A possible mechanism for acoustic triggering of decompression sickness symptoms in deep-diving marine mammals

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Abstract— **An interest in plausible mechanisms for significant acoustic impact on some species of marine mammals at receive levels significantly below that currently anticipated to cause direct physical trauma has arisen in response to questions of how the operation of sonars may have contributed to mass beaching events of beaked whales. Resonance in cavities and other specific structures was at one time proposed as a mechanism, but after some scrutiny this now appears unlikely. Rectified diffusion was posed as another candidate, but has been demonstrated to be significant only at relatively high pressure levels, exceeding receive levels anticipated in observed beaching circumstances. We examine an alternative proposition; that pre-existing micro-bubbles that are normally stabilised and which do not normally permit gas exchange across their walls can be acoustically activated so that continued growth is supported through static diffusion from super-saturated tissues in the absence of an acoustic field. The proposed mechanism would explain why micro bubbles (believed to be normally present in mammalian tissues) do not grow and cause decompression sickness (DCS) in healthy deep divers with super-saturated tissues, why these micro bubbles do not collapse under the Lap lace pressure exerted by surface tension in unsaturated tissues, and why long-duration, deep diving cetaceans such as beaked whales appear to be particularly vulnerable to anthropogenic acoustic exposures. Numerical results for bubble growth modelled according to the treatments of Crum and Mao under tissue super-saturations of 200-300% (an appropriate range for deep-diving marine mammals on surfacing) show that if micro-bubble gas exchange could be activated acoustically, even by only a very brief exposure, this would result in subsequent bubble growth by static gas diffusion so that within 10 minutes their size would be sufficient to cause symptoms of decompression sickness (DCS).**

Index Terms—**Acoustic, Diffusion, Decompression Sickness, Micro-bubble**

I. INTRODUCTION

The stranding of beaked whales in association with naval acoustic activities [1,2,3] has prompted speculation on potential mechanisms underlying the relationship between stranding in this particular family of whales (*Ziphidae*) and certain types of anthropogenic acoustic exposure. The first widely-publicised mass stranding of beaked whales in association with naval operations was that in Greece in 1996 [1] followed by a second mass stranding event in the Bahamas [2], now understood to be associated with the use of mid-frequency tactical Navy sonars [4,5] and then by further incidents in the Canary Islands [3]. There was also stranding of two beaked whales (Ziphius Cavirostris) in the Gulf of California on September 24, 2002. The NSF-supported R/V Maurice Ewing had been conducting a seismic airgun survey in the area at the time. It has since been reported (by Bruce Mate and Daniel Palacios) that the same vessel had been conducting a seismic survey off the Galapagos Islands in April 2000 when four Z. Cavirostris stranded on Santa Cruz Island. No-one highlighted this coincidence until October 24 2002, when Bruce Mate and Daniel Palacios reported it to Roger Gentry of NOAA. R/V Maurice Ewing was not notified at the time, and the stranding was never reported publicly. It has not been established if the R/V Maurice Ewing was a causative factor in these strandings, but the coincidence of two incidents of seismic surveying and beaked whale strandings nearby is unsettling.

Examination of historical records indicates at least 11 mass strandings of beaked whales in the vicinity of naval operations, none of which occurred before the mid 1960's, when mid-frequency tactical sonars became widely deployed. The Bahamas beached whale tissues that were examined exhibited some hemorrhaging similar to some traumas observed in human DeCompression Sickness (DCS) [4]. While it is not possible to ascertain the receive levels for the stranded Bahamas animals, probability analyses based on ship tracks, bathymetry and stranding pattern suggest it is improbable that they were exposed to levels of 180 dB re 1 μPa (all acoustic pressure levels in dB will henceforth be referenced to 1 μPa in this paper) and most probably were exposed only to 160-165 dB. An extensive body of US Navy testing suggests that levels below 180 dB are not anticipated to cause direct hemorrhaging of tissues. It thus appeared that this family, and perhaps others, may in some way be particularly sensitive to acoustic impact, and the search began for a plausible mechanism. If, as this paper suggests, the susceptibility is caused by acoustic triggering of micro-bubble activation, leading to bubble growth and consequent physiological disruption, we might expect the problem to affect marine mammal species that share common crucial diving pattern habits, rather than simply to be related taxonomically.

