates the importance of tissue density frontiers in determining the relationship of the pattern of hemorrhage with regard to the blast wave parameters. In this case the compressive wave results in blood vessel hemorrhage and the rarefactive wave produces a migration of sero-sanguinous fluid outward up to the point that the hemorrhage front encounters the change in density between the two blubber layers. Consequently, a concentric "ring" develops concentrated at and demarcating the density interface (Fig. 5).

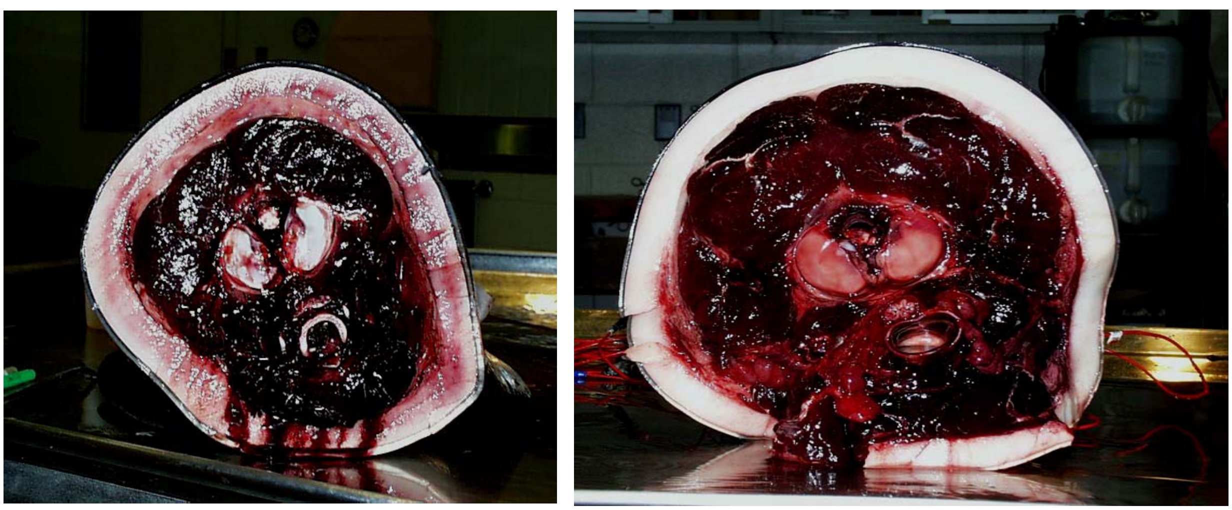

Fig. 5. Ring Hemorrhage is present in the left image in the blubber of a harbor porpoise exposed to *P.* 200 psi peak received surface but absent in the right image of a significantly larger Atlantic white-sided dolphin ***(L. acutus)*** exposed to an equivalent pressure. Ring hemorrhages were also not seen in a porpoise exposed to 100 psi surface peak pressure.

The most significant data for the primary goal of this research were obtained from the inner ear histologic exams and associated gage pressure records. Injuries ranging from severe to mild were found in all cases (Fig. 6) that received >25 psi peak recorded in the near ear or peribullar gages. All specimens exposed to <10 psi internal and 10 psi surface had no detectable mechanical trauma to the inner and middle ears (Fig. 6).

