Report of the Workshop on Acoustic Resonance as a Source of Tissue Trauma in Cetaceans. April 24 and 25, 2002, Silver Spring, MD

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Disclaimer:

Workshop participants and sponsors intend this document to serve as the basis for planning and follow-up actions only. This report summarizes discussions among scientists about processes that may cause beaked whales to strand in the presence of active sonar, and the research projects and other actions that are most needed on this subject. This report should not be cited as peer-reviewed literature and should not be used either as a comprehensive source of facts about stranding events, or as the basis for government policy or regulations. This report represents current thought about a dynamically changing issue. It was reviewed by all participants, and where different opinions or unresolved issues occurred, these have been noted.

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Executive Summary:

The purpose of this workshop was to discuss evidence for the possible mechanisms by which mid-frequency range (2-10 kHz) active sonar may lead to strandings of beaked whales. This workshop focused on the March 2000 stranding of beaked whales in the Bahamas because it produced the best data, but the workshop results apply to other sonar-related stranding events. The most widely discussed hypothesis was that stranding may have resulted from air cavity resonance caused by exposure to mid-frequency active sonar, or to a source with similar operating characteristics (i.e. similar source levels, frequencies, etc.). This group of scientists was specifically chosen to discuss the validity of that hypothesis. Several proponents of that hypothesis were present, and began the meeting by reporting that their own more recent analyses had failed to support the hypothesis. Other participants presented information from experiments with laboratory animals and modeling work that also failed to support the hypothesis.

Workshop participants concluded that resonance in air-filled structures was not likely to have played a primary role in the Bahamas stranding for the following reasons: 1) tissue displacements at resonance are estimated to be too small to cause tissue damage; 2) acoustic pressure attributable to resonance is orders of magnitude less than the ambient pressures during diving; 3) tissue-lined air spaces most susceptible to resonance are too large in marine mammals to have resonant frequencies in the ranges used by either mid- or low frequency sonar; 4) lung resonant frequencies increase with depth, and tissue displacements decrease with depth, so if resonance is more likely to occur at depth it is also less likely to have an effect there; 5) based on experiments with terrestrial mammals, tissue damage is estimated to require higher exposure levels than most wild animals would receive from sonar, especially at the depths where lung resonant frequencies would match the sonars being used; 6) based on terrestrial mammals, the time required for acoustically-induced vibrations to damage tissues is usually longer than animals would experience from short (1 sec) sonar pings; 7) lung tissue damage has not been observed in any mass, multi-species stranding of beaked whales. However unlikely resonance effects may currently seem, the hypothesis is best tested through empirical studies. Suggested studies include measurement of lung resonant frequencies at many depths, the role of tissue shear and tissue acceleration as drivers of tissue damage, nonlinear effects in resonating structures as a function of amplitude, resonance in non-air containing structures (acoustic fats, bone), resonance in beaked whale Eustachian tubes and vestibular structures, and a synergistic effect of resonance acting in concert with diathetic fragility (propensity to bleed).

A second hypothesis the workshop considered as a possible cause of beaked whale strandings was the acoustic activation of nitrogen bubble nuclei in tissues that are supersaturated with nitrogen from respiratory gases after diving. Factors that support the hypothesis include, 1) beaked whales are deep divers with slow descent and ascent rates that promote high degrees of supersaturation which, in theory, should increase their susceptibility to bubble growth, and 2) some trauma in the Bahamas animals was similar to that experienced by terrestrial animals subjected to rapid decompression. Factors that refute the hypothesis include, 1) the resonant frequency of microbubbles is much higher than either low- or mid-frequency sonar, and 2) deep diving mammals that produce intense vocalizations would be expected to have evolved some bubble suppression mechanisms over time. Less is known about acoustically mediated bubble

activation than about any other hypothesized mechanisms for the strandings. Especially important is 1) determining whether marine mammals have bubbles at all when they dive, 2) the lowest sound pressure level that can trigger bubble activation if it occurs, 3) modeling bubble onset (nucleation) and stabilization, and 4) modeling the role of acoustic waves in bubble growth under realistic levels of nitrogen supersaturation. Workshop participants began working on the latter two subjects after the workshop ended, but the results are not included here.