While resonance was initially thought to be a candidate, the opinion of delegates of a workshop held specifically to address this issue in 2002 was that the resonant enhancement factor 'Q' for marine mammal tissues was too low and that there were no cavities or structures that could be identified that would resonate at the appropriate frequency to explain the data [unpublished report, 5]. The workshop participants, who included this author, were unanimous in considering the likelihood that tissue or airspace resonance was involved in the strandings and observed physiological symptoms to be minimal.

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II. BUBBLES IN TISSUES

Bubble formation has the potential to produce emboli, high pressure in localised regions of tissue, tissue hemorrhage and tissue separation. Pain, particularly in the joints, nausea, respiratory difficulties, visual and auditory dysfunction, disorientation and other CNS dysfunctions may result, symptoms that are common to DCS and presumed to be caused by the same mechanism. It is therefore possible that if a mechanism could be identified that produced bubbles in tissues, this could explain the observations, including disorientation through disablement or partial impairment of the vestibular system.

Examinations of beached marine mammals in the UK have revealed a small number of cases with extensive cavities in the liver and kidneys that have been tentatively linked to beached marine mammal pathology from the Canaries [6]. However, the pathology of the Canaries beaked whales and the UK cases differed. The Canaries beaked whales had acute, systemic and widely disseminated lesions consistent with, although not diagnostic of, DCS in humans [7]. The large hepatic cavities found exclusively in the UK cases are atypical of DCS in humans and experimental animals. For logistical reasons, the CNS was only examined in two UK cases and the bones were not examined in any. We cannot therefore confirm or refute the presence of lesions consistent with DCS (or other causes of gas embolism) in these tissues. However, large numbers of gas bubbles (emboli) were seen in portal veins and sinusoids in the livers from all UK cases examined microscopically, consistent with DCS in humans. It is their accumulation and persistence leading to both acute hepatic injuries and progressively fibrosed cavities that differ from human DCS. Since cetaceans also differ from humans behaviourally (as obligate, repetitive breath-hold divers), physiologically (e.g. diving reflex, hypo-coagulable blood) [8] and anatomically (e.g. retia mirabilia, large epidural venous spaces and portal veins, diaphragmatic sphincters) [9], it is perhaps too simplistic to assume that the distribution, severity and chronicity of gas emboli-induced lesions (whatever the cause) will be identical in both human divers and free-living cetaceans. The rete mirabilia will undoubtedly filter arterial gas emboli from the arterial supply to the entire CNS, so we are left with lung and liver as the main organs that would filter venous bubbles, and kidneys that might suffer major insults due to arterial bubbles. The liver lesions in our UK cases were associated with venous bubbles (portal/sinusoidal) and the kidney lesions (in one dolphin) were associated with arterial bubbles.

A. Bubbles and Rectified Diffusion

Rectified diffusion has been considered as a mechanism to create bubbles. Crum and Mao [10] addressed this by modelling bubble growth under conditions of continuous, low frequency exposure at levels of 150–220 dB, for initial bubble radii from 1–10 mm, and for levels of the dissolved gas concentration from 100% to 223% of saturation. They determined that for a Sound Pressure Level (SPL) in excess of 210 dB, significant bubble growth can be expected to occur, and that human divers and marine mammals exposed to these conditions could be at risk. For SPL below about 190 dB, however, significant bubble growth was not predicted to occur by rectified diffusion. Yet the most probable receive levels for both the Greece and Bahamas mass strandings (the only two for which even speculative estimates are possible at this time) are much lower than this (in the region of 160-165 dB), so that the rectified diffusion mechanism is inconsistent with observations.

B. Supersaturation

Houser et al. [11] later modeled the accumulation of nitrogen in the muscle of several cetacean species by using recorded dive profiles and assuming that unstudied physiological characteristics of diving were the same as that monitored in bottlenose dolphins [12], i.e. half-times for the rate of nitrogen accumulation into and out of the muscle and the depth at which lung collapse occurs (70 m). The modeling results suggested that tissue nitrogen supersaturation in certain cetaceans could be substantially higher than that modeled by Crum and Mao. Slow descending / ascending and deep diving marine mammals, such as beaked whales and sperm whales, were predicted to accumulate the greatest amount of nitrogen during diving, e.g. the northern bottlenose whale (*Hyperoodon ampullatus*) was predicted to have tissue nitrogen saturations > 300% ambient when surfacing from a typical series of dives. Greater accumulation would presumably result from 1) longer exposure times to hydrostatic pressures capable of driving nitrogen uptake (slow diving) and 2) exposure to higher hydrostatic pressures (deep diving) up to the point of lung collapse. The results of Houser et al. indicate that if gas bubble growth in tissues can be triggered or driven by anthropogenic sound sources, beaked whales and other inhalation deep-diving marine mammals might experience increased risk to micro-bubble activation and growth under appropriate exposure conditions. Exhalation divers, such as many pinnipeds, exhale most of the exchangeable lung air prior to diving and may not be so susceptible.

