Terms of Reference

Introduction

In this Terms of Reference (TOR) document we emphasize the phenomenon of concern (noise-related strandings) and provide a fairly thorough background discussion of the leading hypotheses about possible causal mechanisms of such strandings. This is intended to provide context for the workshop discussions, but should not be confused with the primary purpose of the workshop, which is to develop practical guidance for immediate stranding response action and ensuing data collection, tissue sampling and preparation for analysis. Devising solutions for the causes of manmade noise related strandings is beyond the scope of this workshop. However, the background provided by the TOR should reduce the tendency to collect data and samples simply because they can be collected, without consideration of their relevance. Keeping the significance of the data in mind, will help guide physiological, pathological, and morphological examinations relative to testing scientific hypotheses about the strandings and should also help identify and resolve possible conflicts in sample needs and collection techniques for different applications.

The effort in the workshop itself will be organized around the themes presented only briefly in this TOR, under the Workshop Goals and Workshop Format. The ultimate goal is an enhanced general procedure for stranding response and tissue collection. We hope that will assist the people involved in stranding response in their role of providing critical data for diagnosis of the event itself and for a better overall understanding of the role of manmade noise in strandings and stranding-related mortality.

The participants should not limit their focus to any particular taxon, sound use scenario or hypothesized etiology. The primary focus of discussions in the TOR and workshop is likely to center on the mass strandings of beaked whales in association with known or inferred naval sonar sound exposure. This emphasis should not, however, preclude discussions about other cetacean species of possible interest, other sound sources (low-frequency active sonars, airgun arrays, explosives, etc.), or other hypotheses about the mechanism by which sound produces the observed effects.

Furthermore, it is critical that diagnosis of any stranding event is approached classically without bias: acquisition of available history, blood sampling as possible, careful external and internal gross examination, radiography as possible, sampling for histology, microbiology and toxicology, and subsequent sample analysis, the steps of which are iteratively driven by the implications of accumulating data. Thus each event must be analyzed without preconception, allowing a full consideration and distillation of a list of differential diagnoses, to ultimately arrive at the most parsimonious conclusion as to likely cause(s) of stranding and/or death.

In order that the biology of acoustic-induced stranding can be placed in the best possible context it is hoped that the protocols developed in this workshop will be applied as practical to single and mass stranded cases of all cetacean species in the future.

Background

Over the past decade the scientific community has produced increasing evidence that manmade sound is a potential factor in marine mammal strandings and mortalities (Cox et al, 2006). This workshop is intended to develop stranding response and tissue collection protocols that will enable us 1) to objectively assess the significance of sound in any particular mortality, and 2) obtain data from stranded animals that are useful for testing hypotheses about the possible causal mechanism(s) of acoustically mediated strandings and mortalities. Ultimately, our aim is to generate improved knowledge about the symptoms and causes of acoustically-mediated strandings, which will in turn lead to more effective actions to mitigate adverse consequences of manmade underwater sound. This in turn will place sound in the list of differential diagnoses along with the necessary data interpretation parameters to logically assign causality.

Cox et al (2006) reviewed a range of possible mechanisms by which animals might be adversely affected by sound, leading to stranding and mortality (ref figure 1 – corrected so that all paths don't lead to death). In this Background section we briefly review the major hypotheses that have been advanced, setting the stage for discussions of diagnostic and research data sampling procedures for stranding responders and researchers.