The scientific community needs a great deal of information before it can satisfactorily explain, 1) why most sonar operations apparently do not cause strandings but some do, depending on the factors present, 2) which taxa are most and which are least susceptible to these sounds, 3) whether the differences between these groups suggest a plausible mechanism of effect, 4) whether there is some as yet unknown physiological effect of exposures much lower than those that cause tissue trauma in laboratory animals, 5) whether animals respond behaviorally to sonar in ways that may increase their exposures, 6) whether mid-frequency sonars affect populations of animals in ways they do not affect individuals (for example, through socially facilitated panic), 7) whether modeling of structures can substitute for experiments that might be considered unethical or unacceptable to perform, 8) what normal vocalizations reveal about susceptibility of marine mammals to sounds of human origin, and 9) how the beaked whale auditory system responds to acoustic exposure. Beaked whale populations occur in many parts of the world where military exercises are held. Stranding events occur in such areas often enough to make them a conservation concern. A program of research and other actions is needed to understand why strandings occur and to prevent them from occurring in the future. Several impediments exist (listed at the end) that could slow progress in such a research program.

Introduction

Beaked whales may be somehow specifically vulnerable to mid-frequency (2-10 kHz) active sonar of the type used by many navies of the world. Active sonars are those that produce a sound; passive sonars are those that do not. Ziphiids (beaked whales) have been reported to mass strand (3 or more animals) in mixed groups (two species) at least six times since 1838.[1] All six cases occurred after 1963 when the present generation of mid-frequency sonars was introduced, and all are reported to have coincided with military exercises. Whether sonar was used in all these exercises is not yet known. The operating characteristics of sonar are not known for all cases in which sonar was known to be used. The closeness of animals to sonar in time and space were not documented in any of these cases, and biological specimens were collected for only three of the six cases. These deficiencies preclude a scientific conclusion about the relationship between mass, multi-species strandings of beaked whales and mid-frequency active sonars (hereafter referred to as mid-frequency sonar). Mid-frequency sonar has been used probably thousands of times without causing a known stranding. Nevertheless, the fact that mass, multi-species strandings are not known except when military activities are reported suggests that an investigation is needed. The preponderance of beaked whales in these events focuses attention on that group of animals. Mid-frequency sonar was used in all the cases thus far associated with active sonars, whereas low frequency sonar (< 500 Hz) was used in only one of the reported cases.[2]

Beaked whale specimens that were fresh enough for detailed examination (five of 14 animals collected from three stranding events) had similar suites of injury. [As of this writing no necropsy results are available for the stranding of beaked whales in the Canary Islands on 24 September 2002]. All showed cerebral ventricular and subarachnoid hemorrhage, small (petechial) hemorrhages in the acoustic fats of the jaw and melon, and blood in the inner ear without round window damage (that is, the blood may have either originated in the inner ear or diffused to it from hemorrhage sites in the subarachnoid space). No conclusion has yet been reached on whether these hemorrhages occurred before or after stranding.

The best documented stranding occurred in March 2000 in the northern Bahamas Islands where a close temporal and spatial match between ship passage and individual strandings (and the absence of any other sound source) strongly suggest the cause was tactical, mid-frequency sonar.[3] Two types of sonar were used, designated SQS-53C (greater source level) and SQS-56 (higher frequency). It is not known which of the two caused the trauma or whether a combination of the two was responsible (pings of a 53C and a 56 often alternated with each other a few seconds apart but never overlapped). Seventeen animals stranded (an estimated 8-17% of the local beaked whale population[4]), and at least seven are known to have subsequently died. The fate of animals returned to sea is unknown. Tissues from several parts of the carcasses were collected and analyzed. The Bahamas population ratio of Blainsville's to Cuvier's beaked whales was about 3:1, but the stranding had the reverse ratio of the two species[4]. The Providence Channel where the sonar exercise occurred was 35 km wide at its narrowest point. Animals would have had to be within 1 km of the ship's tracks to experience the very highest exposures (Figs. 5c and 5d in reference 3).

The specific mechanism by which the sonar signal caused the stranding and/or the tissue trauma is still under investigation. It is still not known whether the trigger was a physiological or psychological event. It is not known whether the sound caused tissue damage which caused the whales to strand, whether certain sounds may cause stranding in the absence of tissue damage, or whether this kind of tissue damage can be caused by non auditory stressors. According to the Interim report, the animals died from the effects of being stranded, not the tissue damage described above. So far the link between tissue damage and stranding is correlational.

The present workshop was convened primarily to assess whether acoustic resonance could have been the cause of any known, multi-species mass stranding event. Discussion focused on the Bahamas event because it was better documented and produced better specimens. Acoustic resonance is enhanced vibration due to a match between an acoustic signal and the media or structure that is vibrating. This match can happen with a string, such as on a guitar, or a bubble (a space containing gas). The frequency at which a bubble resonates depends on its size; a small drum has a higher pitch than a bass drum because it contains a smaller volume of air. The same is true for air containing spaces such as the lungs. Small lungs have higher resonant frequencies than larger lungs; a mouse lung resonates around 325 Hz and a human lung at around 40 Hz. The maximum potential for tissue damage is typically at the resonant frequency because the largest oscillations in the bubble will occur at that frequency for a given energy impinging on the bubble. That is the reason that knowing resonant frequencies is important.

