

**Review of the Mechanisms by which Anthropogenic Noise
may cause Cetacean Strandings**

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UNIVERSITY OF SOUTHAMPTON
INSTITUTE OF SOUND AND VIBRATION RESEARCH
SIGNAL PROCESSING & CONTROL GROUP

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by

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1. Background Motivation

The impact of anthropogenic noise on marine mammals remains poorly understood. The most extreme examples of this impact are the mass strandings of cetaceans, temporally and spatially coincident with the use of mid-frequency military sonars. Initial stranding events occurred before the causal link between sonar and strandings was hypothesised [1]. Subsequent similar events are routinely the focus of investigations. In some cases these investigations have concluded that sonar was directly implicated the cause of the strandings [2-4], whereas in other cases no evidence of a link to sonar has been determined, e.g. [5]. For mass stranding events in which sonar is widely accepted as being a causal factor, the vast majority of animals affected are species of beaked whale. Consequently considerable recent research effort has been dedicated to understanding the mechanisms which potentially lead to beaked whale strandings.

In several cases, necropsies performed on the stranded carcasses reveal the presence of gas and fat emboli [3;4] which are consistent with, but not diagnostic of, decompression sickness (DCS) [6;7]. Historically it has been assumed that marine mammal physiology prevents the generation of bubbles which can lead to DCS [7;8]. Whilst almost universally accepted that evolutionary forces have mitigated the risks to marine mammals from DCS [9], would be overly simplistic to rule out the possibility of *in vivo* bubble generation. Indeed, there is increasing evidence of bubble formation in cetaceans in the absence of evidence of exposure to unusual anthropogenic noise [10;11]. The two cited studies involve damage to bone in sperm whales [10] and gas embolisms identified in animals stranding in the UK [11]. Whilst it is not universally accepted that the bone damage reported in [10] is caused by gas embolisms, the UK based study provides evidence of gas embolisms in cetaceans. It is reasonable to argue that the UK strandings are the result of anthropogenic noise and are caused by the same (or similar) mechanism that caused the sonar related strandings [2-4], however, there is no direct evidence of this. The examples of embolism formation in the UK strandings [10;11] appear to have been the consequence of sustained processes (for example, Jepson *et al* [11] showed example where lesions were surrounded by fibrosis), which is in contrast to the sonar related strandings.

There are broadly two theories as to the mechanisms by which *in vivo* bubble formation occurs in beaked whales leading to DCS. The first is that the acoustic source directly leads to the growth/generation of bubbles [12], i.e., the acoustic field generated by the sonar source causes bubble formation. The second mechanism is that the sonar induces a behavioural response that causes DCS [13]. The diving behaviour of beaked whales has been poorly understood, but recent studies [13-15] have begun to provide data about typical dive profile for three species: Northern bottlenose whale (*Hyperoodon ampullatus*) [15], Cuvier's beaked whale (*Ziphius cavirostris*) [13;14] and Blainville's beaked whale (*Mesoplodon densirostris*) [13;14]. The diving behaviour

of the beaked whales is unusual amongst odontocetes and may be linked in some manner to their propensity to strand in the presence of anthropogenic noise, e.g. sonar. This may be either through a physical or a behavioural mechanism. It is almost certainly the case both mechanisms have the potential to cause in vivo bubble generation. However, it is not apparent which will be the dominant mechanism under realistic environmental conditions in vivo. For example one might consider two Gedankenexperiment scenarios when a beaked whale encounters a sonar field: 1) the animal flees as a consequence of exposure to sonar, before a physical harm is incurred, but in doing such induces DCS; or 2) the animal incurs damage leading to DCS prior to, or in spite of, a behavioural response. The current project aims to assess what are the sound pressure levels, frequencies and exposure times need to directly cause in vivo bubble formation. This will be done using the best estimate of in-tissue conditions (both for the modelling and for what is a practicable in an experimental model). This will be vital in assessing whether these physical mechanisms are likely to occur in practice. In general in animal response studies, behavioural responses are commonly assessed through controlled exposure experiments (CEEs). However, conducting such experiments on beaked whales is both exacting and contentious and does not form part of the current work plan.