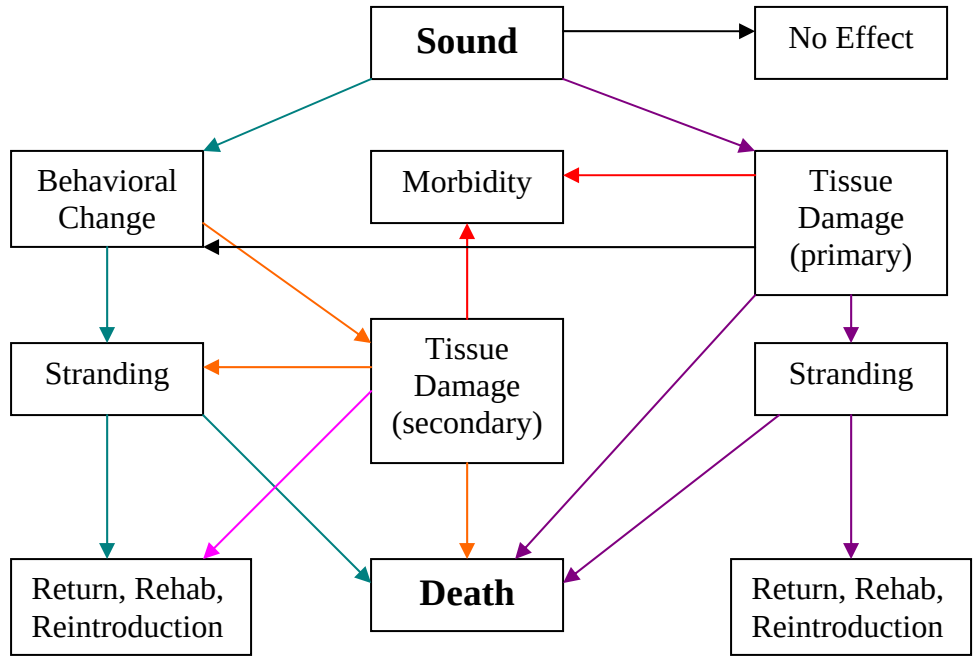

Figure 1. Potential causal pathways leading beaked whales from sonar exposure to stranding. Note that the end result can be stranding followed by return to the open water with or without human intervention, onsite treatment and immediate return to the open water, temporary holding under human care followed by reintroduction to open water, or death due to direct effects from sound or consequences of stranding (hyperthermia, suffocation, agonal movements, etc.). Alternatively there can be sublethal trauma that does not lead to stranding but does lead to long term morbidity such as PTS, (Modified from Cox et al. 2006).

Direct damage to hearing

Much research effort on the potential for anthropogenic sound to affect marine mammals has focused on auditory effects. Potential damage to ears from underwater sound can range from gross tissue damage such as that caused by the detonation of explosive charges underwater (Ketten *et al.* 1993) to a temporary loss of hearing sensitivity (Finneran et al 2005). In the Bahamas stranding there was no evidence of auditory structural damage, but there were bloody effusions into and around the inner ears and hemorrhages in the subarachnoid areas of the brain and in the lateral brain ventricles. It is undetermined whether this was caused, either directly or indirectly, by acoustic exposure (Ketten 2005).

Exposure to sounds of sufficient intensity and duration can cause a reduction in hearing sensitivity (an upward shift in the threshold of hearing) over part or all of the individual's

hearing range. This can be temporary (known as temporary threshold shift (TTS)), with recovery after minutes, hours or even days, or permanent (permanent threshold shift (PTS)) in which hearing loss is permanent over part or all of the individual's pre-exposure hearing range (Kryter 1996).

Non-auditory tissue damage

Non-auditory consequences resulting from exposure to sound have received less attention than direct auditory effects, because the ear is generally assumed to be the physiological system most sensitive to sound and therefore most vulnerable to damage by loud sound. There are a number of plausible ways in which sound might affect anatomical structures and physiological processes other than via the auditory system (Richardson et al 1995). We review only those hypotheses that have been widely discussed in reference to the beaked whale and sonar issue.

Acoustic resonance

The first hypothesis we describe concerns acoustic resonance of tissues and air spaces. Acoustic resonance of an air space (specifically the pterygoid sinus) was suggested early on by Ken Balcomb as a possible explanation of the Bahamian stranding. A workshop on acoustic resonance (Evans *et al*., 2002) challenged this hypothesis, noting that the resonant frequencies of marine mammal lungs are too low for resonance to have been caused by mid-frequency sonar, and that other airspaces like the pterygoid sinus would not experience enough displacement at the sound energy levels and anatomical architectures involved to produce sufficient resonant displacement of tissues. There is still considerable scientific debate about this hypothesis, especially as it relates to the possibility of resonance in other organs or structures. Therefore it deserves further investigation even if it the hypothesis does not have strong support at this time.

Specific relevant stranding response actions might be investigation of especially dense structures (bone) or less dense structures (air spaces and fat bodies) for specific types of tissue damage or petechial hemorrhage at interfaces where impedances mismatch is high, or collection of tissues for characterization of structural dimensions and tissue mechanical properties for modeling.