Initial opinions about resonance and the Bahamas stranding ranged between two extremes. One was that if resonance explained the Bahamas stranding, then other sonars, specifically SURTASS LFA sonar[5], may also cause tissue damage via resonance. The other was that since there is a specific match between a structure and a frequency, sonar operating at a different frequency (like SURTASS LFA, operating at 100-500 Hz)) would be *unlikely* to stimulate resonance in the same structure or species as a mid-frequency sonar would. Scientists with diverse backgrounds gathered to examine the collective evidence, judge whether it reasonably fit the facts, and discuss research that is needed on unresolved questions. The workshop focused more on information sharing and discussion than on research planning.

The background of the workshop is as follows. Resonance was suggested as a possible cause of a multi-species mass stranding of ziphiids in Greece in 1996[2]. Since 1998 research on lung resonance has been conducted in humans, pigs, and rodents[6], and one study was done on lung resonance at shallow depth in the bottlenose dolphin and white whale (J. Finneran, See Appendix 1). In 2001, calculations were made public[7] showing possible resonance in marine mammal lungs using formulae that appeared in the investigation report of the Greek stranding.[2] The present workshop brought together researchers that had been involved in all the above activities, others who had not (including researchers from five nations), and independent observers from the Acoustical Society of America, Society for Marine Mammalogy, and U.S. Marine Mammal Commission.

The workshop began with formal presentations of largely unpublished information from pertinent research that is in progress (see Appendix I). Each paper was thoroughly discussed by the group.

The group spent most of its time discussing resonance effects and the acoustic enhancement of bubble growth[8] in tissues that have become supersaturated with nitrogen during deep, prolonged dives. Discussion on the acoustic activation of bubbles was inconclusive due to lack of empirical data on the circumstances leading to bubble initiation and growth in marine mammals.

Finally, the group discussed the kinds of scientific information that would be needed to clearly answer the question of why marine mammals sometimes strand and die after exposure to mid-frequency sonar. This report details the discussions on resonance, acoustic activation of bubble growth, needed research, and impediments or challenges to that research.

Part Ia. Acoustic Resonance

The group generally agreed that at present it seems unlikely that acoustic resonance in air containing tissues played a primary role in the tissue damage observed in stranding events. The evidence and reasoning that lead to this conclusion are listed below. A secondary role for resonance has not been ruled out (see end of this section).

1. Tissue displacement is too small. Calculations by two workshop participants suggested that for free spherical bubbles with resonant frequencies in the range of mid-frequency sonar, displacements at resonance would be on the scale of a micron to a few microns (approximately the size of cells), and would occur mainly at low ambient pressures (that is, close to the surface). Actual tissue displacements at resonance in living tissue are predicted to be even smaller because damping mechanisms (the tendency of adjacent tissues to reduce resonance effects), which occur in whole animals, were not accounted for in the free bubble model. [The damping coefficient, Q, is usually 3-5 for mammals. Tepley (see Appendix 1) assumed a maximum Q of 10 and still found small tissue displacements]. Laboratory studies show that mouse lungs exhibit tissue displacements of tens or hundreds of microns at most frequencies, and up to 1 mm when driven at their resonant frequency. Even at this displacement, tissue damage was not consistent.

2. The acoustic pressure attributable to resonance is orders of magnitude less than the ambient pressure that tissues experience when animals dive. (However, pressure fluctuations during dives occur on a much slower time scale than the frequency of pressure oscillations of sound waves).

3. Marine mammal lungs and sinuses are too large to have resonant frequencies in the range of mid- (2.7-10 kHz), or low frequency (100-500 Hz) sonars. The SACLANT report[2], page H2, calculates resonant frequencies of beaked whales lungs as 289-291 Hz at 500 m depth without considering that probably all marine mammal lungs collapse by 100 m depth. Resonant frequencies of marine mammal lungs at the surface can be estimated from body weight using a model based on laboratory studies on mice, rats, and guinea pigs. The model is $742\ w^{-.25}$ where w is weight in grams. Extrapolation of this model to marine mammal species assumes that the isometric relationship between the oscillating mass of the water surrounding the lung and the total body mass in terrestrial mammals applies to marine mammals. The model is robust as the following comparisons show. For humans, the model suggests a lung resonant frequency of 42