C. Static Diffusion

Crum and Mao [10] noted that continuous sound exposures were not required to drive the growth of bubbles if tissues were sufficiently supersaturated. Rather, once bubble growth was initiated it would be supported through static diffusion and would continue in the absence of further acoustic exposure. Additional modeling to address the impact of supersaturation on the rate of bubble growth in the absence of an acoustic field, but following activation of a micro-bubble, needs to be performed to address this issue. An evaluation of the Crum and Mao model at tissue saturations predicted to occur in beaked whales was not undertaken in the Houser et al. paper.

D. The problem with micro-bubbles – how are they stabilised?

But this begs the question, how are micro-bubbles, believed to exist in mammal tissues under normal circumstances, stabilised in the first place? In non-supersaturated tissues, the gas pressure inside a bubble must be higher than that outside to balance the so-called 'Laplace pressure' exerted by the surface tension in the bubble walls. This pressure becomes more significant as the bubble size decreases. If the walls of the bubble are permeable, this should result in gas being driven out of the bubble, and the bubble should collapse into solution. But this does not always appear to happen.

Similarly, in supersaturated tissues, the gas pressure outside the bubble exceeds that inside (providing the degree of supersaturation is sufficient to overcome the Laplace pressure) and a permeable bubble wall would lead us to expect that micro-bubbles would always grow in such conditions. But this also does not appear to happen, and human divers habitually exit the water after their dives with 200% supersaturation without incurring DCS.

Underlying Crum and Mao's calculations, and therefore also ours, is this issue of bubble nucleation and stabilisation. When a micro-bubble is prevented from collapse under the Laplace Pressure, it is referred to as 'stabilised'. There is considerable debate as to how stabilisation might be achieved, and Crum and Mao circumvent the problem by simply assuming that micro-bubbles have been stabilised in some way that does not affect their gas permeability and proceed from that starting point. Crum and Mao were primarily concerned with how rectified diffusion might drive bubble expansion directly, assuming that the bubble was initially stable but permeable.

Our proposition is that the mechanism that stabilises a micro-bubble, serving to protect it from collapse under Laplace pressure, is the same as that which prevents inflation under static diffusion

in highly super saturated tissues. The two problems are solved simultaneously if one can identify a mechanism that makes the micro-bubble walls effectively impermeable to gas exchange. Any process that disturbs this impermeability would then 'activate' the micro-bubble, allowing it to exchange gas across its walls. Clearly, a sufficient external overpressure, exerted by highly super-saturated surrounding tissue, would need to be able to overcome the stabilising mechanism. We will come back to this issue in a following section, but first we will develop the model for how activated bubbles might respond to acoustic forcing in highly super-saturated tissues to see if this could provide a mechanism to generate large bubbles capable of causing DCS-like symptoms in a reasonable period of time.

III. A PHYSICAL MODEL OF GAS BUBBLE RESPONSE TO ACOUSTIC FORCING

The physical bubble response model is taken from Crum and Mao [10], who presented a suitable approach to solving the Rayleigh-Plesset equation of motion for a gas bubble embedded in an infinite liquid and subject to a sinusoidally-varying pressure field:

$$R\ddot{R}^2 + \frac{3\dot{R}^2}{2} + \frac{1}{\rho} \left\{ P_0 \left[1 - \left(\frac{R_0}{R} \right)^{3\eta} \right] - P_A \cos \omega t + \rho R_o \frac{{\omega_0}^2}{\omega} \left(b_t + b_r + b_v \right) \dot{R} \right\} = 0 \tag{1}$$

where R is the instantaneous bubble radius and R_0 the equilibrium radius. The density of the liquid in which the gas bubble resides is given by ρ and P_0 is the pressure inside the bubble at equilibrium radius given by the sum of the 'at infinity' pressure P_∞ and the 'Laplace surface tension' pressure so that $P_0 = P_\infty + 2\sigma / R_o$, where σ is the surface tension coefficient. The bubble is driven by an acoustic wave of pressure amplitude P_A with angular frequency ω compared to the resonant bubble frequency of ω_0. The damping terms are incorporated by the b_t, b_r and b_v terms representing the thermal, radiative and viscous damping.

