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Can Diving-induced Tissue Nitrogen Supersaturation Increase the Chance of Acoustically Driven Bubble Growth in Marine Mammals?

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The potential for acoustically mediated causes of stranding in cetaceans (whales and dolphins) is of increasing concern given recent stranding events associated with anthropogenic acoustic activity. We examine a potentially debilitating non-auditory mechanism called rectified diffusion. Rectified diffusion causes gas bubble growth, which in an insonified animal may produce emboli, tissue separation and high, localized pressure in nervous tissue. Using the results of a dolphin dive study and a model of rectified diffusion for low-frequency exposure, we demonstrate that the diving behavior of cetaceans prior to an intense acoustic exposure may increase the chance of rectified diffusion. Specifically, deep diving and slow ascent/descent speed contributes to increased gas-tissue saturation, a condition that amplifies the likelihood of rectified diffusion. The depth of lung collapse limits nitrogen uptake per dive and the surface interval duration influences the amount of nitrogen washout from tissues between dives. Model results suggest that low-frequency rectified diffusion models need to be advanced, that the diving behavior of marine mammals of concern needs to be investigated to identify at-risk animals, and that more intensive studies of gas dynamics within diving marine mammals should be undertaken.

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Introduction

Awareness of effects that anthropogenic sound may have on marine mammals, particularly cetaceans, has increased over the last decade. Concern reached even higher levels in March of 2000 when at least 17 whales representing several species stranded in the Bahamas coincident with naval exercises (OPR, 2000a). Previous mass strandings and entrapments concurrent with naval exercises (Frantzis, 1998; Simmonds & Lopez-Jurado, 1991) and acoustic industrial activity (Todd

et al., 1996) have been reported. These observations have been taken as evidence that, at extreme exposure levels (> 200 dB re : 1 μPa), low-frequency signals (< 5 kHz) like those emitted by some naval sonar systems (Watts, 1999) may detrimentally affect cetacean behavior or physiology.

Research that relates high-intensity, low-frequency sound to impacts on cetaceans has focused principally on potential auditory effects and behavioral disruptions. Auditory effects include temporary or permanent reductions in hearing sensitivity termed threshold shifts (Schlundt *et al.*, 2000). Disruptions of behavior include induced deviation from normal activity,

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startle and avoidance (Richardson et al., 1995). Although never demonstrated in cetaceans, there is ample evidence for humans and laboratory animals that extremely loud sound or prolonged continuous exposure to moderately loud sound may produce damage to the auditory system and cause deafness (Saunders et al., 1991). Non-auditory effects resulting from exposure to high-intensity sound have received much less attention (Ridgway, 1997) though adverse neurologic symptoms have been reported in human divers continuously exposed to water-borne sound at sound pressure levels (SPL) of 160 dB re: 1 µPa at a duration of approximately 12 min (Steevens et al., 1999). It has been hypothesized that non-auditory effects resulting from sound exposure may occur through stimulation of the central nervous system (CNS) via a cochlear path (Landström et al., 1983), vibration of CNS tissue (McIntosh et al., 1989), or through vestibular stimulation (Parker et al., 1978). Another mechanism has only recently been considered. Sound exposure can stimulate bubble growth within biological tissues, particularly if tissues are supersaturated with dissolved gas (Crum & Mao, 1996). In vivo bubble growth in excess of a certain size (~ tens of microns) can result in embolism, tissue separation, and high pressure in localized regions of nervous tissue. Joint pain, disorientation, visual and auditory dysfunction, and other CNS deficits may result, symptoms that are associated with decompression sickness and are presumably caused by similar mechanisms (Moon et al., 1995).

RECTIFIED DIFFUSION AND LOW-FREQUENCY SOUND

In vivo bubble growth caused by ultrasonic exposure has been documented (ter Haar et al., 1982) and its occurrence supported both theoretically (Crum et al., 1987) and experimentally (Daniels et al., 1987). This process, known as rectified diffusion, was hypothesized to be capable of causing bubble growth in human divers and marine mammals exposed to high-intensity, low-frequency sound (Crum & Mao, 1996). Rectified diffusion occurs as a bubble oscillates around its equilibrium radius in response to an acoustic pressure fluctuation to which it is exposed. As the bubble radius expands, the concentration of gas

within the bubble decreases, gas diffuses into the bubble, and effectively increases the bubble volume. As the bubble contracts, the reverse process occurs and gas leaves the bubble. Since diffusion is proportional to the bubble surface area, more gas enters the bubble than exits over an acoustic cycle. This process is augmented by an increase in gas concentration around the bubble during radial expansion, a process known as the "shell" effect (Hsieh & Plesset, 1961). Thus, growth occurs over each acoustic cycle and continues with the duration of the sound exposure.

Crum & Mao (1996) modeled rectified diffusion in mammals based upon the assumption that stabilized nuclei (i.e. microscopic bubbles), ranging in size from 1 to 50 µm, existed *in vivo*. A number of stabilization models have been developed (e.g. crevice and organic skin model) and the reader is referred to Crum & Mao (1996) for a listing of publications pertaining to this subject. Crum and Mao predicted the growth of insonified nuclei by application of the Rayleigh–Plesset equation with specific treatments to the equation according to the intensity of the sound being modeled [see Crum & Mao (1996) for references]. Models used frequencies from 300 to 500 Hz, SPLs from 150 to 220 dB (re: 1 µPa), and dissolved gas saturations from 100 to 200%.

Model results for nuclei with a semi-impermeable organic skin predicted that SPLs of 180 dB could induce bubble growth in solutions both saturated and slightly supersaturated, though growth was modest and occurred at continuous sound exposures on the order of minutes. In contrast, SPLs of 220 dB were predicted to cause a 30-fold increase in bubble size (from 1 to ~ 30 µm) in as little as 10 s of continuous exposure. Supersaturated body fluids were predicted to contribute to bubble growth after acoustic activation of nuclei such that growth would continue after sound exposure ceased. Under such conditions bubble growth would be restricted by tissue boundaries and could potentially exert enough pressure to cause cell and tissue trauma. Crum and Mao concluded that human divers or marine mammals exposed to SPLs greater than 210 dB were potentially susceptible to bubble growth and that those with gas supersaturated body fluids would be susceptible at much lower SPLs.