Hz (compared to actual value measured at the surface of 40 Hz). For bottlenose dolphins it predicts 32.3 Hz (vs. 34 Hz measured), and for white whales it predicts 27.4 Hz (vs. 28 Hz measured). This model suggests that at the surface the lungs of mid sized marine mammals would not have resonant frequencies in the range of mid-frequency sonar. For large whales it predicts a lung resonant frequency at the surface of 11 Hz, too low to be excited by low frequency sonar. As animals dive the increasing hydrostatic pressure compresses the lung volume and increases the resonant frequency until the depth at which the lungs collapse (100 m or less in dolphins from past research[9]). Thus for dolphins, lung collapse would be expected to occur well before the lung volume was small enough to produce a resonant frequency in the 2-4 kHz range. The change of resonant frequency with depth has not been directly measured in any marine mammal. Since diving animals do not remain long at any one depth they would be expected to quickly pass through the depth at which lung resonance would occur, thus limiting the exposure duration.

4. The window of exposure would likely be too narrow for lungs to receive sufficient excitation to cause tissue damage. Ensonification would have to occur within 100 m of the surface, the depth at which pinniped and dolphin lungs collapse (and most likely deep-diving beaked whale lungs as well). Tissue displacements tend to be greatest at shallow depths, so if resonance is more likely to occur at greater depth it is also less likely to be of a magnitude sufficient to cause trauma there.

5. Tissue damage appears to require higher exposure levels than most animals would receive from mid-frequency sonars, especially at depths where lung resonant frequencies would match the sonar frequency. The onset of tissue damage was reported to occur in mouse lungs after continuous exposure to 5 minute tones (at the resonant frequency) with a sound pressure level of 184 dB re 1 µPa. Humans reported dizziness and impaired balance at 182 dB re 1 µPa for 4 sec exposures of 0.9 to 2.2 kHz sweeps, but showed no tissue damage even at 191 dB for 4sec. Pig spinal cords showed myelin sheath swelling at 197 dB SPL at 1.2 kHz, and 181dB SPL at 2.4 kHz[10]. Whether tissue damage in marine mammals results from similar exposure factors is not yet known. The Bahamas report suggests that beaked whales were not likely to experience very many seconds of exposure to high amplitude sound except within 1 km of the ship. Logically it would seem unlikely that 17 animals would have received prolonged, high amplitude exposures given the dimensions of the Providence Channel and the small number of beaked whales reported there.

6. Resonance in air spaces is not instantaneous but takes time to develop. Mice, rats, and guinea pigs tested at lung resonant frequencies in the laboratory showed increasing amounts of lung damage as exposures increased from one to five minutes. Sonar in the Bahamas produced about a 1sec ping approximately every 24 seconds.

7. If resonance in lungs were an important route of tissue damage in the Bahamas and other sonar-related events then trauma in lung tissue would be expected. None of the beaked whales examined to date has shown any lung tissue trauma of the type that would be expected from resonance.

A secondary role for acoustic resonance in air spaces has not been ruled out. For example, resonance might combine with diathetic fragility (propensity to bleed), or a resonating air space may trigger a psychological effect (panic) which may cause animals to strand. No evidence yet exists for either of these cases.

Part Ib. Research Needed on Acoustic Resonance.

The workshop participants agreed that whereas the preceding logic makes it seem unlikely that acoustic resonance in air spaces played a primary role in tissue trauma in the Bahamas and other events, nevertheless the only acceptable basis for testing this hypothesis is empirical data. Research was suggested to follow these lines.

1. Natural resonant frequencies of the lungs of marine mammals are now available for a bottlenose dolphin and a white whale near the surface (See J. Finneran in Appendix 1). Data are needed on lung resonant frequencies during natural (unforced) dives at many ambient pressures as the lungs undergo various stages of collapse, and on a wider range of species and sizes of individuals. Data and models are also needed on the resonant frequencies of other air containing structures.

2. Small tissue displacement is an inadequate basis upon which to dismiss resonance. Research has not yet determined whether it is tissue displacement, velocity, or acceleration that best correlates with the onset of tissue damage. It was reported at the workshop that it was the sound pressure and not the sound velocity that was the key aspect of the sound that resulted in lung damage. Assuming that this holds true for other air containing spaces, it still does not indicate the actual mechanism of damage. In a separate analysis[11] tissue shear was indicated as the mechanism of damage, but the transformation from sound pressure to shear is not clear. At certain amplitudes of exposure the resonating structure may become highly nonlinear as higher modes are excited. This would increase the difficulty of extrapolating effects from one taxa to another.

3. The stresses caused by the constraint of adjacent structures (especially rigid ones) on air containing tissues are likely to be high. Knowing these stresses is central to the development and evaluation of predictive models for tissue trauma.