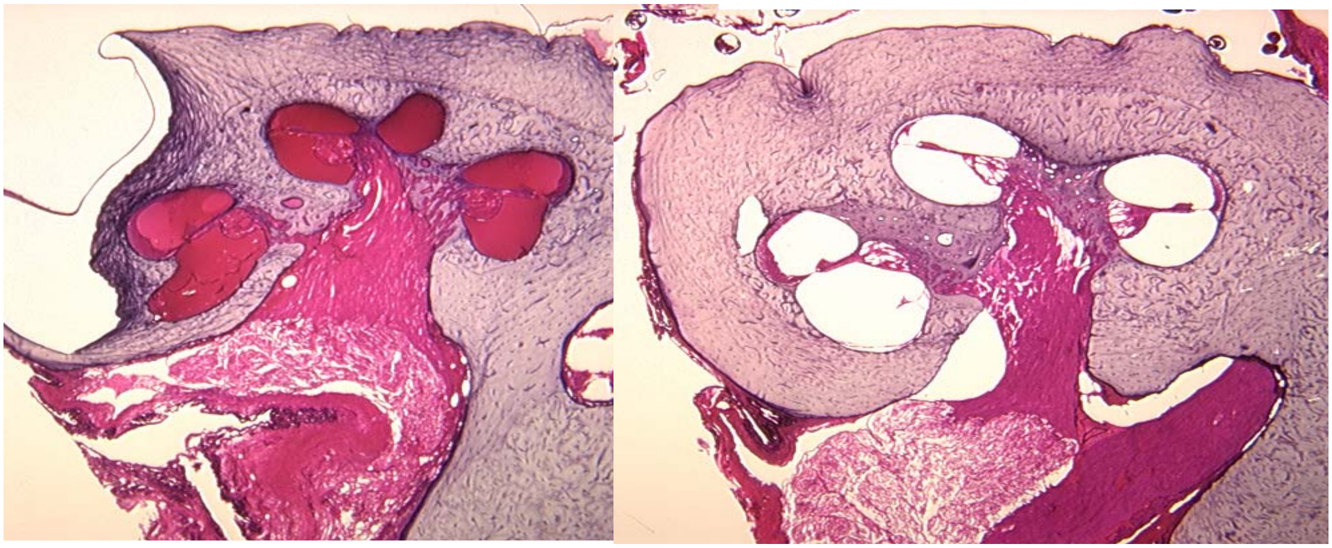

Fig. 6. The inner ears of two specimens are shown, both of which were verified through CT scanning to have normal, uncompromised inner ears prior to blast testing. Left: Histologies of the inner ear of a harbor porpoise post-exposure to 100 psi peak pressure external shows evident hemorrhage throughout the cochlea. Right: The inner ear of a second porpoise that received a 10 psi peak pressure exposure has an intact and fully normal inner ear with no evident trauma.

CONCLUSIONS

A trend emerged suggesting that the severity and number of impacted tissues clearly decreases with decreased psi but injuries are inversely proportional to mass; i.e., smaller animals sustain greater trauma than larger at equivalent received psi (Table 3). Further, the same suite of trauma will, on average, occur in animals of double the mass at double the psi compared to a smaller mass of the same or similar species. This is consistent with mass-dependent predictions from previous studies on land mammals. None of these traumas were reported for any control specimen.

A cascade of impacts (Table 3) was found to range from lethal to no significant impact that was consistent with received peak pressure. The severity of the injury at equivalent exposure was dependent upon body mass. The majority of injuries were consistent symptomatically with air blast injury (e.g., blast lung, liver lesions, segmental gut hemorrhage, inflated cerebral ventricles, hemorrhage), however, some injury patterns were related to cetacean specific anatomy (ring hemorrhage).

The current results also suggest that different elements of the shock wave and its effects may preferentially impact or exacerbate impacts on certain tissue groups. Examples of this are traumas to the lung and middle ear which appear to behave differently according to air to soft vs. soft to consolidated tissue proportions that exist pre-exposure. There are also issues about near and far-field effects that cannot be addressed based upon this experimental paradigm. Both of these questions can best be addressed by employing a tapered charge in lieu of the charges we have employed in order to limit measures to controlled, received peak pressure. Consequently, now that we have in hand a data set that addresses peak pressure effects, it is recommended, in order to fully understand all elements of impact scenarios and mechanisms, that this series of experiments be followed by a parallel series using tapered charges.

