COMMENTARY

Improving estimates of diving lung volume in air-breathing marine vertebrates

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ABSTRACT

The air volume in the respiratory system of marine tetrapods provides a store of O_2 to fuel aerobic metabolism during dives; however, it can also be a liability, as the associated N_2 can increase the risk of decompression sickness. In order to more fully understand the physiological limitations of different air-breathing marine vertebrates, it is therefore important to be able to accurately estimate the air volume in the respiratory system during diving. One method that has been used to do so is to calculate the air volume from glide phases – periods of movement during which no thrust is produced by the animal – which many species conduct during ascent periods, when gases are expanding owing to decreasing hydrostatic pressure. This method assumes that there is conservation of mass in the respiratory system, with volume changes only driven by pressure. In this Commentary, we use previously published data to argue that both the respiratory quotient and differences in tissue and blood gas solubility potentially alter the mass balance in the respiratory system throughout a dive. Therefore, near the end of a dive, the measured volume of gas at a given pressure may be 12–50% less than from the start of the dive; the actual difference will depend on the length of the dive, the cardiac output, the pulmonary shunt and the metabolic rate. Novel methods and improved understanding of diving physiology will be required to verify the size of the effects described here and to more accurately estimate the volume of gas inhaled at the start of a dive.

KEY WORDS: Respiratory physiology, Lung function, Diving physiology, Gas exchange

Introduction

Imagine it is a warm summer day and you are getting ready to dive down to the bottom of a deep pool. You prepare mentally, take a deep breath and kick off from the surface. For a human, with limited breath-hold capacity, it may seem obvious that you would dive with your lungs filled with air in order to maximize the amount of O_2 to sustain your dive. However, for air-breathing vertebrates that continuously have to leave the surface to obtain food underwater there are conflicting reports regarding whether they inhale or exhale before diving to depth. The general idea is that cetaceans and otariids inhale, whereas phocid seals exhale before diving (Ponganis, 2011). In theory, diving on inhalation does increase the O_2 stores, which would help to extend the duration of the breath hold; however, the increasing pressure during descent compresses

the lungs to the limit of collapse, which prevents gas exchange and limits O_2 uptake (Kooyman and Sinnett, 1982; McDonald and Ponganis, 2012). In addition, more air in the lungs also means more N_2. When N_2 is taken up into the blood stream and tissues under pressure, there is an increased risk that bubbles will form during the ascent, causing 'the bends' or decompression sickness (see Glossary). Thus, for ecophysiologists, understanding how marine mammals breathe while at sea and before diving has long been of interest, although it has been difficult to study.

In this Commentary, we briefly summarize current methods that have been used to estimate the air volume in the respiratory system of air-breathing marine vertebrates while diving. We first provide details of a method that has been developed to estimate the diving respiratory air volume (DRAV; see Glossary) from data obtained from biologging data loggers (see Glossary) in diving birds and marine mammals during periods when the animal does not add thrust but movement is produced by changes in buoyancy (Miller et al., 2004; Sato et al., 2002, 2011). We then use results from previously published theoretical studies on how lung and tissue gas content changes during breath-hold dives to show how the respiratory quotient (RQ; see Glossary) and differences in gas solubility in gas and liquid phase result in temporal changes in the mass balance of gas in the respiratory system. When considered together, these two different approaches provide improved estimates of the air volume in the respiratory system during diving; this presents new possibilities for future studies that will help enhance our understanding of the physiological limitations of these marine species. Although most of this Commentary is about marine mammals, we refer to these gas volume estimates as 'respiratory air volume' (RAV; see Glossary) rather than 'lung volume', to highlight the fact that similar mass-balance changes would occur during dives in other air-breathing species (such as diving birds, which have air sacs as well as parabronchi).

Tidal volume and total lung capacity in air-breathing marine vertebrates

Early measurements of lung function and respiratory capacity in marine mammals were performed by Scholander during forced diving experiments in the grey seal (*Halichoerus grypus*) and harbor porpoise (*Phocoena phocoena*) (Scholander, 1940), and in a semi-restrained bottlenose dolphin by Irving et al. (1941). These early studies, and those that have followed, have shown that the respiratory capacities and breathing strategies of marine mammals are very different from those of terrestrial species: marine mammals have lower breathing frequencies and higher tidal volumes (see Glossary) (Fahlman et al., 2020a, 2017; Irving et al., 1941; Mortola and Limoges, 2006; Scholander, 1940). Studies on respiratory function and tidal volumes in other air-breathing marine vertebrates (e.g. penguins and turtles) are few in number, but suggest that total lung capacity (TLC, liters; see Glossary) is greater than that of both terrestrial and marine mammals, while the tidal volume and

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Glossary

Biologging
The tracking of individual animals by attaching a data recorder that archives the data.