4. The possibility of resonance in structures that do not contain air was not ruled out by the discussion above, and remains a viable hypothesis. Two attempts to gain some of this information were reported at the workshop. A study of pinniped cadaver wet bone did not reveal any large resonances. A study of human cadavers indicated that fairly sizeable resonance, on the order of 10-15 dB, could be obtained for the skull. A major difference between the two studies was the much higher bone density in the human study. It was suggested that any further studies be done with species that are kept in laboratories so that any data or modeling can be experimentally verified before being applied to more exotic species for which few data are available.

5. Measurements are needed of beaked whale Eustachian tubes and vestibular structures and the ways they interact with the environment, including resonant effects. Beaked whales have unique adaptations in these organs compared to other marine mammals. Some of the Bahamas animals showed vestibular atelectasis (collapse of the tissues lining the vestibule).

6. It is possible that resonance can have a larger effect in air containing structures if it acts in concert with diathetic fragility (a predisposition to bleeding, such as is caused by leukemia). Beaked whale blood could be examined for Fletcher's factor and/or Hageman's factor, which are indicators of this disorder.

7. Non-resonant acoustic effects on other air spaces, like the pterygoid sinuses, have not been ruled out.

Part IIa. Acoustically Mediated Bubble Growth/Formation

One mechanism of tissue damage that has not been considered to date in the Bahamas investigation is sonic-induced bubble formation or growth in tissues that are supersaturated with nitrogen. The amount of gas dissolved in tissues is a function of dive depth (hydrostatic pressure), dive duration, descent and ascent rates, and the depth at which gas exchange is precluded by alveolar collapse. In humans and some other mammals, especially those breathing compressed gas, sudden decompression causes nitrogen to come out of solution and form bubbles (termed decompression sickness, caisson disease, or the "bends"). Two possible means of bubble growth initiation have been hypothesized; 1) activation of bubble nuclei (microscopic bubbles) that are stabilized within the tissues, and 2) acoustic or mechanical cavitation (see [8,12] for models that have been proposed). The workshop discussion focused more on the potential for acoustic fields to cause bubble nuclei to grow than on proposed mechanisms of nuclei stabilization.

One potential acoustically mediated mechanism of bubble growth is rectified diffusion. During rectified diffusion a small bubble suspended in an ensonified tissue that is supersaturated with nitrogen rapidly shrinks and grows in radius as the successive compression and rarefaction portions of a sound wave pass. During the radius expansion phase the gas concentration inside the bubble decreases, and gas diffuses into the bubble causing its volume to increase. Since gas diffusion rate is proportional to surface area, more gas enters than leaves the bubble during a single cycle of a sound wave. Exposure to continuous sounds may result in significant bubble growth. This mechanism likely does not explain the Bahamas stranding because the sound exposures there were too short to result in substantial bubble growth. However, once bubbles are acoustically activated they would be expected to continue to grow by static diffusion in the absence of further sound as long as the tissues remain supersaturated,[8,12] and the bubbles are not constrained by rigid boundaries. In that case rectified diffusion would play no role in tissue damage, and acoustic activation of bubble nuclei would play a primary role.

Given the observed degrees of supersaturation it is conceivable that the acoustic activation of bubble nuclei could begin at fairly low sound exposures. But, this would be important only if no bubbles of a different origin (such as from mechanical cavitation) already existed. If they did

exist, and this would not be surprising given the degrees of supersaturation observed, they would grow by static diffusion and might produce tissue damage even without the contribution of acoustically triggered bubble nuclei. So, the question of whether sound can cause tissue damage through nitrogen bubble activation may turn on the question of whether diving mammals have preexisting bubbles. At present there are no data with which to answer this question.

One workshop presentation drew parallels between the conditions under which bubble formation and growth occur in tissues and the behavior and ecology of beaked whales. For example, the degree of supersaturation in tissues should be greatest in those species that perform long, deep dives and have slow descent/ascent rates. Beaked whales have this form of diving. Also, bubble growth should be greatest in lipid structures (like acoustics fats and blubber) because lipids have a greater affinity for nitrogen than other tissues. Lipid structures that maintain low rates of blood flow during diving, like blubber, should be especially susceptible to bubble growth. No blubber trauma was observed in the Bahamian beaked whales, but acoustic fats had small petechial hemorrhages. The presentation also drew comparisons between the types of tissue damage seen in decompression sickness and in the Bahamas specimens. For example, inner ear trauma without round window rupture, trauma to myelin sheaths of nervous tissue and various fatty bodies and glands, and vestibular, auditory, and visual dysfunctions are all seen in decompression sickness.[13,14,15,16] Some parallels to these forms of trauma were noted in the Bahamas specimens, but are too detailed to repeat here.