The resonance frequency, ω_0, is given by the familiar formula:

$${\omega_0}^2 = \left[3\eta P_0 - \frac{2\sigma}{R_0} \right] \Big/ \rho {R_0}^2 \tag{2}$$

where η is the 'polytropic coefficient' of the gas, which handles the thermal response of the bubble in terms of adiabatic versus isothermal bubble oscillation.

The thermal, radiative and viscous damping coefficients are obtained from:

$$b_t = 3(\gamma - 1) \left[\frac{X S_+ - 2C_-}{X^2 C_- + 3(\gamma - 1) X S_-} \right]$$
$$b_r = \frac{\rho {R_0}^3 \omega^3}{3\eta c \left(P_\infty + \frac{2\sigma}{R_0} \right) \left(1 - \frac{2\sigma}{3\eta R_0} \left[P_\infty + \frac{2\sigma}{R_0} \right] \right)} \tag{3}$$
$$b_v = \frac{4\mu\omega}{R R_0 \rho {w_0}^2}$$

where γ is the ratio of specific heats of the gas and c is the speed of sound in the liquid and the coefficient of viscosity of the gas is given by μ. The following hyperbolic trigonometric substitutions have been used:

$$S_\pm = \sinh X \pm \sin X$$
$$C_- = \cosh X - \cos X \tag{4}$$

where X characterises the ratio of bubble size to boundary layer thickness in terms of the thermal diffusivity of the gas and the acoustic forcing displacement, given by

$$X = R_0 \left(\frac{2\omega}{D_1} \right)^{1/2} \tag{5}$$

with D_1 representing the thermal diffusivity constant of the gas.

Finally, the polytropic exponent, η, is given by

$$\eta = \frac{\gamma \left(1 + {b_t}^2 \right)}{1 + \frac{3(\gamma - 1)}{X} \left(\frac{S_-}{C_-} \right)} \tag{6}$$

The value of η= 1 in the isothermal limit (applicable to small bubbles) whereas $\eta = \gamma$ in the adiabatic limit (applicable to large bubbles).

Once a solution for R as a function of time has been obtained by solving the Rayleigh-Plesset equation (usually by numerical integration), it can be used to evaluate the time-evolution of the equilibrium radius from Eller and Flynn's equation (given in [10]) governing the diffusion of gas into and out of the bubble. This equation can be solved numerically, but at the cost of two numerical integration processes (including the one for the Rayleigh-Plesset equation). If we are prepared to accept a truncated Taylor series expansion of the solution to Eqn. 1, expanded in powers of P_A / P_∞. Crum and Mao [10] have shown that the evaluation of the time-averaged terms can be greatly simplified. For a received acoustic intensity level of 200 dB (=0.1 atm amplitude), ignoring third-order terms and higher in the Taylor expansion will incur an error on the order of 1%. Lower acoustic intensities will incur a lesser error. Since we are interested in the possibility of acoustically-mediated bubble growth at moderate acoustic receive levels (<180 dB) this is acceptable and so we adopt Crum's truncated Taylor series solution method for this work.

A. Validating the model against prior results

Before moving on to compute how an activated micro-bubble might behave in a moderate or zero acoustic field with highly super-saturated surrounding tissues, we first ran our computational model of bubble oscillation based on the equations given above to confirm that the results agreed with the Crum and Mao results in [10] for parameter values close to theirs.

Several comparative tests were run, and the results were indeed very similar, only minor differences arising, possibly out of slightly different choices for some of the tissue parameters and/or rounding errors. As a final check, and one which is particularly sensitive to the balance between the Laplace overpressure, degree of supersaturation and the action of rectified diffusion in 'pumping up' the bubble, our numerical model was run to compare results for acoustic levels at 200 dB re 1 μPa and below to those of Crum and Mao's Figure 7 to see if the predicted extinguishing of micro-bubbles matched their results.