Acoustically mediated bubble formation and growth and decompression sickness

Another hypothesized, non-auditory link between strandings and sonar exposure is bubble formation and growth through either 1) acoustically induced behavioral changes that result in gas bubble formation (left pathway of Figure 1) or 2) mediated bubble growth from the direct exposure of bubble nuclei to sound (e.g. rectified diffusion) within tissues that are supersaturated with dissolved nitrogen gas (right pathway of Figure 1) (Crum and Mao, 1996). Such bubble growth/formation could result in gas emboli forming and damaging multiple organs or interfering with normal physiological function, similar to decompression sickness (DCS) in human divers.

Scientists have assumed that marine mammals have evolved anatomical, physiological, and behavioral adaptations that presumably guard against nitrogen bubble formation during rapid and

repeated decompressions during repetitive and prolonged deep dives (Ridgway 1972, 1997; Ridgway and Howard, 1979, 1982; Falke *et al*., 1985; Kooyman et al. 1972; Kooyman and Ponganis, 1998, Ponganis *et al*., 2003; Zapol et al. 1979; Davis et al. 1983). However, some scientists believe that the gas emboli and associated lesions found in cetaceans in the Canary Islands and in the UK (Jepson *et al*., 2003; Fernandez *et al*., 2004, in press) could be the result of animals exceeding the limitations of these evolutionary adaptations to deep diving when in the presence of manmade sound sources.

Scientists have predicted that those cetaceans that dive deep and have slow ascent/descent speeds could have tissues that are more supersaturated with nitrogen gas than other marine mammals (Cox et al, 2005). For example, dive profiles of some beaked whales (Cuvier's and Blainville's beaked whales and bottlenose whales, *Hyperoodon ampullatus*; Hooker and Baird 1999; Cox et al. 2005) indicated that they may accumulate nitrogen in a manner similar to human "saturation divers" (Hooker and Baird 1999; Houser et al. 2001) and may be more susceptible to acoustically mediated bubble formation than originally predicted by Crum and Mao (1996).

Jepson and colleagues have speculated that observed chronic intravascular gas –filled vesicles in liver, kidney, spleen, and lymph nodes of four Risso's dolphins, four common dolphins, one harbour porpoise (*Phocoena phocoena*), and one Blainville's beaked whale that stranded in the United Kingdom (Jepson et al. 2003; Jepson et al. in press; Fernández 2004; Fernández et al. 2004; Cox et al. 2005) may be related to episodic gas bubble formation in these animals, and thus linked to the gas and fat emboli found in ten beaked whales that died as part of a mass stranding of 14 beaked in the Canary Islands mass strandings event linked to an international naval exercise (Neo Tapon)in September 2002 (Jepson et al.2003, Fernández 2004; Fernández et al. 2004; Cox et al. 2005).

The gas bubble hypothesis is relatively new and has received much recent theoretical attention and evidence; however there has as yet been little opportunity for systematic data collection or directed scientific testing of the hypotheses.

Specific stranding response actions responsive to this hypothesis might involve systematic investigation of stranded animals for gas bubbles in tissues and fluids, data on bubble location, numbers and size, and sampling of gases from bubbles, both those animals known to have stranded when sound sources of concern were present as well as those thought to have been free of recent exposure to loud sound.

Hemorrhagic diathesis

Another hypothesis forwarded to explain some of the observed hemorrhaging in stranded animals is hemorrhagic diathesis; a tendency to bleed more than might normally be expected as a result of either the depletion of clotting factors (disseminated intravascular coagulation, or DIC), the hereditary deficiency of one or more blood clotting factors, or platelet dysfunction or thrombocytopenia. Research in humans with the hereditary deficiency indicates that they develop hemorrhages in regions similar to those of the beaked whales (*i.e.*subarachnoid spaces and the inner ear; Palva et al. 1979). These hemorrhages may cause headaches, nausea and vomiting, confusion, ataxia, dizziness, loss of consciousness, and even death (Hart et al. 1995). If beaked whales are subject to hemorrhagic diathesis, their stress response to sound, or to stranding in

general may exhibit itself in similar intracranial hemorrhages, disorientation, a subsequent inability to navigate, and eventually stranding (NOAA 2001).

While nothing is known currently about clotting abilities or DIC in beaked whales, a lack of clotting factors specifically the absence of Hageman's factor, Fletcher factor activity and Factor IX, are common to all of the limited number of cetacean species studied to date (Saito et al. 1976; Lewis et al. 1969; Robinson et al.1969). Given the lack of multiple clotting factors in cetaceans, it is not clear why beaked whales exposed to sonar might be more susceptible to the effects of this hemorrhage than other species. Further studies of taxonomic differences in cetacean blood chemistry might be supported by samples and observations of stranded animals.