Table 3: CETACEAN BLAST TRAUMA CASCADE

SEVERE DAMAGE	
300 psi	***Ring blubber hemorrhage***
	Orbital blowout - fractures
	Temporal bone fractures
	Ossicular fractures
	G-I hemorrhage - perforation
	Kidney - Liver - Spleen ruptures
200 psi	***Melon hemorrhage***
	CNS-Pulmonary emboli
	Orbit/Retinal emboli
	Intraocular hemorrhage
100 psi	**Airways - Edema/hemorrhage**
	Mandibular fat hemorrhage
	RW/OW rupture- Laryngeal
50 psi	**Middle/Inner ear hemorrhage**
25 psi	***Mild damage peribullar***
10-12 psi	**AUDITORY SYSTEM INJURY LIMIT**

In summary, the test pond has been fully mapped; all apparatus tests were completed; and twenty full specimen tests were completed with replicates at each of the major pressures proposed. The specimen tests show graded damage that is inversely related to specimen mass (Figure 7). Suites of damage (number of organs involved, severity, etc) are consistent with received pressure and orientation of the specimens. In addition, some organs, including blubber, jaw fats, and melon, which are unique to cetaceans, are differentially impacted based on received psi and may serve as diagnostic correlates for blast injuries. We expect that not only will these experiments provide conservative estimates of auditory trauma, but also that the data may provide a basis for calculating continual dose-damage curves for multiple marine species.