Section 2 discusses the physical mechanism by which an acoustic field is likely to induce bubble growth. Section 3 provides a brief discussion of the diving behaviour and physiology of beaked whales, since this directly impacts on the physical conditions under which exposure takes place. Section 4 describes the numerical and experimental testing that has previously been undertaken. Conclusions are presented in Section 5.

2. Mechanisms for Acoustically Mediated Bubble Growth

It is well established that an acoustic field can be used to enhance bubble growth through a process referred to as rectified diffusion [12;16;17]. Rectified diffusion is a consequence of the asymmetry in the exchange of gas between the surrounding medium and the bubble during the expansion and compression phases of a bubble oscillation. The amount of gas that diffuses into or out of the bubble is proportional to its surface area and the pressure difference between the bubble and the surrounding medium. During expansion more gas diffuses into the bubble than diffuses out of it during compression, leading to bubble growth. This is primarily due to two effects: the ‘area effect’ relies on the fact that during the expansion phase the surface area of the bubble is greater than it is during the compression phase; ‘shell affect’ is the result of cyclical changes in the diffusion gradient.

In order to model rectified diffusion one needs to model the bubble oscillations in response to an acoustic field; this can be achieved with varying levels of sophistication [16]. These models can then be combined with models of diffusion, via Fick’s law, to predict rates of bubble growth in response to an acoustic field. There are again varying degrees of simplification that can be applied to the coupling between the bubble dynamics and diffusion process [16].

Rectified diffusion assumes a pre-existing population of bubbles and such a population requires some explanation. In the absence of external factors a bubble will eventually dissolve. This is because at rest surface tension requires that the pressure inside the bubble be slightly greater than the pressure in the surround medium, this excess pressure is referred to as the Laplace pressure [16]. According to Fick’s law, the Laplace pressure will ensure that gas will gradually diffuse out of the bubble into the surrounding medium. Hence for a population of bubbles to pre-exist either new bubbles must be continually generated or the existing bubbles need to be stabilised in some fashion. Mechanisms that can cause bubble stabilisation include surfactants that change the properties of the bubble wall allowing a “skin” to form on small bubbles [16;18] and microscopic crevices in which stable bubble can exist [16]. Characterising the background population of pre-existing bubbles is a challenging task in benign environments, and remains an open question in complex environments such as blood in a living organism. This uncertainty regarding pre-existing populations remains a fundamental limitation of predictions based on rectified diffusion. However the existence of such bubble nuclei in biological tissue is widely accepted [19].

In a supersaturated medium bubbles above a threshold radius are unstable in that they will grow through a process referred to as static diffusion. This threshold radius occurs, for a spherical bubble, when the pressure inside the bubble, as predicted through Henry’s law, equals the Laplace

pressure. For bubbles larger than this threshold radius the internal pressure is greater than the Laplace pressure leading to bubble growth.

Rectified diffusion combined with static diffusion presents the most like mechanism by which bubble growth may occur. Specifically, rectified diffusion may cause small, stable nuclei to grow above the static diffusion threshold, so become unstable. At which point the effect of the acoustic field is negligible as the bubble growth is dominated by static diffusion. Once this condition is reached the acoustic field can be turned off and bubble growth will unfettered.

3. Diving Behaviour and Physiology of Beaked Whales

One obvious comparison to draw when considering DCS in cetaceans is with human divers. There is considerable experience and expertise in the effects of DCS in human divers, but even here there remain many unanswered questions. Human divers breathe air (or other gas) under pressure¹. Air in the divers lungs is absorbed, causing levels of nitrogen supersaturation in the body to increase. If the diver surfaces rapidly without giving time for the excess nitrogen to exsolve out of the tissue and bubbles form leading to DCS. A fundamental difference between the human diver and a marine mammal is that the marine mammal's lung collapses at some depth [20]. This depth has been measured for the bottlenose dolphin (*Tursiops truncatus*) as being approximately 70m [8]. It is frequently assumed that the structural strength of the alveoli is similar for all cetacean species and thus a depth of lung collapse of 70m is assumed for all species. The raison d'être of the lung is to facilitate the diffusion of gas into the blood, a process that primarily takes place in the alveoli. Alveoli have evolved to possess a large surface area and single cell membranes which make for efficient gas transfer, making them ideal sites at which nitrogen uptake to occur. It is believed the collapse of the lung of a marine mammal effectively halts the transfer of gas. During lung collapse the alveoli are compressed, forcing air from the peripheral airways into the larger central airways, such as the trachea. These larger airways have relatively small surface areas and are thick walled, so only permit slow transfer of gas into the tissue. Hence whilst beaked whales dive to significantly greater depths than human divers, it is probable that the most significant gas transfer happens in upper-most 70m of a dive.