For this example, Crum and Mao calculated that a super-saturation of 126.9% was required to balance the Laplace

overpressure, and ran their model for a super-saturation of 126%, thus precipitating a marginal bubble collapse unless rectified diffusion could drive the bubble growth at a sufficient rate to counteract the slight shortfall in static pressure balance required to maintain the micro-bubble. We obtained almost identical results for acoustic intensity levels of 150-200 dB and a super-saturation pressure of 127.6%, slightly higher than their result, but well within expected tolerances given the highly non-linear sensitivity of the result to saturation pressure. These numerical modeling results are shown in Fig. 1.

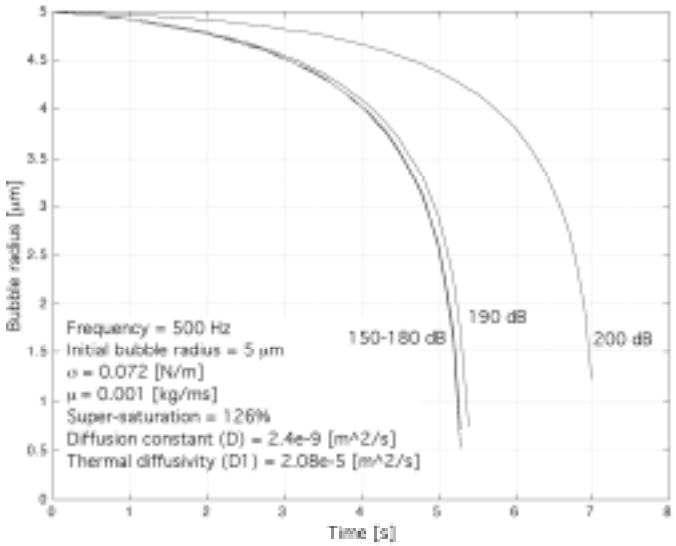

Figure 1. Numerical results for micro-bubbles that extinguish due to a shortfall in super-saturation pressure compared to the Laplace overpressure, despite rectified diffusion

IV. NEW RESULTS FROM OLD EQUATIONS

We are interested here in how static diffusion, in the absence of an acoustic field, would inflate an 'activated' bubble in a highly super-saturated tissue such as found in a deep and slow-diving marine mammal on surfacing. The role of an acoustic field in our hypothesis is to trigger de-stabilising of the micro-bubble or nucleus, not to drive the expansion itself. We are concerned only with the possibility that the acoustic field might activate the micro-bubble in some way to respond to the high gas pressure differential across its walls to enable it to accept gas diffusing inwards, thereby inflating it. The inflating pressure itself is provided by the deep-diving profile of the animal. The Crum and Mao results in [10] do not cover activated micro-bubble behaviour in highly super-saturated tissues. The next step is therefore to fill in the gap for highly-super saturated tissues by running the model for saturation values of 200-300% for low and zero acoustic field amplitudes to determine how long it would take activated micro-bubbles of various sizes under different acoustic intensities to expand to sizes that would begin to become a problem. Accepting that the results of our modeling approach acceptably match those of Crum and Mao for higher acoustic intensities up to 200 dB and lower saturation levels, some micro-bubble evolutions of interest for moderate acoustic intensities and in high super-saturation environments have been calculated. Crum and Mao have already shown that, if the super-saturation is 200%, the acoustic intensity has an insignificant effect on the bubble growth rate, provided the bubble is activated and can accept growth by static diffusion. This confirms that rectified diffusion is not required for bubble growth in highly super-saturated tissues, but rather the role of the acoustic insonification is only in the activation of the bubble to permit static diffusion to operate effectively.

Houser et al. [11] calculated that super-saturation values in the muscle of some deep diving marine mammals (a tissue taken to be close to seawater for our physical modeling purposes) can reach values in excess of 300%. Therefore, a simulation was run in which this level of supersaturation was included. The model was run using the lower surface tension figure (which we consider more likely for real tissues, although it makes very little numerical difference to the results) and the lower diffusion coefficient (the most conservative scenario). Frequency was varied from 500 to 2500 Hz but had negligible impact on the results, as the presence of an acoustic field at all is somewhat irrelevant once the bubble is activated in a super saturated tissue. Results for initial micro-bubble radii of 1-10 μm in tissues of super-saturation levels of 150-300% are shown in Figure 2 for an acoustic intensity of 160 dB re 1 μPa.