Behavioral responses and possible physiological damage.

Behavioral responses may range from changes in surfacing rates and breathing patterns to active avoidance and flight. Such responses may include staying at depth longer than normal; remaining at the surface longer than normal; or an altered dive profile such as changes in ascent or descent rates and changes in the "bounce" dives at the end of a deep dive and these could lead to hypoxia, hyperthermia, cardiac arrhythmia, hypertensive hemorrhage, or other forms of trauma (Cox *et al*., 2006). Many now hypothesize that behavioral responses to acoustic exposure rather than a direct physical effect of acoustic exposure may lead directly to stranding, such as swimming away from a sound into shallow water. (Jepson *et al*., 2003; Fernández *et al*., 2004, in press; Cox *et al*., 2006)

Another behavioral response scenario may involve the vestibular system. Marine mammals could become disoriented due to a vestibular response to sounds. Tullio's phenomenon, or dizziness induced by sound, has long been known in humans (Tullio1929). The peripheral vestibular system of beaked whales may be affected by sound, affecting their ability to navigate. Beaked whales, which are usually found in deep waters, might, if disoriented, move into shallow waters and be unable to navigate back to deeper waters.

Behavioral observations of live stranded animals, or animals in shallow water near shore might therefore reveal possible symptoms of vestibular problems such as difficulty in maintaining upright orientation, nystagmus (rolling or rapid twitching motions of the eyeball), nausea or other abnormal behaviors.

Scientific Questions

Further research is needed on behavioral and physiological (non-auditory responses of deep-diving cetaceans to low- and mid-frequency sonars) as there are still major uncertainties and difficulties in estimating the impacts of sonar and noise generally on marine mammals. Based on the hypotheses presented above, there are several areas of investigation:

1. Are there anatomical data or samples that might indicate whether acute or chronic exposure to sonar transmissions results in TTS and/or PTS in beaked whales or deep-diving small cetaceans?
2. Does anthropogenic sound exposure result in tissue shear or damage from acoustic resonance? Are there data we can obtain from stranders about anatomy and tissue mechanical properties that will enable us to develop accurate, realistic models of acoustic propagation in the animal's body?

3. Are beaked whales and other deep-diving small cetaceans susceptible to gas bubble formation, either as a function of altered behavior or as the direct impact of sound/sonar transmissions (of low, mid or high frequency) on existing bubble nuclei in tissues assuming the tissues are sufficiently supersaturated with nitrogen and the received sound pressure levels are of sufficient intensity? What data should be collected from stranded marine mammals to better understand gas bubble frequency and formation?
4. Are beaked whales more susceptible to hemorrhaging in stressful situations such as exposure to sound/sonar transmissions due to some type of coagulopathy? What can we learn from stranded animals about their general blood chemistry and possible predisposition to hemorrhage?
5. Are there behavioral observations that can be made at the scene of the stranding that might offer clues about the possible behavioral effects of sound exposure?
6. Are there air blood interfaces in the beaked whale respiratory tract of sufficient brevity other than the presumably collapsed alveolar wall that might be susceptible to gas transfer at depth?

Needed Actions

These unanswered questions point to a need for:

- a better description of normal gross and microscopic anatomy of healthy beaked whales and other deep-diving small cetaceans as collected from "normal" strandings;
- better description of pathological changes in stranded small cetaceans especially beaked whales exposed to sound;
- standardization of gross and histopathological examination protocols for all beaked whale strandings;
- better descriptions of blood flow patterns in the vicinity of tissues potentially sensitive to sound; and
- better description of the anatomy and function of tissues and organs involved in hearing in beaked whales.

The MMC workshop participants recommended detailed necropsies be conducted of all freshly dead beaked whales using rigorous protocols and standardized gross and histopathological examinations (Cox et al, 2006).

There are two intertwined but distinct agendas:

1. Augmentation of routine diagnostic procedure to enhance our ability to discriminate between acoustic and other causes of mortality

2. Pursuit of specific fundamental research questions that will enhance our ability to interpret future strandings using 1 above. Such research needs have been identified and discussed in the report of the Baltimore Meeting cited below.