Several aspects of the nitrogen bubble question make it an attractive subject to pursue. First, an earlier paper on this topic[12] concluded that significant, rapid bubble growth did not occur until sound pressure levels reached about 200 dB, and exposures lasted hundreds of seconds. These conclusions were based on an estimated supersaturation rate of 100% or higher, with thresholds of activation decreasing as greater levels of saturation (223%) were approached. A more recent paper[8] suggested that 300% may be a more realistic level to model. Bubble growth is expected to begin at lower sound exposure levels as supersaturation increases. Second, the threshold for rectified diffusion is lower near the resonant frequency of the bubble than it is for other frequencies[17]. But since these resonant frequencies may be several tens to hundreds of kHz, they are much higher than the frequencies used in either mid- (2-10 kHz) or low-frequency (<500 Hz) sonar.

Several aspects of the acoustic activation of bubble growth make it a questionable subject to pursue. Trauma from bubble formation/growth seems unlikely given the natural history of these animals. They have evolved adaptations for frequent compression/decompression cycles, high degrees of nitrogen supersaturation, and intense vocalizations (> 235 dB re 1 µPa in sperm whales). Logically, evolution should have produced adaptations that prevent or reduce bubble formation/growth in animals having this combination of traits. [It is possible that the exposures they receive from human sources exceed their evolved tolerance limits, or that they limit vocalizations to situations of low risk]. Also, bubble growth should become a problem only near the surface where supersaturation levels are greatest. Finally, at normal saturation activation of bubble growth is less likely to be supported by transients, such as sonar pings, than by longer duration sounds. We still do not know the conditions for activation and subsequent growth of bubbles by static diffusion under conditions of 300% supersaturation.

Part IIb. Research on Acoustically Mediated Bubble Growth

Since the only papers on acoustically mediated bubble growth in marine mammals are modeling efforts focusing on long duration exposures associated with rectified diffusion, many questions need answering before it is clear what role, if any, bubble formation/growth played in the Bahamas stranding. This section contains a list of the major issues.

1. Do *any* marine mammals experience bubble growth on decompression? A demonstration of bubble formation in any marine mammals (but especially beaked whales) would aid this debate. Demonstration of bubble growth could come about by *in vitro* experiments, using animals that are trained to dive, or by applying to beaked whales the hyperbaric test methods that are applied to humans, taking care to use a normal cycle of compression and decompression, not one that is atypical.

2. The tissues usually damaged by bubble growth/decompression sickness need to be carefully checked against the suite of injuries in the beaked whales studied to date. The apparent parallels that were pointed out in this workshop, while interesting, were not convincing to some participants.

3. Information is needed on the role of acoustic waves in enhancing bubble nucleation and activation in tissues that are supersaturated to upwards of 300%. The Crum and Mao model[12] needs to be rerun using higher supersaturation values. Also, it is not known what controls nucleation of bubbles and the growth and stability of bubbles in supersaturated marine mammal tissues. At present we do not know how much of a role rectified diffusion plays in initial bubble formation. We also do not know the extent to which sonic activation of microbubbles in highly supersaturated tissues and their subsequent growth by static diffusion is a feasible mechanism of tissue damage. A model and empirical tests of nucleation are needed. Some of the post workshop discussion on bubble activation, and on rectified and static diffusion, are available at reference[18].

4. Methods should be devised to acquire/preserve/test tissue samples from stranded animals so that the presence of bubbles in tissues can be investigated.

5. If beaked whales show bubble growth from any cause then the most essential questions are, "What is the lowest sound pressure level at which it can be triggered," and "Which sonars have the transmission characteristics most likely to trigger bubble growth?"

Part III. Information Needs on the Overall Question of Beaked Whales Stranding in the Presence of Mid-Frequency Sonar

Leaving aside the specific questions of whether resonance or the acoustic activation of bubble growth can explain the tissue damage observed in the Bahamas specimens, what are the larger unanswered questions about military sonar and marine mammals? These questions cannot be answered by the kind of *ad hoc* approach that followed the Greek and Bahamian strandings. In the third portion of this workshop, participants identified the information needs that could lead to

scientifically based conclusions about sonar's effects on marine mammals. They agreed that it is necessary to simultaneously conduct studies on live animals and cadavers, and to conduct modeling studies because no one of these approaches is complete without the others. In the live animal studies, behavior and physiology need to be studied at the same time because they are linked. Sonar-related strandings are an international problem. The resources to study these events and the people involved may come from many countries, as appropriate. The workshop identified these major questions:

1. Which marine mammals are most susceptible to sonar-like sounds? Two taxa of beaked whales (*Ziphius* and *Mesoplodon* spp) stranded in Greece, Madeira, Puerto Rico, the Bahamas, and the Canary Islands after military exercises. In the Bahamas event two minke whales also stranded and then refloated. Methods of statistical inference can be applied to past marine mammal survey data to identify susceptible species, but the data are not adequate for definitive answers. An alternative approach is to conduct systematic species surveys before and after sonar operations and also to look for disabled animals. It is equally important to determine which species are exposed to sonar sounds in the same area and time but do not strand when beaked whales do. Comparison of these groups could provide insight into the anatomical or behavioral traits through which acoustic effects are mediated.

2. Do marine mammals, especially beaked whales, have some as yet unknown response to sound exposures far below those associated with tissue trauma in laboratory animals? Both the Greek and Bahamian strandings gave hints that more of the local populations were affected than would be expected by animals encountering the narrow zones where exposures would have been greatest. Do susceptible species experience some sub-damaging, unpleasant physiological effect that triggers a large magnitude behavioral response (stranding)? The general principles of behavioral aversion, avoidance, and approach/withdrawal responses are largely unknown in marine mammals and are difficult to assess in the wild. Perhaps laboratory studies could offer insights into these processes. Are nonlinear tissue responses involved, or does resonance in the vestibular system trigger stranding? Finite Element or similar mathematical modeling of material and structural properties of the animal, might predict effects that are based on anatomy (but not behavior-related effects).

3. How do animals respond behaviorally to sonar, and do these responses somehow increase their exposure times or levels? One approach is to measure marine mammal behavior at and below the surface while sonars, or their equivalent, are used in a Controlled Exposure Experiment (CEE) format. New instruments (Woods Hole D tags, Greeneridge Sciences Acoustic Data Loggers) are available that will record the acoustic exposure animals receive and the behavioral responses these exposures elicit. These tags can be used to detect low level effects that might precede riskier effects, such as stranding. CEE studies typically begin with scaled exposures needed to perfect techniques, often using calibrated research sound sources like the Navy J series projectors. In the CEE format the sound sources are under the control of experimenters because otherwise it is difficult to deliver the desired sound levels and exposure patterns, or to quickly terminate exposures when needed. The CEE approach has some risk because we do not yet know whether beaked whale stranding is a graded response or has an abrupt, unpredictable onset. CEE studies could include observations from high-flying aircraft

because the spatial and temporal scale of some effects might exceed the range of observation from surface or shore platforms alone. Studying animal responses during actual military operations might be more realistic, but such operations are too complex and variable for field workers to observe systematically.

4. What are the demographic effects of military sonar on marine mammal populations or local stocks? Some exposed animals probably do not strand either because they are far from land or they swim away from land after exposure. Do such animals survive, drown, succumb to predators, or move away? Without this information the larger scale impacts of sonar cannot be judged. Injured and disabled animals could be found by surveying marine mammal populations before and after sonar operations, and they could be captured for study or tagged and followed to determine their fates.

5. Modeling can answer some questions about physiological response faster and cheaper than the field and laboratory studies already mentioned. Mathematical models, such as Finite Element Models, could be constructed first for the species that are commonly kept in laboratories so the models could be verified. The verified models could then be used to make predictions about beaked whales and others that are more difficult or impossible to take into the laboratory. Specific models for the acoustic activation of bubbles using realistic supersaturation levels, and on nuclei formation were mentioned previously.

6. What can an animal's sound production characteristics reveal about its possible responses to human sound? Do animals have mechanisms that protect them from the intense sounds they or their neighbors produce? How do animals respond to natural sounds? The instruments mentioned under CEE experiments are also useful for this purpose.

7. How does the beaked whale auditory system respond to sonar? These animals have shown a clear susceptibility to sonar, but it is still uncertain whether the route of effect is through the auditory system. Anatomical responses to high exposure levels could be studied by using beaked whale cadavers if fresh specimens can be found. An audiogram for beaked whales would be highly desirable. The most precise method would be a behavioral audiogram, which requires having trained animals in the laboratory (*Berardius* is a candidate because of its size and availability, but might not fairly represent *Ziphius* and *Mesoplodon*). Evoked potentials (monitoring auditory nerve and brainstem responses using skin electrodes) are increasingly useful in measuring hearing thresholds[19] and TTS. Therefore, they might be used to assess the effects of sonar and other sound sources on marine mammal auditory systems. A rapid field response team (Stranded Whale Acoustics Team, or SWAT) has been started that would use Auditory Evoked Potential (AEP) techniques on stranded animals under field conditions.