NITROGEN SATURATION IN MARINE MAMMALS

Given that (1) models predict bubble growth to be a potential hazard to diving mammals exposed to SPLs equivalent to those currently produced in the ocean by anthropogenic sources, (2) that non-auditory neurological disturbances have been identified in human divers exposed to SPLs as low as 160 dB, and (3) that the cause of strandings and entrapments coincident with naval exercises and industrial activity have yet to be determined, further investigation into the potential for bubble growth in marine mammals is warranted. Predictions of bubble growth in marine mammals are largely dependent upon knowing the tissue gas concentration of animals at the time of exposure. Nitrogen is of primary concern because it constitutes almost 79% of the atmospheric air taken in with each breath and it is inert, i.e., it cannot be metabolized and easily accumulates in tissue. Blood nitrogen tensions have been obtained for various pinnipeds (phocid and otariid seals) under forced and free dive conditions (Falke *et al.*, 1985; Kooyman *et al.*, 1972) and muscle nitrogen tensions and nitrogen washout were measured with a mass spectrometer in dolphins trained to perform a series of dives (Ridgway & Howard, 1979). Ridgway and Howard found that two dolphins performing sequential dives to 100 m depths had intramuscular nitrogen tensions of 1300 and 1600 mm Hg at the conclusion of diving, tensions 224% and 276% of the respective initial tension (580 mm Hg). By assuming that nitrogen washin and washout were symmetric, Ridgway and Howard were able to modify equations used in decompression schedules (Workman, 1965) to predict intramuscular nitrogen tensions after a known repetitive dive schedule. In this paper, we use the same model to predict the intramuscular nitrogen tension of several representative cetacean species. We then compare the calculated nitrogen tensions to the results of the Crum and Mao model in order to estimate the potential for acoustically driven bubble growth in these species, under these conditions.

Materials and Methods

The equation used by Ridgway & Howard (1979) is based upon Fick's law for diffusion across a membrane but is integrated with respect to the driving pressure of the gas. This modification was made to account for the rapid ascent and descent rates of the dolphin as opposed to the slow ascending/descending rates of human divers for which the Workman schedules were derived. By assuming that rates of ascent and descent were constant, an assumption reasonably consistent with observed dolphin dive time, the equation then became

$$P_{N_2} = H_0 + R(t - k^{-1}) + e^{-kt}(P_0 - H_0 + Rk^{-1}),$$

where P_{N_2} is the final gas tension in the muscle, P_0 is the initial gas tension, t is time, H_0 is the initial pressure of alveolar nitrogen where $H_0 = 0.78 \times$ (initial hydrostatic pressure $- 46$ mm Hg), R is the rate of change of the driving gas pressure, $R = 0.78 \times$ descent rate (negative for ascent rate) and $k = (\ln 2)/h$, where h is the nitrogen half-time for muscle. Reduction of the initial hydrostatic pressure in the calculation of H_0 accounts for the vapor pressure of water in alveolar air. Initial hydrostatic pressure was assumed to be the pressure exerted upon the lungs at ~ 1 m depth.

Figure 1(a) demonstrates the change in muscle nitrogen tension that occurs during a series of 9 dives to 100 m with a dive time of 1.5 min and surface intervals (SIs) of 1 min (Ridgway & Howard, 1979). Average rate of ascent/descent was thus 2.2 m s^{-1}. Nitrogen half-time used in the calculations was 5.9 min, the average of the half-times observed by Ridgway and Howard, and the initial muscle nitrogen tension was 584 mm Hg. A depth of 70 m was implemented as the depth at which gas exchange with alveolar gas ceased to occur due to lung collapse. Ridgway and Howard calculated this depth as the one providing the best fit between model predictions and the observed nitrogen tension data of the diving dolphins used in their study. Note that by the end of the first dive nitrogen tension is 140% of the pre-dive value and the muscle is supersaturated [Fig. 1(b)]. The degree of supersaturation increases logarithmically with repetitive diving and dissolved gas concentrations are at 267% normal saturation by the end of the ninth dive. Intramuscular nitrogen tension was calculated for two other cetacean species, the northern bottlenose whale (*Hyperoodon ampullatus*), a beaked
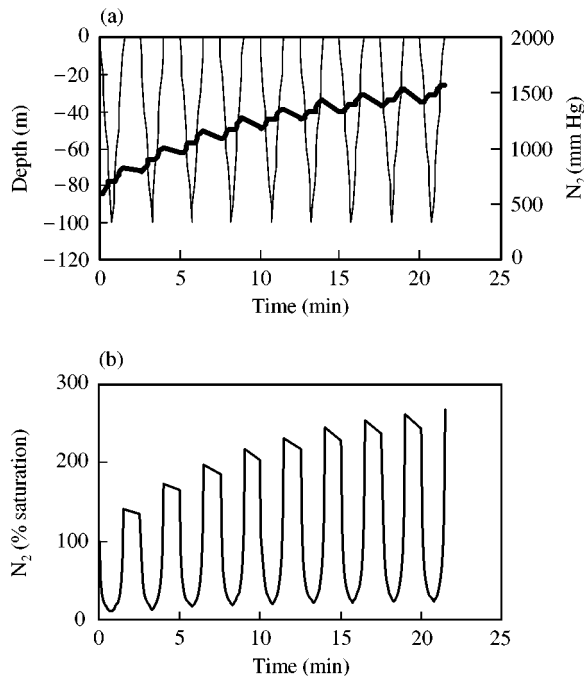
FIG. 1. (a) Dive profile and change in muscle nitrogen tensions for a dolphin completing nine sequential dives to a depth of 100 m; (b) change in muscle nitrogen saturation corresponding to the dive profile given in (a). (——) depth; (▬▬▬) N_2 tension.
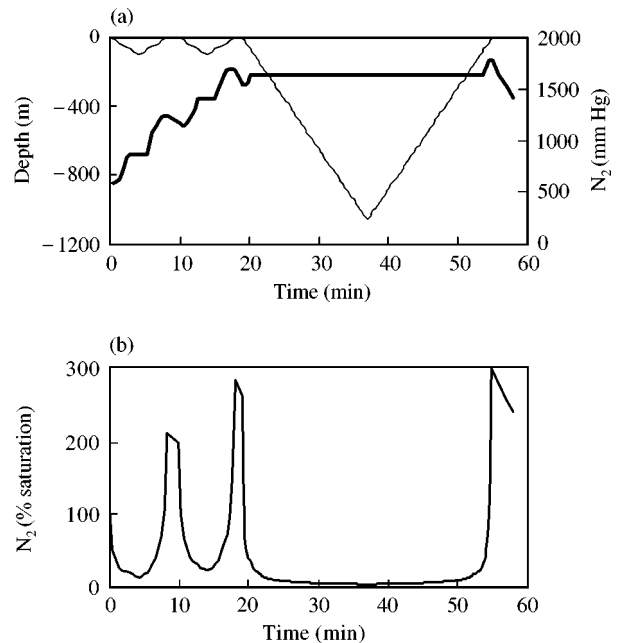