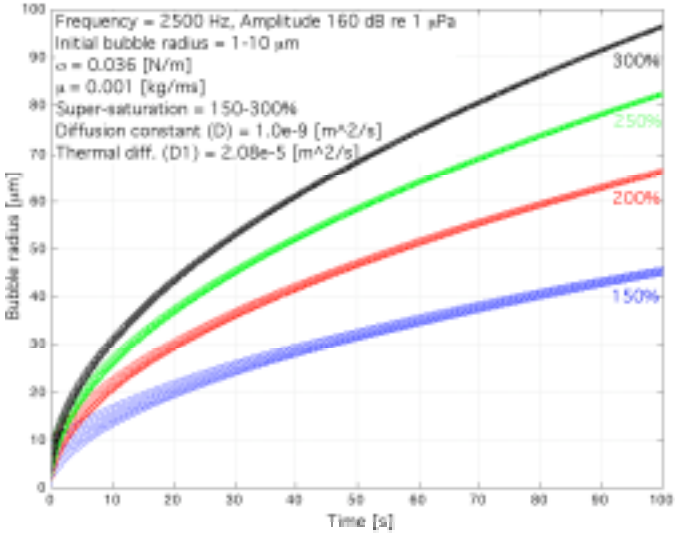

Figure 2. Growth rates for micro-bubbles of 1-10 microns in super-saturations of 150-300%

Providing the micro-bubble can be activated to accept inflation by normal diffusion, it becomes apparent that neither the acoustic frequency nor the intensity has any significant effect on the bubble growth rate because the evolution of the bubble becomes independent of any acoustic forcing once it is activated. This agrees with the model predictions of Crum and Mao, that acoustically driven rectified diffusion is negligible under moderate acoustic intensities provided sufficient supersaturation exists. Furthermore, Figure 1 demonstrates that after 100 seconds or so of growth, the starting radius has also become relatively unimportant. Bubble growth increases monotonically and eventually converges regardless of the initial bubble radius. After 100 seconds' of growth, the size of the bubbles and their growth rate most strongly reflect the degree of supersaturation in the tissue. This is therefore the overriding factor of importance in how large the bubbles will grow in a given time. Bubbles of 40~100 μm are predicted to appear in tissues with a 150-300% supersaturation value in 100 seconds. This is large enough to cause DCS-like symptoms in mammals. Even if the growth were slower, due to inaccuracies in our estimated parameter values or modelling, the growth is monotonic and it would simply take a little longer before the bubbles became large enough to become constrained by other physiological factors, associated with disruption of physiological processes leading to DCS-like symptoms. Even in mildly super-saturated tissues at 150%, bubble growth is predicted to stabilize at 15 μm every minute, resulting in problematic bubbles in only a few minutes after activation. The key is therefore micro-bubble activation. Once activated in super-saturated tissues, DCS-like consequences are almost certain to occur.

We have thus fulfilled our first objective, to model bubble growth at zero to moderate acoustic intensities and high degrees of super saturation, at level predicted to exist in deep, slow diving marine mammals on surfacing. We have found that, provided a micro-bubble nucleus is activated, it expands in a matter of minutes to a size that could cause serious difficulties for the animal.

We now turn to address what might stabilise micro-bubble nuclei and how an acoustic field might disrupt this stabilisation mechanism.

V. MICRO-BUBBLE STABILISATION

The issue of micro-bubble stabilisation is a complex one, and many mechanisms have been proposed to explain how this might occur. One popular hypothesis proposes that micro-bubbles are stabilised in crevices [13,14,15,16] and this may indeed be an operative mechanism without invalidating others operating in parallel. Another idea is that the micro-bubble walls become coated with some material, perhaps a biological surfactant, that inhibits gas permeability across the walls [17,18,19]. As Houser et. al point out [11], "the presence of such coverings seems reasonable as biological fluids contain a number of surface active elements capable of reducing the surface tension of nuclei and models incorporating such concepts match mammalian pressure-reduction data rather well" One role that surfactants on the bubble walls may play is in reducing the surface tension, so that the Laplace pressure no longer acts so strongly to 'squeeze' gas from the bubble. This could help explain why micro-bubble nuclei do not dissolve into solution in tissues, but not why the same micro-bubbles fail to inflate in supersaturated tissues, which clearly they do not (or at least not to the extent that our numerical model results in Fig 2 predict) since large bubbles that would inevitably lead to major physiological disruption would be the result.