Whilst the broad consensus is that gas exchange is halted by lung collapse, but there are structures in the cranial sinuses, e.g. pterygoid sinus, which permit limited gas exchange. Given the long duration of the dives and the high pressures, say 30 mins at 1 km depth, then gas exchange via this route may provide a significant mechanism by which gas can continue to be taken up even after lung collapse.

Beaked whales are known to dive to great depths to forage using echolocation [21;22]. There are physiological and behavioural adaptations that aid deep diving. In addition the use of lung collapse, these measures include extreme vasoconstriction and changes in heart rate and blood perfusion to selected tissues [20].

The profiles for two northern bottlenose whales (*Hyperoodon ampullatus*) are reported in [15], recorded off Nova Scotia. Note that there is no evidence of this species being involved in strandings involving sonar. Typical dive sequences for these animals consists of a deep dive to depths of around 1000m, lasting 30-40mins, followed by a series of shallow dives predominantly <200m. The animals spent 30-40% of their time at depths <40m.

¹ This statement does not include human breath-hold divers.

Similar dive profiles have been recorded for two further species (Cuvier's beaked whale and Blainville's beaked whale) [13;14] at total of three sites: the Ligurian sea, Canary islands and off Hawaii. The mean depths of the deep dives, for these species were 1080m and 835m respectively, with the mean durations 58mins and 46mins. These results are based on more animals than those for northern bottlenose whale study, specifically 10 animals [13] and 6 animals [14].

These studies also show that beaked whale dives are consistently characterised by an ascent which is slow in comparison to the descent [13-15]. There are ecological disadvantages to employing slow rates of ascent. This is because the animals only feed at depth [13;21], so extra time spent transiting to and from the surface, would appear to be detrimental; the benefit presumably accrues through a mechanism relating to manner in which the animals physiology deals with the demands of deep diving.

The results in [13] suggest that the diving of Cuvier's and Blainville's beaked whale is atypical among cetaceans, in that they seem to exceed their Aerobic Dive Limit (ADL), whereas the dives of the majority of other species of marine mammals appear to be close to the ADL². For example, the sperm whale (*Physeter macrocephalus*) has been observed performing consecutive deep dives with only short periods on the surface (mean surface time 9mins) [25]. The calculation of ADLs [13] are based on extrapolating ADLs measured for Weddell seals [26], so should be treated with some caution. The estimated ADLs for Curvier's and Blainville's beaked whales are 33mins and 25mins respectively, which are roughly half the mean times of the observed deep dives. It is suggested [13] that the reliance of the beaked whales on an anaerobic metabolism is the reason for the long periods between deep dives. It is argued that this period could be used to purge metabolites, such a lactic acid; a process that is enhanced by moderate exercise.

It is interesting to note that the mean dive durations for the northern bottlenose whale reported in [15] are close to the extrapolated ADLs of Cuvier's beaked whale. Northern bottlenose whales are slightly larger than Cuvier's beaked whales³ so, everything else being equal, should have a slightly longer ADL. Hence there is no evidence that the animals observed in [15] were exceeding their ADLs, unlike the animals studied in [13]. Yet the animals in [15] continued to exhibit the same long intervals between deep dives, in contrast to the sperm whales in [25] whose dives time approximately match their supposed ADLs and do not require the animals to spend extended periods near the surface. Given that these observations regard animals of different species, at different times and in different locations general conclusions need to be treated with

² Other marine mammal species that seem to regularly exceed their ADLs are: sea lions [23] and fur seals [24].