FIG. 2. (a) Predicted dive profile for the northern bottlenose whale and calculated changes in muscle nitrogen tension associated with the dive pattern; (b) change in muscle nitrogen saturation corresponding to the dive profile given in (a). (——) depth; (▬▬▬) N_2 tension.

whale, and the blue whale (*Balaenoptera musculus*), a baleen whale. In each of these predictions it was assumed that the starting intramuscular nitrogen tension and nitrogen half-time were the same as that observed in the dolphin (Ridgway & Howard, 1979) and that alveolar collapse also occurred at 70 m. These assumptions are certainly incorrect to some degree but are necessary since this information is unknown for all cetaceans except the dolphin. For the bottlenose whale, rate of ascent and descent were determined as the average of ascent and descent rates of a TDR-tagged individual (individual 2) monitored by Hooker & Baird (1999). Since Hooker and Baird categorized dives into two groups (short and shallow/long and deep), two sets of ascent/descent rates were used, one for each dive type. Ascent/descent rates for short and shallow dives were $0.6\,\mathrm{m\,s^{-1}}$ while those for long and deep dives were $1.2\,\mathrm{m\,s^{-1}}$. Short and shallow dives had a mean maximum depth of 108 m, while long and deep dives had a mean maximum depth of 1060 m. Figure 2(a) shows a series of three dives similar to the type

observed for a beaked whale (Hooker & Baird, 1999). The dive series consists of two dives to a depth of 100 m followed by a dive to 1060 m. One-minute SIs were used between each of the 100 m dives and the SI was extended to 3 min following the 1060 m dive. Considering all three dives, average dive duration was 13.4 min and the average rate of ascent/descent was $1\,\mathrm{m\,s^{-1}}$.

Diving information on blue whales was obtained from a satellite-tagged individual off the coast of California (Lagerquist *et al.*, 2000). This whale (DEP-1) spent $\sim 78\%$ of its time diving in water $< 16\,\mathrm{m}$ deep and $\sim 1\%$ of its time at depths $\geqslant 98\,\mathrm{m}$. In general, other monitored blue whales displayed similar dive characteristics (Lagerquist *et al.*, 2000). For the model, rate of ascent/descent for the blue whale was determined as the mean duration of dives $> 1\,\mathrm{min}$ (mean $= 6.9\,\mathrm{min}$) divided by the mean depth of dives $> 16\,\mathrm{m}$ (mean $= 105\,\mathrm{m}$); mean rate of ascent/descent was $0.5\,\mathrm{m\,s^{-1}}$. Figure 3(a) displays a series of dives generally consistent with the observed dive behavior of the blue whale: a series of 10 shallow dives to a depth of 10 m with alternating SIs of 0.1 and 0.5 min, a single deep dive to 100 m
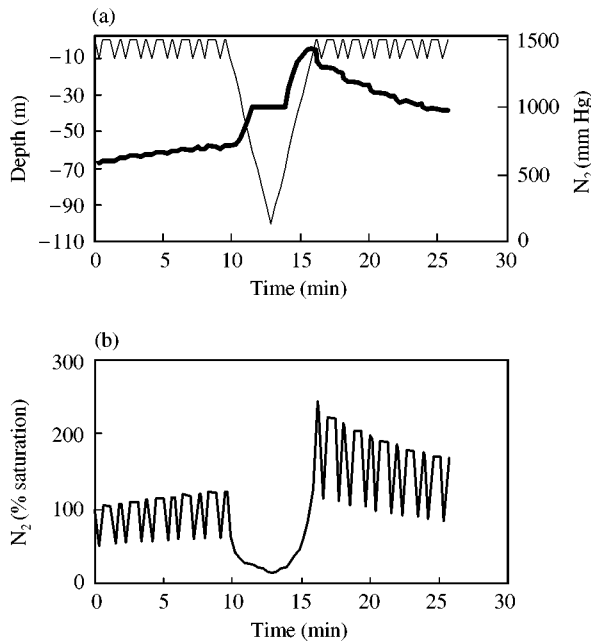
FIG. 3. (a) Predicted dive profile for the blue whale and calculated changes in muscle nitrogen tension associated with the dive pattern; (b) change in muscle nitrogen saturation corresponding to the dive profile given in (a). (——) depth; (▬▬) N_2 tension.
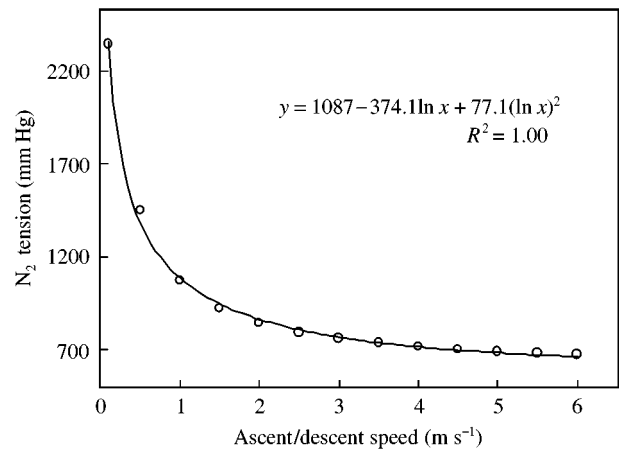