Breathing frequency
The number of breaths over a given duration, normally in breaths min^{-1}.

Decompression sickness
Symptoms seen in divers if the ascent rate is too fast, causing bubbles to form in the blood as the gas solubility decreases faster than the gas.

Diving respiratory air volume (DRAV)
The volume of air in the respiratory system of a mammal, sea bird or turtle during a dive.

Field metabolic rate (FMR)
An animal's metabolic rate that includes all different activities of their daily life.

Pulmonary shunt
A measure of the efficiency of gas exchange across the alveoli/parabronchi/falveoli and pulmonary capillary. No shunt means that the blood gas tension when leaving the capillary is the same as the partial pressure inside the alveoli/parabronchi/falveoli. A complete shunt means that there is no exchange of gas.

Respiratory air volume
The volume of air in the respiratory system.

Respiratory quotient (RQ)
The ratio $\dot{V}_{CO_2}/\dot{V}_{O_2}$, which provides an index of the fuel source used in aerobic metabolism.

Total lung capacity (TLC)
The maximal volume of air in the respiratory system.

Tidal volume
The volume of gas inhaled or exhaled during each breath.

Vagal tone
The activity of the vagus nerve, an important nerve in the parasympathetic nervous system.

Vital capacity
The maximal volume of air that can be exhaled after a full inhalation.

breathing frequency (see Glossary) appears similar to that of marine mammals (Chappell and Souza, 1988; Halsey et al., 2008; Lutcavage et al., 1989; Ponganis et al., 2015; Portugues et al., 2018; Tenney et al., 1974; Wilson et al., 2003).

Estimates of TLC have been made from experimentally inflated excised lungs in a number of species of both shallow- and deep-diving marine mammals (e.g. harbor porpoise and pilot whale, respectively); from these data, the equation to derive estimated TLC (TLC$_{est}$) was given as:

$$TLC_{est} = 0.135 M_b^{0.92}, \qquad (1)$$

where M_b is body mass in kg (Fahlman et al., 2011; Kooyman, 1973). In contrast, Scholander (1940) made preliminary measurements of TLC from excised lungs of a deep-diving bottlenose whale (*Hyperoodon rostratus*), and estimated that the mass-specific TLC was approximately 29 ml kg^{-1}. This is considerably smaller compared with predictions made using the TLC$_{est}$ equation.

Inflating excised lungs provides some estimate of TLC, but there are limitations to the ability of this method to estimate the true TLC in live animals. Although these studies have advanced our understanding of respiratory physiology in air-breathing marine vertebrates (Fahlman et al., 2017, 2011; Kooyman, 1973; Mortola and Limoges, 2006; Piscitelli et al., 2013), measurements of respiratory volume on free-ranging animals during diving have been limited. In addition, such measurements are mostly based on the assumption that the tidal volume is close to the vital capacity (see Glossary) for most breaths; however, this assumption contradicts

current estimates of respiratory volume, at least in marine mammals, both from studies from animals in professional care and from studies on free-ranging dolphins and sea lions (Fahlman et al., 2016, 2019a, 2020a,b; McDonald and Ponganis, 2012). In the California sea lion, it appears that the DRAV increases with dive depth, suggesting that they do not always dive after a full inhalation (McDonald and Ponganis, 2012). Consequently, methods to estimate tidal volume in air-breathing marine vertebrates would be welcome, in order to help resolve these issues regarding estimates of DRAV.