³ In [27] Cuvier's beaked whale length is quoted as 7m as opposed to the 9.5m for the Northern bottlenosed whale.

considerable care. However, the results from [15] run counter to the claim in [13] that the long interval between deep dives is a consequence of the need to purge metabolites built up during deep dives that rely on an anaerobic metabolism.

An alternative theory for these long inter-dive intervals, in particular the shallow dives, is that they are part of the decompression process, purging nitrogen [9]. It is noted in [13] that that the long periods beaked whales spend at shallow depths, reported in all studies [13-15], would lead to an increased risk of emboli. This is based on the assumption that lung collapse halts nitrogen uptake and so shallow dives result in an increase in the nitrogen level, since during these dives the lung is mainly open and nitrogen saturation will increase. Conversely if one accepts that gas exchange happens at depth, then the role of the shallow dives may be as mechanism to purge nitrogen and reduce the risk of DCS.

The behavioural hypothesis of in vivo bubble generation is based on the assumption that the use of sonar disrupts this diving behaviour and prevents the animal from appropriately compensating for the effects of deep diving, leading to DCS [13].

4. Modelling of In Vivo Bubble Growth

In April 2002 a Workshop on Acoustic Resonance as a Source of Tissue Trauma in Cetaceans was convened by the National Oceanic and Atmospheric Administration (NOAA) in Silver Spring, Maryland. It considered the evidence regarding the possible physical mechanisms that could lead to acoustic trauma; subsequently a report of this workshop was published [28]. The two major physical mechanisms considered were resonances of air filled structures and rectified diffusion. Whilst unable to completely dismiss the resonant cavity theory, the report concludes that rectified diffusion is the most likely potential mechanism by which tissue damage may occur.

Later work [29] demonstrated, through the use of finite element models constructed using data collected from CT scans of the head of a neonate Cuvier's beaked whale, that direct thermal heating from an acoustic field caused negligible rates of temperature increase: $O(10^{-3}Ks^{-1})$ at 180 dB re $1\mu Pa^4$.

Numerical models of rectified diffusion have been used by [12;18] to predict rates of bubble growth in supersaturated media. The earlier study [12] considered supersaturation levels in the range 100%-223%, and sound pressure levels 150-220 dB. It is assumed that the bubbles are small ($10\mu m$) and are excited far below resonance. This is based on the fact that the sound fields under consideration having frequencies less than, say, 10 kHz, for which the bubbles that are resonant have diameters in the free field under 1 bar static pressure of around 600 microns (although this will decrease with increasing depth). If the assumption is made that the bubbles under consideration for growth by rectified diffusion are microscopic, then these bubbles will be insonified at much less than their resonance frequencies, and so there will be no strong frequency-dependent (i.e. across-resonance) effects in the dynamics of the bubbles in question. As a result, in this regime and under these assumptions, the rate of bubble growth would be expected to be independent of the frequency. These almost ubiquitous assumptions in this topic will be re-evaluated as part of this project. The results of the study which applies these assumptions [17] are simulated for a range of excitation frequencies 300-500Hz and assumed to be valid over the range of the range of sonar frequencies of interest (50Hz-5kHz). It is predicted that a $10\mu m$ bubble can grow to a size to block small vessels in the body in time scales of a few seconds (although as this limit is approached the bubble dynamics would differ markedly from the free-field condition assumed in the model [30]). However, to achieve this, the sound pressure level of the acoustic field needs to be approximately 210 dB. Such SPLs are unlikely to be experienced by a marine mammal exposed to mid-frequency sonar.

⁴ All sound pressure levels in this report are assumed to be "re $1\mu Pa$ " and the reference pressure will not be explicitly stated throughout the remainder of this document.