FIG. 4. Relationship between ascent/descent speed and muscle nitrogen tension upon surfacing from a dive to 100 m. All variables except ascent/descent speed were equal to those used in the initial bottlenose dolphin model (Fig. 1).

followed by an SI of 1.0 min, and a repeat series of 10 m dives. The average SI for the dive pattern was 0.35 min and the average dive duration was 0.9 min.

Variations of the dolphin nitrogen uptake model are presented in order to illustrate the influence that each variable has on nitrogen dynamics. The reader is referred to Fig. 1(a) and (b) to contrast results of the parameter variation with the initial dolphin model. Rates of ascent/descent were varied from 0.1 to 6.0 m s^{-1} in order to determine the relationship between ascent/descent rate and nitrogen uptake. Muscle nitrogen tension and nitrogen saturation for a dolphin ascending/descending at 4.4 and 1.1 m s^{-1} were modeled as a demonstration [Fig. 5(a) and (b)]. These rates of ascent/descent are, respectively, twice and one-half the rate used in the initial bottlenose dolphin model (Fig. 1). Similar exercises are presented to demonstrate the relationship between the depth at which lung collapse occurs and nitrogen uptake and between SI duration and nitrogen clearance from muscle tissue. For the former, the depth of lung collapse was varied between 10 and 90 m over a single dive

and the amount of nitrogen upon surfacing compared to the depth of lung collapse. For the latter, the SI was varied between 0.1 and 10.0 min and the amount of nitrogen washout over the interval plotted as a function of interval duration. In each of these exercises, all parameters except the one explicitly varied were equal to those used in the initial bottlenose dolphin model (Fig. 1).

Results

Changes in intramuscular nitrogen and nitrogen saturation that accompany the beaked whale dive profile are presented in Fig. 2(a) and (b), respectively. Hydrostatic pressure increases during dive descent and increases the degree to which muscle tissue can dissolve nitrogen. A slower rate of nitrogen uptake relative to increasing nitrogen solubility, in combination with the elimination of nitrogen uptake below 70 m (lung collapse), reduces relative tissue saturation during descent. The effect of lung collapse is clearly demonstrated in the nitrogen uptake profile of the beaked whale [Fig. 2(a)] as a horizontal line at ~2, 8 and, most apparently, at 13 min into the dive sequence. At dives below the depth of lung collapse, the absolute amount of nitrogen in muscle tissue remains constant but the relative tissue saturation declines. During ascent, the equilibrium level of saturation declines with decreasing hydrostatic pressure, but the loss of

nitrogen from muscle tissue remains dispropor-
tionately slow. Supersaturation results as evid-
enced by the spike in saturation percent during
surfacing. Rapid washout during the SI occurs as
muscle nitrogen tension declines towards surface
equilibrium levels.

During the deepest portions of diving, when
pressure and gas solubility are the greatest, nitro-
gen tissue saturation is $\sim 20\%$ (100 m dives) and
$\sim 3\%$ (1060 m dive) of the equilibrium tension.
However, muscle tissue is saturated within 10 m
of the surface upon ascent from the first dive and
is supersaturated at the surface [213% satura-
tion, Fig. 2(b)]. Accumulation of nitrogen con-
tinues on subsequent dives, and during ascent
from the second dive, saturation is approached
20 m below the surface (98%) and supersatura-
tion is apparent both at 10 m (148%) and at
the surface (287%). Following the deep dive to
1060 m, intramuscular nitrogen tension at the
surface is $>300\%$ the saturation level. Decline
in tissue saturation at the surface is rapid;
saturation declines from $>300\%$ to $\sim 240\%$
within the first 3 min of surfacing from the final
dive.

Changes in intramuscular nitrogen and nitro-
gen saturation that accompany the blue whale
dive profile are presented in Fig. 3(a) and (b),
respectively. In contrast to the beaked whale, the
blue whale does not exceed the assumed depth of
lung collapse on any but one of the dives. Al-
though nitrogen tissue saturation fluctuates with
he periodicity of the first 10 dives [Fig. 3(b)],
alveolar gas exchange is permitted throughout
the dive series and nitrogen tension gradually
increases [Fig. 3(a)]. Prior to the 100 m dive,
supersaturation reaches a level of $\sim 120\%$. Dur-
ing the 100 m dive a rapid increase in nitrogen
tension occurs up to the assumed point of lung
collapse, at ~ 12 min into the dive, and resumes
at ~ 14 min into the dive. Upon surfacing,
muscle nitrogen saturation is double than exist-
ing prior to the dive. Gas saturation declines
quickly as shallow diving is resumed because of
a reduction in pressure near the surface.