Part IV. Impediments to research

Throughout the workshop difficulties were identified that could impede the collection or interpretation of data. They are listed here for the benefit of those that plan to do research in this field.

1. Exposing animals to actual mid-frequency sonar is the most direct route to answering the question about its effects. However, using high level exposures runs the risk of inducing tissue damage or causing some catastrophic reaction (e.g., activating bubble growth). Such studies would likely be challenged because of the suspected role of sonar in past stranding events. Conversely, using low level CEE to simulate a sonar would risk failing to trigger the event (such as resonance or bubble growth) that is of interest. In such cases, "no response" or mild behavioral aversion may not be connected to the amplitudes or frequencies that cause stranding behavior, and/or physical damage. Finally, aversive behavior does not always precede physiological effects such as TTS, and therefore TTS used without reference to behavior may not be "safe ceilings" for field exposures.

2. The physiological damage limit of sound can only be determined by exposing animals to sound sufficient to cause tissue trauma and then examining for it. Behavioral responses cannot be used as a substitute for causing tissue injury. It is not realistic to plan research that is injurious to marine mammals within the U.S. because public opposition would likely stop any request for a research permit. Therefore, the research that is needed the most is virtually precluded. Cadavers can be used to study subjects that involve static, structural properties of tissues. But for subjects that involve dynamic properties, such as lung function, or for properties that change after death, such as stiffness or damping, live animals will have to be used and sacrificed. In these cases surrogates for marine mammals would have to be found. Surrogates of the beaked whale vestibular system are especially important, but whether any exist is unknown.

3. The paucity of beaked whale specimen material is a problem for many questions the group formulated. There is no steady, predictable source of high quality beaked whale carcasses in the U.S. such as would be needed to support a regular program of research. Specimens might be obtained from whale fisheries in Japan or the Faroe Islands. Beaked whales are occasionally recovered by the U.S. stranding network. However, network members need training in collection and preservation methods and basic diagnosis, as well as sensitization to the high international priority for acoustic work on beaked whales. The U.S. stranding network does not operate in many countries where beaked whale strandings have occurred in the past. The SWAT team that is prepared to conduct AEP work on stranded cetaceans has a permit for U.S. areas only, which could limit their ability to respond to sonar-related strandings.

4. Forensic pathologists have not yet met to discuss the patterns of tissue trauma seen in the Bahamas stranding. Therefore, there is still no hypothesis about the temporal sequence of trauma relative to stranding, or the mechanism that caused the animals to strand. Forensic pathologists are scheduled to meet in spring 2003.

5. Research funds will always be limited such that not all research ideas can be implemented. Highest priority for funding should be given to those projects that are 1) about the most likely candidate mechanism, 2) the most feasible to complete, and 3) the most likely to have impacts. The present workshop did not have the time or the necessary participants to perform this kind of evaluation. These considerations need to be taken into account in any future programmatic planning on this topic.

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Appendix 1: Formal Presentations Given at the Workshop

Ken Balcomb. Possible Resonance Effects in the Bahamas Stranded Animals

Lee Tepley. Physics of the Free Bubble Model, and Neglected Factors in Discussing Resonance in Cetaceans

Rick Love. Fish swimbladder Resonances: Laboratory and Unpublished Field Experiments

Joel Garrelick. The Influence of Adjacent "Structure" on Ensonified Resonant Cavities

Eddie Mercado. Using Target Strength as an Estimator of Resonance

Ed Cudahy. Measurement of Lung and Skull Resonance in Human Divers

Steve Parvin. Underwater Sound Exposure Tests in Human Divers

Darlene Ketten. Beaked Whale Trauma Suites: Pros and Cons for Acoustic Impacts

David Mountain. Experimental Structural Vibration Analysis of the Odontocete Head

Ted Cranford. Acoustic and Biomechanical Properties of Odontocete Tissues

Sean Wiggins. Acoustic and Structural Modeling of Marine Mammals

Jim Finneran. Whole-Lung Resonant Frequency Measurements in Odontocetes

Patrick Miller. A Technique to Measure Total Air Carried by Deep-Diving Whales

Aaron Thode. Change in Spectrum of Sperm Whale Clicks with Depth as Possible Evidence of Resonance

Dorian Houser. Tissue Damage in Decompression Sickness and Rectified Diffusion

Bob Gisiner. Developing a Program of Research to Address Unanswered Questions About Acoustic Resonance of Biological Structures: Current Projects and Possible Future Directions for the Office of Naval Research Marine Mammal Program