To test the hypotheses briefly described above, or to test additional hypotheses that may arise from increased understanding of the issue, a number of additional routines within existing gross and histopathology necropsy investigations may need to be established. The hypotheses may need to draw from systematic standardized collection of information from live stranded and dead

beaked whales and deep-diving small cetaceans. This workshop will discuss how to test these hypotheses and from that generate a suite of observations and samples that should be made as far as practical in future strandings.

Workshop Goals

Diagnostic Procedures

1. Develop a standardized procedure for gross and histopathologic examinations that allows stranding network respondents to collect evidence to determine the cause of death of a stranded beaked whale or deep-diving small cetacean, to either support or refute the role of anthropogenic sound as the proximate cause of death, and ultimately move toward a case definition for anthropogenic sound.
2. Further adapt the procedure for various conditions or states of decomposition (Code 1-5) so that it becomes a routine part of the investigation. The procedures should be able to span the various stranding situations from basic minimal information to be collected by untrained stranding network responders under compromised conditions (e.g. no refrigeration or freezer, the animal is too big or too far from required tools or facilities to work with easily) to trained stranding network responders with supplies, possibly proper facilities, and the ability to undertake a thorough necropsy and to collect a suite of tissue samples.
3. Characterization of potential handling or tissue collection/preservation issues that might generate artifacts that could affect interpretation of the data, and provide guidance to minimize such artifacts, or where unavoidable, to develop techniques for avoiding erroneous conclusions based on data variance and uncertainty.
4. Develop a decision tree/triage matrix for large stranding events to prioritize necropsies and tissue and sample collection. The triage or matrix set of standardized necropsy and tissue collection protocols would incorporate new acoustics-related procedures into existing general procedures that have been developed to respond to mass stranding situations. Limitations of time, personnel, training/skill level, specialized equipment and other considerations may limit the type and number of new specialized procedures that can be incorporated into any given stranding response scenario. Recommended data collection and sampling activities for sound related assays may themselves place conflicting demands on limited tissue sample availability, time, and staff capabilities. This workshop should help identify and resolve such conflicts to maximize the overall benefit from a given effort.
5. The following table is suggested as both an organizing tool and as a summary of our discussion during the workshop.

STANDARDIZED NECROPSY PROCEDURE OR TISSUE COLLECTION	**TISSUE/PROCEDURE BY CODE**				**MASS STRANDING TRIAGE RESPONSE**	**RATIONALE/INTERPRETATION OF SPECIFIED NECROPSY PROCEDURE OR TISSUE COLLECTION**
	Code 1	Code 2	Code 3	Code 4		

Fundamental Research Agendas – To frame the workshop participants will review, consider, and expand the list of Scientific Questions shown above, recognizing that the answers to those questions will augment our ability to undertake routine diagnostic evaluation of mortalities. The scientific questions will generate a list of projects that might be served by specific sampling protocols during future stranding events—therein they provide the rationale for specific tissue collection protocols and prioritization.

Workshop Format

During a three-day workshop (summer 2006) participants will review and evaluate the proposed straw man standardized necropsy procedure and tissue collection protocols for their potential use in investigating possible cause and effect relationships between small cetacean strandings and anthropogenic sound. In each of these situations a standardized necropsy and tissue collection protocol will be developed to facilitate gross and histopathological examinations of marine mammal strandings.

The first day the workshop will have background presentations to familiarize participants with the various protocols and triage procedures currently used by stranding networks respondents and researchers. The group will evaluate, comment on and revise sections of the strawman for necropsy procedures and the triage matrix. There may be smaller work groups to further develop specific sections of the strawman work document. The work groups will then present their work to the plenary for discussion and inclusion into a synthesized workshop report.

Workshop Participants

Approximately twenty scientists and stranding network respondents from a diverse range of relevant disciplines (*e.g.,* human diving physiology and medicine, marine mammal ecology, marine mammal anatomy and physiology, veterinary medicine, pathology, stranding response, rescue, and rehabilitation, and acoustics) have been invited to evaluate existing protocols and develop a matrix-style set of standardized protocols to investigate the role of anthropogenic sound in marine mammal strandings.

Workshop Outcomes

A standardized procedure for gross and histopathologic examinations for all condition codes and mass stranding situations that allows stranding network respondents to collect evidence to determine the cause of death of a stranded beaked whale or deep-diving small cetacean