An alternative role for surfactants is one of reducing the permeability of the micro-bubble wall that tends to prevent gas exchange in either direction. This would explain both sides of the conundrum. It could also provide a clue as to how an acoustic field might interact and disrupt this function. The important role of the surface agent would then be in decreasing the permeability, rather than the surface tension, in the bubble walls. If protein platelets or some other biological material that might be concentrated on the bubble walls were less permeable, they may also be less flexible, introducing some mechanical modifications to the bubble response to an acoustic field that would otherwise 'pump' the bubble in a 'breathing' mode response.

If a plausible surfactant candidate could be found, the next question might therefore be, what are it's mechanical properties? If the candidate were relatively stiff as a material, and/or if the material were cohesive, then the action of an oscillating acoustic pressure field could open up 'cracks' in the cohesive protective surface on expansion, and close them again on compression. In this scenario, there would be fresh areas of the micro-bubble walls, unprotected by the relatively impermeable surfactant, in the expansion phase of the bubble response. These would permit gas exchange into the bubble by static diffusion, inflating the mean bubble radius. On the compression cycle, the gaps would close and even if the micro-bubble were compressed to the point of increasing the internal pressure above that in the tissues outside, no gas would diffuse out due to the compressed surface layer. The result could be a new kind of rectified diffusion mechanism, driven not by the different areas exposed in the expansion and compression stages of the oscillation, but by the different properties of the surface film.

After a number of such inflationary cycles, the bubble would have increased in size, perhaps to the point where there were always permeable parts of the bubble walls, even at equilibrium radius. Once the micro-bubble had reached this point, the micro-bubble would continue to grow in a super-saturated tissue, even if the acoustic field were discontinued. The micro-bubble would have been 'activated'.

It is here that this speculation requires more input, in establishing plausible biological agents to form a relatively impermeable surface layer, and in modeling the likely mechanical response of this hypothesised layer to an oscillating acoustic pressure field.

VI. BEHAVIOURALLY-INDUCED MICROBUBBLE GROWTH

There is another possibility for inducing DCS-like symptoms in a deep-diving marine mammal that does not require any physical disruption or activation of micro-bubbles. As we have previously noted, a series of short, shallow dives would improve the safety margin for mammals with high supersaturation levels of Nitrogen in their bodies after a long, deep dive. Effectively, they would be de-saturating under a higher ambient pressure that reduces the degree of supersaturation. It is also known that strenuous exercise shortly after diving can induce DCS in humans, possibly through creating cavitation in tissues. What if the presence of a moderately loud acoustic signal changed the behaviour of a marine mammal in such a way that mitigating behaviours were modified to the detriment of the animal?

A loud sound could induce an energetic swimming response ('flight' response), attempting to escape the sound, or just being excited about it in some way. A sound source could also discourage a marine mammal from performing a series of shallow dives, since the Lloyd's mirror effect would likely reduce the intensity very near the surface, and the surface also offers the opportunity for the animal to place its hearing in air rather than in the water, greatly reducing the received sound level. A series of leisurely shallow dives after a long, deep dive would reduce the risk of DCS-like symptoms by providing the marine mammal with the nearest equivalent to decompression stops used by human divers.

Beaked whales are observed to be calm at or near the surface for a while after a long, deep dive, and have been observed to take a number of short, shallow dives after deep diving (pers. comm. K. Balcombe). These activities are consistent with reducing the risk of micro-bubble growth, implying that these animals may be close to the limit of where this can be expected to occur, an unsurprising conclusion given the very high estimates of their supersaturation levels after such activity.

There are entirely plausible mechanisms that might tend to discourage these animals from performing these natural behaviours, and this would be expected to increase their risk. It is therefore plausible that an acoustic field might be a significant factor in triggering micro-bubble growth via a behavioural mechanism.

VII. CONCLUSIONS

Sizeable bubbles may be created in a period of a few minutes by static diffusion, given sufficient super-saturation levels of 150-300% (typical of deep-diving marine mammals on surfacing) and destabilisation of *in vivo* bubble nuclei. The accumulation and aggregation of such bubbles may be sufficient to cause emboli and high, localised pressures in tissues that could result in DCS-like symptoms and at least some of the observed damage in stranded marine mammals exposed to sonar. If micro-bubbles can be destabilized, the impact is likely to be significant in highly gas supersaturated tissues rather than in mildly supersaturated tissues, resulting in DCS-like symptoms. Slow, deep diving marine mammals are therefore expected to be at greater risk of acoustically triggered micro-bubble growth. This is consistent with the observations of deep-diving marine mammals being selectively stranded after receiving moderate acoustic intensities loosely estimated to be in the region of 160-165 dB re 1 μPa.