The levels of supersaturation for beaked whales have been estimated by Houser *et al* [19] based on the dive profiles in [15] (the only data on beaked whale dive profiles available at the time of Houser's publication [19]). This model yielded predictions for levels of supersaturation of nitrogen of up to 300%. It is worth highlighting that of the three species of beaked whale for which there is dive profile data; the data regarding the northern bottlenose whale has the shortest mean duration for its deep dives. One might legitimately speculate that levels of supersaturation in the other two species (Cuvier's and Blainville's beaked whales) could even exceed the 300% value predicted in [19]. The higher levels of supersaturation predicted by Houser were used in later work [18] where rates of bubble growth are predicted. These predictions suggest that exposure of an animal, whose tissues are 300% supersaturated when exposed to a 160dB sound field can cause bubble growth that produces bubbles of size anticipated to cause DCS in <100s. A key point in this that this bubble growth does not assume that the acoustic field is present throughout the bubble growth period. It is suggested [12;18;19] that once growth is initiated the level of supersaturation ensures that the bubble continues to grow through static diffusion.

Potter [18] also discusses stabilisation mechanisms for small bubbles. However it is well-known that there are entropic and other mechanisms by which bubbles can be chemically stabilised against dissolution [16]. Potter [18] discusses how, if the bubble walls become coated by some impermeable biological material (protein palettes are proffered as a candidate source), then this could stabilise the micro-bubbles. This leads Potter to propose what he refers to as a new mechanism for rectified diffusion, which upon inspection is a modest modification of existing theory.

The only apparent experimental work considering bubble generation in biological material in the context of marine mammal DCS is described in [17]. In this work the results of three experiments are reported.

In the first fresh bovine blood is supersaturated by exposing it to a static pressure equivalent to a depth of 40m-70m for a period of 1h. This blood is then exposed to an acoustic field at 37kHz at what is referred to as a "low pressure" 50kPa (equivalent to 214dB) and bubble generation is observed. It is noted that spontaneous bubbles do not form in the absence of an acoustic field.

The second experiment involved liver tissue immersed in bovine blood at pressures equivalent to 50m for 3h. Note the exposure time in this experiment exceeds the known dive times for any beaked whale. The liver tissue is then exposed to a high level of ultrasound 400kPa (232dB) and left, it is noted that after 30min an ultrasonic scan of the tissue reveals the presence of bubbles within the tissue, these bubbles are absent in a control sample prepared in the same manner but not exposed to the ultrasonic field.

The final experiment employed a porcine kidney, this was immersed in bovine blood and pressured to the equivalent of 40m depth for a total of four hours. Two hours into the procedure the kidney was removed from the pressure chamber and some of the blood surrounding the kidney was injected into the kidney via the renal artery, to simulate blood perfusion. After this perfusion the kidney is replaced in the pressure chamber for a further two hours. After pressurisation the kidney is divided into two halves and one is subjected to an ultrasonic sonic field. Finally, after a further 30min, the kidney halves are frozen, sliced and then examined under a microscope where bubbles can be seen to have formed the sample subjected to the ultrasonic field.

In neither of the last two cases are full details of the ultrasonic field provided. It seems reasonable to assume that the same ultrasonic transducers and acoustic parameters are used as in the first experiment. However it is very important to note that bubble growth can depend on far more than simply the duration, amplitude, and frequency of the sound field, but also on other factors such as whether the field is continuous-wave or pulsed and, if it is pulsed, the associated duty cycle and start-up characteristics of each insonification: certain pulsing conditions can give more bubble activity than continuous-wave insonification at the same frequency and amplitude over the same total period, even though the actual ‘on-time’ of the pulsed field is less than that of the continuous-wave field [16].

The study by Crum et al. [17] demonstrates that it is feasible to demonstrate enhance bubble generation in excised tissue. The parameters of the acoustic fields employed are not realistic simulations of those one anticipates that beaked whales might be exposed to during the operation of a sonar operation.

5. Conclusions

The mechanisms by which bubbles can form in vivo in cetaceans remain the subject of considerable controversy. Rectified diffusion remains the most likely candidate mechanism for enhanced bubble growth. Models for process of rectified diffusion are well developed, but there is considerable uncertainty regarding the source of stable bubbles which are necessary for rectified diffusion to take place. There is also uncertainty regarding the level of nitrogen supersaturation in beaked whales after a sequence of deep dives. There is very little experimental verification of bubble growth under circumstances that are representative of realistic interactions between sonar and beaked whales.

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