The rate of ascent/descent is inversely related
to nitrogen uptake, as demonstrated in Fig. 4.
For a single dive with an initial muscle nitrogen
tension of 584 mm Hg, nitrogen uptake logarith-
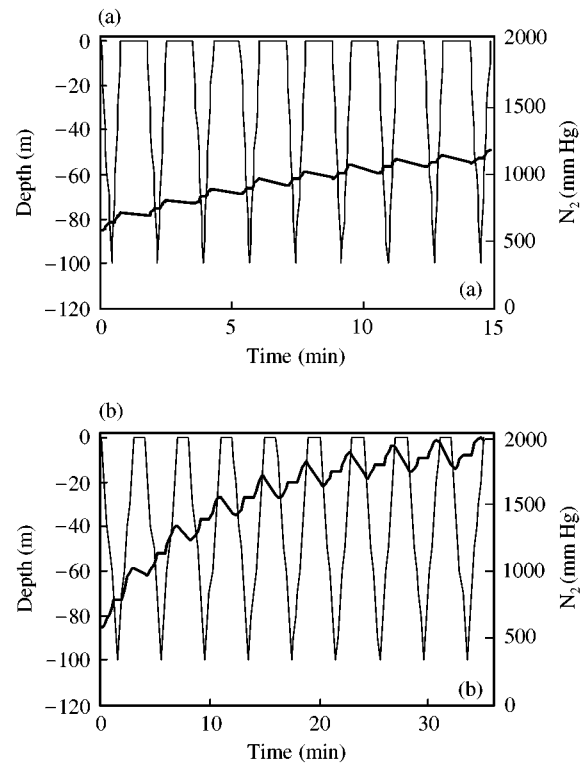mically increases with decreasing rates of

FIG. 5. Demonstration of the rate of nitrogen uptake for
a series of bottlenose dolphin dives when the rate of as-
cent/descent is (a) increased to 4.4 m s^{-1} and (b) decreased to
1.1 m s^{-1}. All variables except ascent/descent speed were
equal to those used in the initial bottlenose dolphin model
(Fig. 1). (——) diving; (▬▬▬) N$_2$ tension.

ascent/descent. At an ascent/descent rate of
4.4 m s^{-1}, nitrogen tension at the conclusion
of the dive bout is approximately double the
pre-dive nitrogen tension [Fig. 5(a)]. Although
muscle tissue is still supersaturated (201% satu-
ration), the saturation level is considerably less
than that achieved when rates are 2.2 m s^{-1}
(267% saturation, Fig. 1b) and 1.1 m s^{-1} [340%
saturation, Fig. 5(b)]. Slower rates of ascent/
descent increase the residency time between the
surface and the depth of lung collapse. Thus,
depending on the degree of nitrogen saturation at
the beginning of the dive, exposure to hydrostatic
pressures capable of driving tissue uptake may be
prolonged.

Lung collapse at depths greater than 70 m
increases the range of hydrostatic pressure pro-
moting nitrogen uptake. The relationship is near
linear; a 10 m increase in the depth of lung col-
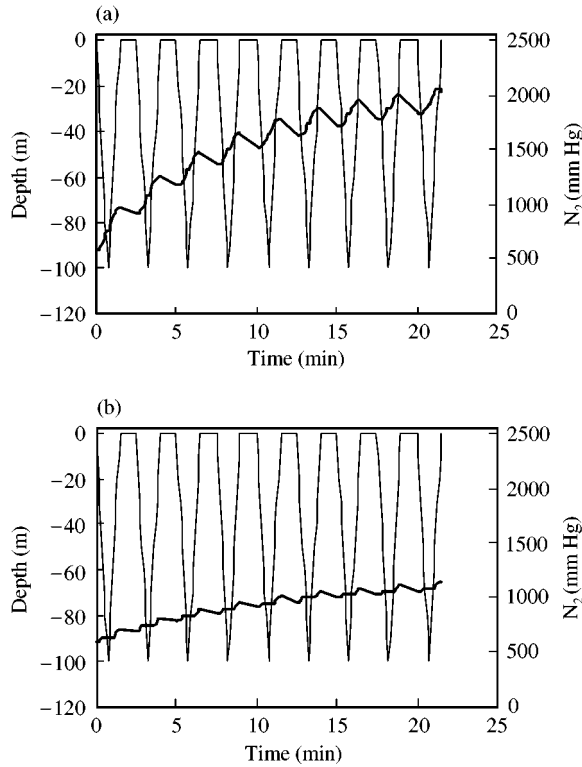lapse increases the equilibrium level of saturation

FIG. 6. Demonstration of the rate of nitrogen uptake for a series of bottlenose dolphin dives when the depth of lung collapse is (a) increased to 90 m and (b) decreased to 50 m. All variables except the depth of lung collapse were equal to those used in the initial bottlenose dolphin model (Fig. 1). (——) depth; (━━━) N_2 tension.
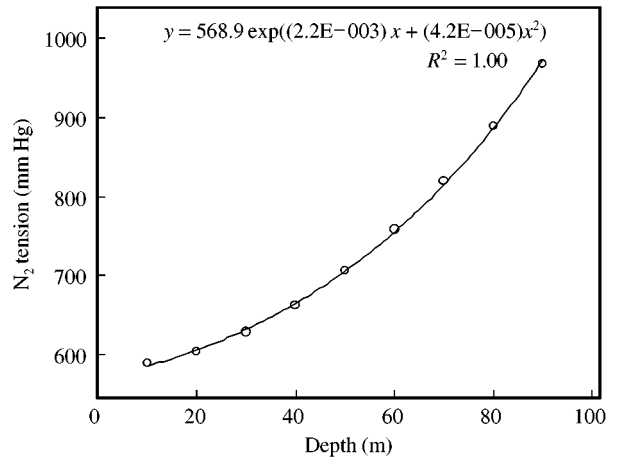

FIG. 7 Relationship between the depth of lung collapse and muscle nitrogen tension upon surfacing from a dive to 100 m. All variables except the depth of lung collapse were equal to those used in the initial bottlenose dolphin model (Fig. 1).
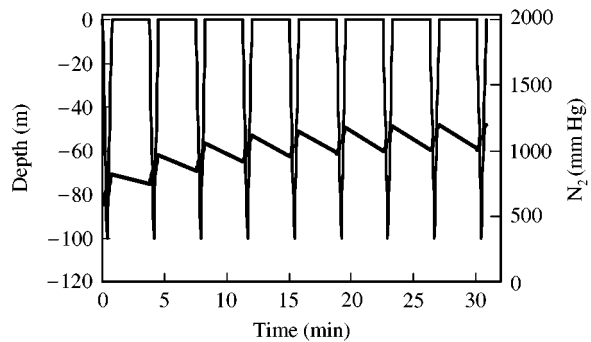