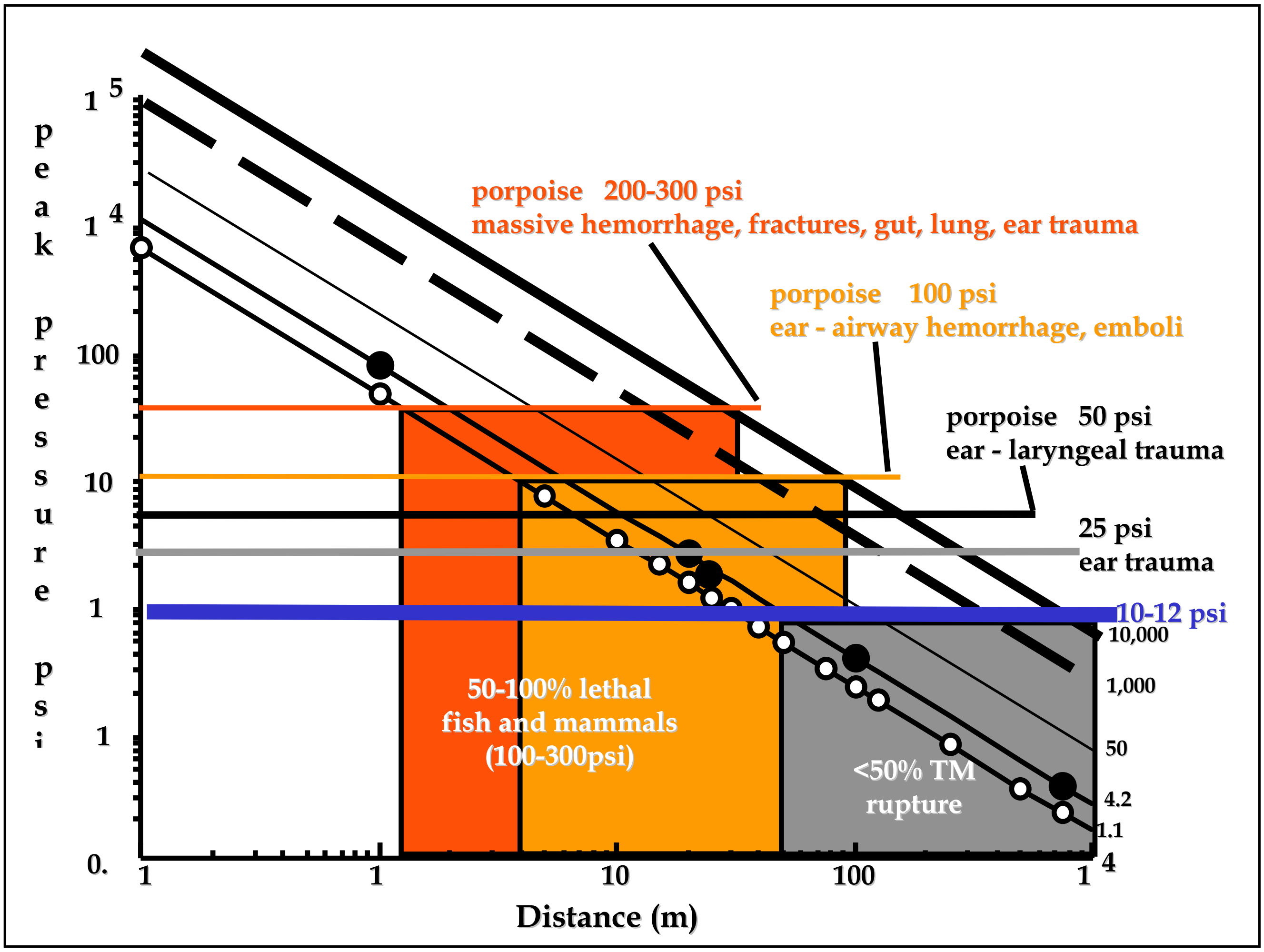

Figure 7 – Theoretical trauma transition zones derived from received peak pressure in cadaveric cetaceans compared with published data on trauma vs estimated received psi in other species in air and water.

IMPACT/APPLICATIONS

The Navy is required to mitigate impacts on marine mammals from blasts for ship shock trials as well as other explosive and impulse sources. Currently, mitigation zones are set by inference from land mammal experiments because we lack explicit data on pressure effects in marine mammals. As noted above, ears are vital sensory organs that are also primary indicators for pressure damage. By directly measuring and monitoring pressure damage in marine mammals and determining the endpoints for pressure-induced trauma, this project will provide the navy with needed baseline data for estimating accurately aquatic mitigation zones.

TRANSITIONS

Until the completion of control and all repeat experimental trials, these results are considered preliminary. The results to date have been reported informally to ONR and N45 and have been presented at international scientific meetings including the Acoustical Society and the Biennial Meeting for the Marine Mammal Society. Upon completion, a full report will be released in addition to publication of the results by conventional, peer reviewed publication. The published data are expected to contribute documentation for environmental impact statements and for actual planning of ship shock trials.

RELATED PROJECTS

A project at Aberdeen was also underway during the funding period which referred to this experimental method and specimen preparation procedures for designing a parallel human cadaveric study and for development of a computerized finite element model of blast trauma.

SUMMARY

Ears are the most pressure sensitive mammalian tissues, but cetacean and land mammal ears differ in stiffness, mass, vascularization, and pneumatization. Therefore, we cannot arbitrarily extrapolate underwater mitigation zones from land mammal responses. Rosowski et al, (1990, Ann. Otol. Rhin., Laryn.) showed live and post-mortem ears have isomorphic mechanical properties. Blast traumas are essentially mechanical responses, therefore blast effects are inducible and measurable in post-mortem specimens. Our objective is to determine at what pressure frontier we find no evidence of inner ear trauma; i.e., what pressure defines the “onset of damage zone” for underwater explosions in marine mammals and sea turtles.

In this study, fresh post-mortem specimens were subjected to controlled blasts to determine how mechanical damage to ears and major organs varies with received peak pressure. Stranded dolphins and porpoises that died naturally in rehabilitation centers or were euthanized for medical reasons were submitted as test subjects for this research. The fresh “cadaveric” subjects were implanted with pressure gages, CT scanned, and exposed to underwater blast pressures of 10-300 psi. Following exposures, specimens were rescanned and necropsied by a team of mammal pathology and blast specialists who were blinded to test pressures. The blast pathologies are being reviewed by AFIP personnel who work on human blast trauma as well. All experimental procedures were documented by UW video and still photography at the NSWC, Carderock, MD.

Analyses of test and control specimens show both the severity and number of impacts are mass-dependent and correlated with received psi in dolphins and porpoises. Classic blast damage was found in all organ suites in the abdomen and head. Further, some organs unique to cetaceans; e.g., blubber, jaw fats, and melon, have distinct damage patterns that may provide diagnostic markers in suspected UW blast cases. The data show for the smallest cetacean species, the harbor porpoise, that safe margins are reached in the 10-12 psi received pressure range although for larger species these ranges may increase to 20-25 psi.

Further, it is important to note that these data reflect only received peak pressure. Now that the model has been demonstrated to be operational, several important issues can be tested for a comprehensive analysis of blast effects. Key elements that can be tested include:

Near field vs. far field loading effects,
Exponential vs sinusoidal bursts
Contributory effects of rate of pressure increase, peak pressure, waveform and duration

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