Crum and Mao did not need to concern themselves with issues of stabilisation since they were interested in exploring the conditions under which rectified diffusion might inflate micro-bubbles, whether in supersaturated tissues or not. Their results showed that rectified diffusion (unassisted by normal diffusion) would not be effective at inflating micro-bubbles below receive acoustic levels of 210 dB re 1 μPa or so. This brings us to the crux of the problem. If the micro-bubbles are somehow stabilised, preventing the Laplace pressure from collapsing them, why do they not always grow in super-saturated liquids? Simple diffusion should be active, driven by the positive pressure gradient into the bubble, causing it to inflate.

We propose that the mechanism by which micro-bubbles are stabilised by blocking the diffusion of gas across the bubble surface interface between the gas and the surrounding liquid, thus preventing bubble collapse under the excess Laplace pressure. In this case, the same mechanism would also tend to prevent super-saturated tissues with a positive pressure gradient into the bubble from inflating it. This proposal thus solves two problems with one proposition; both why micro-bubbles exist in tissues without collapsing, and why micro-bubbles do not inflate until limited by other constraints in mildly super-saturated tissues. If this mechanism, perhaps a biological surfactant layer on the bubble surface, were to be disrupted by an acoustically-driven oscillation of the bubble walls, this could permit ordinary diffusion to inflate the micro-bubble even in the absence of further acoustic impact. Once a micro-bubble becomes marginally inflated, breaking down the stabilising mechanism's ability to prevent gas diffusion across the boundary (i.e. 'activated'), it would continue to grow.

It is known that movement of tissues (such as articulating joints and contracting muscle fibres) can precipitate bubble formation in humans. Proposed mechanisms directly related to bubble formation as a result consist of cavitation effects and, as here, destabilisation of bubble nuclei. SCUBA divers are advised against strenuous exertion during ascent from a dive and after prolonged and deep dives for this reason. One might expect that deep-diving marine mammals would also avoid excessively energetic manoeuvres during ascension through depths at which tissues become supersaturated as well as shortly after a deep dive sequence, observations that have anecdotally been observed. If micro-bubbles can be activated by physical activity, presumably by creating tensions in the tissues, could an acoustic pressure wave act in a similar manner? If so, one might expect vulnerable species to avoid vocalising strongly after deep-diving. This is not known and further research on such behavioural clues would be welcome.

If an acoustic source were to provide a destabilising force to micro-bubbles so that they become able to absorb gas by diffusion across their boundary walls, deep-diving marine mammals with highly super-saturated tissues would be expected to experience bubble growth, the degree of which should vary with the degree of saturation. Pain, disorientation and hemorrhaging (possibly causing vertigo and other vestibular dysfunction) known to occur under certain degrees of bubble formation and growth could then reasonably be expected to cause manifestations similar to that observed in DCS. If experienced, it seems possible that such induce such animals to beach, and to exhibit the kind of injuries found in the Bahamas beached whales.

If there is a mechanism acting approximately in the way this paper suggests, this may explain part of the mystery as to why beaked whales appear to strand when exposed to 'moderate' levels of sound (160-165 dB). While it is true that other inhalation deep-diving marine mammals (such as Sperm whales) have not been observed to beach, this could simply be due to a difference in habitat and behaviour that makes their response more likely to result in sinking in deep water and not being seen. There is also a plausible explanation for non-echolocating marine mammals not to be affected in the same way, because they do not necessarily maintain an operating gas volume at high pressure and depths as echolocating marine mammals are required to do to hunt for prey at depth.

The next step in investigating this potential mechanism is to develop a biological model for the material that might accumulate on the outside of micro-bubbles in marine mammal tissues, especially blood. Experiments with live blood samples in pressure chambers with and without acoustic insonification could also shed considerable light on whether acoustics might play a role in micro-bubble destabilization.

ACKNOWLEDGMENTS

The author is pleased to thank Dorian Houser and many others too numerous to name who have given valuable insight into the biology of marine mammals and the encouragement for this work to have been brought thus far.

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