FIG. 8. Demonstration of the rate of nitrogen uptake for a series of bottlenose dolphin dives when the SI is extended to 3 min. All variables except the SI duration were equal to those used in the initial bottlenose dolphin model (Fig. 1). (——) depth; (━━━) N_2 tension.

by ~ 585 mm Hg. For example, using the dolphin diving pattern presented in Fig. 1 but extending lung collapse to a depth of 90 m, the rate of nitrogen uptake on any dive increases [Fig. 6(a)]. By the end of the dive series, muscle nitrogen saturation at the surface is 2043 mm Hg, or $\sim 350\%$ of surface equilibrium levels. Conversely, if lung collapse occurs at a shallower depth, the potential driving pressure is reduced and the rate of nitrogen accumulation is slowed. Reducing the depth of lung collapse to 50 m in the dolphin model reduces muscle nitrogen tension at the end of the dive series to ~ 1136 mm Hg [Fig. 6(b)], or 195% of the surface equilibrium saturation level. The relationship between the depth of lung collapse and nitrogen uptake at the end of a single dive (dolphin model) is demonstrated in Fig. 7. For the type of "bounce" dive from which these calculations are drawn, nitrogen saturation upon surfacing increases as a

second-order exponential function of the depth of lung collapse.

Extending the duration of the SI after a dive increases the amount of nitrogen cleared from the muscle prior to a subsequent dive. This is demonstrated in Fig. 8 where the SI of the initial dolphin model is extended to 3 min. Nitrogen tension at the end of the dive series is 1198 mm Hg, or 205% of the surface equilibrium level. By way of comparison, the initial dolphin model, in which the SI was 1 min, produced a nitrogen tension of 1559 mm Hg. Although nitrogen clearance increases as a logarithmic function of SI duration, clearance is obviously asymptotic (Fig. 9). Muscle

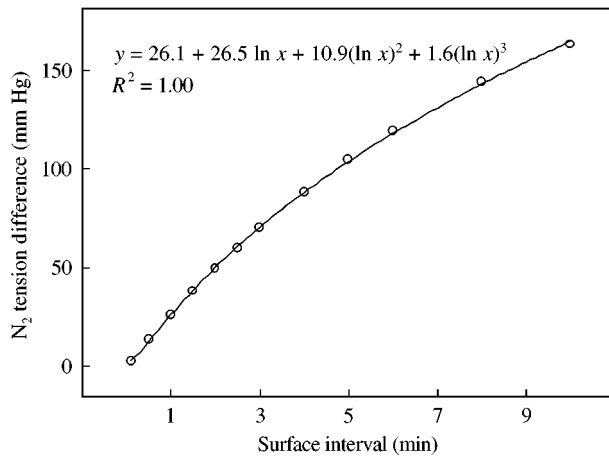$$y = 26.1 + 26.5 \ln x + 10.9(\ln x)^2 + 1.6(\ln x)^3$$
$$R^2 = 1.00$$

FIG. 9 Relationship between SI duration and the difference in muscle nitrogen tension upon surfacing from a single dive and just prior to a subsequent dive. For the single dive, all variables were equal to those used in the initial bottlenose dolphin model (Fig. 1).

nitrogen tensions will not fall below the surface equilibrium tension. The rate of clearance during an SI is thus heavily dependent upon the difference between muscle nitrogen tension at surfacing and the surface equilibrium tension.

Discussion

The proposition that bubble nuclei exist in biological tissues is generally accepted (Vann & Clark, 1975). Although it has been argued that diving marine mammals may crush bubble nuclei through repetitive diving, the issue remains unresolved (Mackay *et al.*, 1982). The mechanism of nuclei generation also remains unresolved, though tribonucleation (Hayward, 1967) and homogeneous nucleation under gas supersaturation (Weathersby *et al.*, 1982) have been offered as candidate mechanisms. Furthermore, previous commentary has indicated the potential for acoustic pressure fields to cause cavitation in mammalian body fluids (Mackay *et al.*, 1982). Assuming the nuclei exist in marine mammals, Crum & Mao (1996) investigated the potential for rectified diffusion under a number of conditions. This discussion will be limited to their investigations in which a semi-impermeable covering of the nuclei was assumed. 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 (Hemmingsen, 1978) and models incorporating such concepts match mammalian pressure-reduction data rather well (Weathersby *et al.*, 1982).

Crum & Mao (1996) demonstrated that at normal fluid saturations bubble growth could be initiated by SPLs as low as 180 dB but that significant growth and rapid growth rate were not demonstrated until exposure levels $\geqslant 200$ dB. At exposures < 200 dB a significant amount of continuous exposure time (100s of seconds) was necessary to achieve modest growth. At exposures $\geqslant 200$ dB the continuous exposure time necessary to cause a significant increase in bubble size declined dramatically with increasing SPL. Bubble size approximately doubled at an SPL of 210 dB and a continuous exposure time of ~ 100 s and quintupled at an SPL of 220 dB and continuous exposure time < 30 s. Moderate supersaturation under the same conditions resulted in considerable bubble growth at lower SPLs and shorter exposure times. For example, a 1 μm bubble (nucleus) existing in a fluid with a dissolved gas concentration of 125% and exposed to a continuous 500 Hz driving frequency was predicted to increase its diameter 20-fold in ~ 20 s with exposure levels ranging from 150 to 190 dB. Herein lies the greatest potential danger for diving marine mammals—at levels of supersaturation, bubble growth was predicted to be driven by static diffusion and could continue in the absence of an acoustic field. Thus, activation of nuclei by an acoustic signal could have severe consequences for a marine mammal with gas-supersaturated tissue as bubbles will continue to grow until restricted by tissue boundaries. Such bubbles can exhibit high local pressures resulting in vascular blockage and tissue damage that may ultimately lead to symptoms of decompression sickness.