Estimating DRAV using biologging tools
Recent technological advances in biologging have provided new innovative tools to enhance our understanding of physiology and lung function in general, and to estimate RAV in particular. Breathing frequency can be determined from high-resolution acceleration or audio-recording tags, where breathing events are clearly identifiable, and this gives an idea of the metabolic demands for different activities (Isojunno et al., 2018; Roos et al., 2016; Rojano-Donate et al., 2018). Breathing frequency has also been used in several studies to estimate field metabolic rate (FMR, see Glossary), although it has been suggested that the ability to also estimate O$_2$ exchanged and tidal volume of breaths is vital to more accurately assess metabolic costs from respirations (Fahlman et al., 2016; Roos, 2015). In Humboldt penguins, breathing frequency and tidal volume have been estimated using magnetic sensors attached to the beak (Wilson et al., 2003). In these penguins, beak opening angle correlates with inspiratory tidal volume (Wilson et al., 2003). For free-ranging penguins, both the estimated flow and breathing frequency follow a U-shaped pattern during the surface interval: they are initially high, then decrease and then increase again. It was suggested that these changes initially help maximize O$_2$ recovery and then help remove CO$_2$ before the next dive (Wilson et al., 2003). Such studies in other taxa are currently lacking, but would help to define physiological drivers and limitations to diving and foraging.

In an alternative approach, tidal volume in the bottlenose dolphin has been estimated using the respiratory sinus arrhythmia (RSA) (Cauture et al., 2019). The RSA is the change in heart rate associated with respiration, and it is also seen in other mammals, including humans; it is thought to improve gas exchange efficiency (Hayano et al., 1996). The RSA appears to correlate with breath-hold capacity in seals (Castellini et al., 1994a,b). As the RSA is affected by vagal tone (see Glossary), validation studies are required before it can be used to make predictions of DRAV, in order to determine how autonomic tone, breathing frequency and volume are related (Cauture et al., 2019). Although the study by Cauture et al. (2019) measured RSA in dolphins at rest, this method of estimating lung volume still appears promising, as heart rate-recording tags have been used in free-swimming animals (Andrews et al., 1997; Halsey et al., 2007; McDonald et al., 2018, 2020; McDonald and Ponganis, 2014; Ponganis et al., 1997; Thompson and Fedak, 1993; Williams et al., 1993; Young et al., 2011).

Phonospirometry is a different approach that has been used in a number of studies; it involves estimating breathing frequency and/or tidal volume from recordings of respiratory flow noise (Rojano-Doñate et al., 2018; Sumich, 2001; Sumich and May, 2009). This method has been used: (1) in gray whales to estimate tidal volume (Sumich, 2001; Sumich and May, 2009); (2) in harbor porpoises to estimate breathing frequency and FMR by assuming a constant energetic value for each breath (Rojano-Doñate et al., 2018); and (3) in free-ranging, near-shore bottlenose dolphins to estimate breathing frequency and inspiratory tidal volume (Van der Hoop, 2016). Thus, phonospirometry provides another promising method

to estimate respiratory dynamics in wild marine species. Some of the limitations of this method include movement of the tag on the body (e.g. sliding, tag oscillation) and interference from water flow noise, but detailed calibration suggests that it provides robust results (Van der Hoop, 2016).

We focus here on another method for estimating DRAV that uses the change in speed during glides that are performed during the ascent or descent portion of dives, with the majority of DRAV estimates being done during the ascent phase (Aoki et al., 2017; Miller et al., 2016, 2004; Narazaki et al., 2018; Sato et al., 2002, 2011). This method is interesting as it could potentially estimate DRAV during both the descent and ascent phases and could therefore help determine whether RAV changes throughout the dive. We will focus on this method in the rest of this Commentary, beginning by discussing some of the underlying assumptions.

Estimating DRAV using changes in gliding speed

The method to estimate DRAV from glide portions of dives is based upon the principle that the movement of a gliding body is affected only by the drag and net buoyancy forces acting upon it. The value of other physical constants, such as the drag coefficient or surrounding seawater density, also need to be estimated or measured to derive an estimate for DRAV (Miller et al., 2016).

Crucial for this Commentary is that the method as applied to date has assumed that the DRAV, after accounting for changes in pressure, remains constant throughout the dive. There are at least two potential problems with this assumption, which we show here could result in an underestimate of the DRAV at the beginning of the dive ($DRAV_{pre}$) of 12–50%. Reducing this underestimate will be important to allow us to better understand how air-breathing marine vertebrates manage gases during diving to support metabolism and prevent diving-related issues such as decompression sickness.