Intramuscular nitrogen tension calculated for the dolphin, beaked whale, and blue whale all exceed the level of supersaturation (200%) for which rectified diffusion was modeled by Crum & Mao (1996). In the case of the beaked whale, gas concentrations could exceed 300% by the conclusion of a dive sequence. Once activated by an acoustic signal, supersaturation to this degree would drive rapid bubble growth via static

diffusion. Crum and Mao were not aware of the earlier study of Ridgway & Howard (1979) and did not make calculations using available cetacean data. As a result, their models did not include gas saturations approaching 300% and relatively little is reported for concentrations > 127%. It would be beneficial to extend the Crum and Mao model to predict the potential for the acoustic activation of nuclei and the effect of time-dependent acoustic exposure on bubble growth when gas concentrations are at levels predicted for diving cetaceans. This is particularly important since an intuitive extrapolation of their model predictions suggests significant tissue damage within cetaceans is possible if nuclei are activated while surfacing from a dive or dive bout.

Species-specific dive behavior has a profound effect upon tissue-gas concentration and should influence the susceptibility of species to the potential effects of acoustic exposure. The depth of dives, up to the depth at which lung collapse occurs, and ascent and descent rates are the primary factors determining susceptibility of a species on a given dive. The surface interval is important when multiple dives are considered as time at the surface is directly related to gas clearance and the initial gas tension on subsequent dives. For instance, the rate of ascent/descent for the northern bottlenose whale is approximately three to four times slower than that of the bottlenose dolphin allowing a greater amount of gas exchange with alveolar gas to occur for a given distance of travel. Depth of diving for the bottlenose whale also exceeds the depth at which lung collapse is presumed to occur on a consistent basis. Thus, the dive pattern of the northern bottlenose whale is such that muscle nitrogen tension is assumed to be high at the end of any typical dive, and extremely high while surfacing from a bout of dives that does not contain an extended SI. This cetacean could be a high-risk candidate for acoustically mediated bubble formation/growth, as could other beaked whales engaging similar dive behaviors. The sperm whale (*Physeter catodon*) also falls in this category. Sperm whales consistently dive to several hundreds of meters, certainly beyond depths at which lung collapse occurs, and demonstrate diving rates comparable to the northern bottlenose

whale (Watkins *et al.*, 1993). High intramuscular nitrogen tensions would be expected upon surfacing, but sperm whales regularly undertake prolonged SIs (>8 min) such that much of the nitrogen would be cleared before a subsequent dive (Watkins *et al.*, 1993). Thus, accumulation of nitrogen would not be compounded from dive to dive to the same extent as it would in species demonstrating shorter SIs.

Most other odontocete species should be at a lesser potential risk than the beaked and sperm whales. Research on free-ranging dolphins (Davis *et al.*, 1996), porpoises (Otani *et al.*, 1998), and belugas (Martin & Smith, 1992) indicates that, although they may dive beyond the assumed 70 m depth at which lung collapse occurs, these animals spend much of their time at depths less than 30 m. Furthermore, ascent and descent rates of these animals are generally much faster than that reported for beaked and sperm whales (Martin & Smith, 1992; Otani *et al.*, 1998; Ridgway & Howard, 1979). Thus, the animal would on average experience a smaller absolute driving pressure for gas uptake (i.e. shallower depth) and spend less time at a depth at which gas uptake would occur (i.e. faster diving). Relative to the beaked whale, tissues would have a lesser degree of gas supersaturation and the potential for bubble growth resulting from acoustic exposure would be lower.

Blue whales (a baleen whale) spend most of their time within 70 m of the surface with deeper diving events occurring infrequently (Lagerquist *et al.*, 2000). Similar diving patterns are generally accepted for baleen whales as a group (Gaskin, 1982). As with most other toothed whales, baleen whales that spend more time close to the surface will accumulate less nitrogen in muscle tissue than those diving near the depth at which lung collapse occurs. However, rates of ascent/descent recorded in diving baleen whales can vary dramatically across species [e.g. mean rate of 0.5 m s^{-1} for blue whale reported by Lagerquist *et al.* (2000) vs. a mean rate of 2.7 m s^{-1} for the fin whale as reported by Panigada *et al.* (1999)] making a generalized prediction about baleen whales difficult. In order to make species-specific estimates of bubble growth potential under acoustic exposure, studies are needed to determine the species-specific dive behavior of baleen

whales in oceanographic regions likely to be exposed to high intensity, low-frequency sounds.

The gray whale (*Eschrichtius robustus*) is known to feed on benthic prey at depths less than 50 m (Oliver *et al.*, 1984). This cetacean is likely to spend more time at depths above which lung collapse occurs than do deep diving cetaceans that rapidly exceed lung collapse depth while diving. Such diving behavior should promote considerable nitrogen uptake, potentially in excess of cetaceans making repetitive dives to deeper depths. Obtaining saturation at depth (e.g. at 50 m saturation ~ 3483 mm Hg) is theoretically possible, but whales would have to spend a considerable amount of time at depth. For example, maintaining the assumptions used in the initial dolphin model, a gray whale foraging at 50 m depth would need to spend more than an hour at depth to approach complete saturation. This is necessary because the magnitude of the difference gradient between muscle and alveolar gas declines with the progression of nitrogen uptake. Still, any whale spending even several minutes at depths above the depth of lung collapse, but deep enough to experience a strong driving pressure for gas uptake, will have gas-supersaturated muscle upon surfacing. This reinforces the importance of species-specific dive behavior in evaluating the potential risk of rectified diffusion to insonified diving cetaceans. Understanding how species-specific behaviors supporting individual and population survival (e.g. foraging and breeding) are reflected in diving patterns, and how these behaviors occur both spatially and temporally, are significant components of this risk assessment and should not be underestimated.

It is worth noting that the cetaceans predicted to be most susceptible to rectified diffusion, the beaked whales (Family Ziphiidae), are the prominent species observed to have stranded coincident with naval activity. Twelve Cuvier's beaked whales (*Ziphius cavirostris*) and a Gervais' beaked whale (*Mesoplodon europaeus*) stranded in the Canary Islands in 1985, and in 1988, three *Ziphius* spp. and a northern bottlenose whale stranded in the same region (Simmonds & Lopez-Jurado, 1991). Naval maneuvers were observed in the vicinity near the time of the stranding events, although no confirmation of acoustic activity by participating vessels was made in either case. In 1996, 12 Cuvier's beaked whales stranded along the coasts of the Kyparissiakos Gulf (Frantzis, 1998), and in 2000, six Cuvier's beaked whales and a dense beak whale (*Mesoplodon densirostris*) stranded and died in the Bahamas (OPR, 2000a). In these latter two cases, respectively, military testing of low-frequency active sonar (Frantzis, 1998) and the use of tactical sonar systems for military exercises (OPR, 2000a) were reported to have occurred around the time of the stranding event. With respect to the hypothesis presented here, the correspondence of these events with the stranding of beaked whales should be handled cautiously. The potential for rectified diffusion to cause tissue trauma, or possibly symptoms similar to decompression sickness, is one of several hypotheses explaining the potential relationship between anthropogenic acoustic activity and cetacean stranding events. It is currently unsupported by empirical evidence. However, observation of tissue damage in non-auditory organs of the beaked whales that stranded in the Bahamas (OPR, 2000b) argues for further investigation into non-auditory mechanisms of sound-induced trauma.

Several assumptions based on studies of the bottlenose dolphin are made in applying the nitrogen tension model to other cetaceans. First, it is assumed that lung collapse occurs at a depth of 70 m. Lung collapse in deep diving marine mammals is generally accepted and has been supported by studies of arterial blood nitrogen tension under dive conditions (Falke *et al.*, 1985; Ridgway & Howard, 1979), hyperbaric compression of an odontocete carcass (Hui, 1975), through biophysical models (Brown & Butler, 2000) and photo identification of the phenomenon (Ridgway *et al.*, 1969). Dolphins are known to dive with a lung full of air (Ridgway *et al.*, 1969). If some species exhale part of their lung air before diving, this would effect a shallower lung collapse. The depth at which lung collapse occurs will also be affected by the structural characteristics of the respiratory system, the anatomy of which varies significantly from species to species [for example, see Wislocki & Belanger (1960)]. The depth at which lung collapse occurs affects nitrogen uptake during a dive and erroneous assumptions about it can profoundly affect the outcome of the nitrogen tension model. The

second major assumption in the nitrogen tension model is that nitrogen half-times are the same across all species. Species variability in muscle nitrogen half-time will be affected by the degree of muscle perfusion and the lipid content of the muscle, the latter affecting its nitrophilic nature. Within an animal, nitrogen half-time will also vary between tissue compartments resulting in differential rates of nitrogen accumulation and, consequently, different potentials for rectified diffusion. It is unlikely that anything immediate can be done to address lung collapse in non-odontocete cetaceans, but tissue-specific rates of nitrogen washin and washout are addressable using trained cetaceans (Ridgway & Howard, 1979). This information would improve nitrogen uptake models, allow them to be tissue-specific, and provide an estimate of the variability in nitrogen half-times across a number of species.

Determination of whether bubbles spontaneously occur in deep diving marine mammals would provide support for the bubble growth by rectified diffusion hypothesis, their occurrence proving either the pre-existence of nuclei prior to diving or nuclei generation during diving. The occurrence of bubbles following a dive can be determined through the application of diagnostic ultrasound. Ultrasound is clinically used on humans to diagnose the presence of gas bubbles within the blood stream and other tissues and application to trained marine mammals following the performance of a deep diving routine is wholly feasible. In addition, portable diagnostic ultrasound scanners could be used to detect bubbles in stranded marine mammals. Realistically, the ultrasound would need to be available for use at the time of the stranding and the stranded cetacean would need to be sufficiently small for the ultrasound to penetrate to tissues and organs of interest. However, accomplishing this task on a stranded cetacean that is suspected of having been exposed to an intense acoustic event might provide the most conclusive evidence as to whether rectified diffusion is a deleterious phenomenon in acoustically exposed cetaceans.

Predictions of the potential for rectified diffusion to occur in marine mammals exposed to water-borne sound would benefit from an extension and reevaluation of the Crum & Mao model

(1996). Their original work addressed continuous signals but indicated the possibility for bubble growth in a gas-supersaturated medium once bubble nuclei were activated. Most sonar signals (Watts, 1999) and many other anthropogenic signals are short in duration and intermittent and it would be appropriate to include signals of these types into the Crum and Mao model in order to predict their potential for activating nuclei. Furthermore, in calculations where bubble nuclei were considered to be covered with surface-active elements of biological origin, the surface tension on the nuclei was considered to be zero. The surface tension experienced by bubble nuclei within an organism more likely lies somewhere between zero and that of nuclei in water. This factor should be of particular consideration given that biochemical adaptations in cetacean blood, for example a potent heparin, have been proposed to protect cetaceans from bubble growth and embolism (McCormick et al., 1973). Certainly the prospect for rectified diffusion exists, particularly given the expected degree of gas supersaturation after some diving event, but the application of model predictions to real-world scenarios would benefit from reevaluation under more realistic conditions.

The diving behavior of a cetacean at the time of acoustic exposure may be the greatest indicator of the potential for rectified diffusion. Diving speed and depth of diving are the primary determinants of tissue nitrogen accumulation, with slower rates of ascent/descent and deeper dives (within lung collapse depths) resulting in greater amounts of gas supersaturation. Acoustic activation or generation of nuclei prior to surfacing, or at the surface, can theoretically result in rapid bubble growth driven by both the degree of supersaturation and continued sound exposure. Bubble growth has the potential to cause tissue damage and vascular blockage and may underlie cetacean stranding events associated with acoustic exposure. It is currently unknown whether bubble formation and growth occurs in acoustically exposed cetaceans or if the size of formed bubbles is significant enough to have detrimental consequences. However, the theoretical basis for its likelihood, the observance of non-auditory effects in human divers exposed to high-intensity sound, and the stranding of cetacean species

coincident with intense acoustic events, both industrial and military, prompts further investigation into the subject.

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