The first problem is that the difference in metabolic consumption of O_2 ($\dot{V}_{O_2}$) and production of CO_2 ($\dot{V}_{CO_2}$) depends on the fuel source used for aerobic metabolism, resulting in an RQ that may vary with time. The RQ [which, at steady state, is the same as the respiratory exchange ratio (RER)] is a measure of the number of molecules of O_2 exchanged for CO_2. In an animal metabolizing glucose, for every molecule of O_2 used for aerobic metabolism there is one molecule of CO_2 produced, and the RQ is 1. When an animal switches fuel source, this ratio changes – the RQ is 0.7 for fat and 0.8 for protein (Schmidt-Nielsen, 1997). Unless the RQ for air-breathing vertebrates is always 1, this will result in an error in the estimate of lung volume. Considering the high protein and fat content in the diet of most marine mammals and penguins, RQ is likely never 1. This bias may not be considerable – it would amount to a maximum of 6.3% if the RQ was 0.7 throughout the dive and all the O_2 in the lung was used for aerobic metabolism. This error would vary with the duration of the dive, with the diving metabolic rate and with the exchange of gas between the respiratory system and blood/tissues.

The second, and potentially greater, bias results from the large difference in gas solubility of the various respiratory gases: CO_2 is 24 and 46 times more soluble in plasma as compared with O_2 and N_2, respectively (Weathersby and Homer, 1980). The universal gas law states that the number of molecules in a given volume of gas at a certain temperature and pressure is the same. In contrast, the solubilities of gases in liquids are very different, which will result in changes in the conservation of mass inside the lungs throughout the dive. Fick's law of diffusion states that the rate of diffusion across a membrane is directly related to the partial pressure gradient and the surface area, and inversely related to the diffusion distance. Thus, the partial pressure gradient of the gas will determine the diffusion

across the lung–blood barrier in the lung, and it will take 46 times more molecules of CO_2 to change the tension an equal amount to that of N_2, resulting in a change in the mass balance inside the lung. The exchange between O_2 and CO_2 is similar, but more complicated given the increased solubility of O_2 in blood due to hemoglobin. The overall effect is that there will be an imbalance between the number of molecules of O_2 and N_2 that are taken up by blood from the lung versus the number of molecules of N_2 and CO_2 that enter the lung from the blood. This imbalance will increase temporally depending on the level of gas exchange, blood flow and metabolic rate. This effect would cause DRAV to differ throughout the dive when the animal is at the same depth. Following a dive, the recovery of the O_2 stores is faster than the removal of the CO_2 produced during the dive; it has been suggested that this difference in O_2 and CO_2 dynamics following a breath hold is due, in part, to the transport of produced CO_2 from the tissues to the lungs for exchange (Boutilier et al., 2001; Fahlman et al., 2019a, 2008; Reed et al., 1994, 2000). Of course, the variation in blood and tissue gas solubility may also be responsible for the observed differences in gas exchange dynamics.

Using a gas dynamics model to estimate changes in DRAV

To illustrate the potential difference between $DRAV_{pre}$ and the diving respiratory air volume at the end of the dive ($DRAV_{post}$) owing to the changes in gas mass balance, we use a previously published gas dynamics model, where gas exchange is driven by the partial pressure of the gas (Fahlman et al., 2009, 2018b; Kvadsheim et al., 2012). The gas dynamics model divides the body into different tissue compartments of varying size and tissue characteristics, e.g. gas solubility differs between tissues (Fahlman et al., 2006). Each tissue receives arterial blood with a determined blood flow rate, and the gases (O_2, CO_2 and N_2) are exchanged assuming partial pressure equilibrium when the blood leaves the compartment and enters the venous circulation. The venous blood circulates back to the respiratory system, where the gas is again exchanged. During a breath hold, the respiratory system does not exchange gas with the atmosphere. The specific metabolic rate of each compartment determines the rate of O_2 consumption and CO_2 production, and thereby the RQ. In the marine mammal, a pulmonary shunt (see Glossary) develops as the lung compresses; this shunt increases until the alveoli collapse and gas exchange terminates (Bostrom et al., 2008; Fahlman et al., 2009). Following alveolar collapse, blood flow continues and exchange of the gases between tissues continues as does the metabolism of O_2 and production of CO_2. During ascent, the alveoli are again recruited and gas exchange resumes, allowing gases to enter the lungs depending on the partial pressure gradients.

Here, we present previously published dive data and model results from the bottlenose dolphin for a 80–200 s (short) or 320–440 s (long) dive to 20 m [3 atmospheres absolute (ATA), shallow] or 140 m (15 ATA, deep) (Fahlman et al., 2018a). These model results indicate that the lung volumes were 20%, 12%, 38% and 16% lower during the ascent ($DRAV_{post}$) as compared with during the descent ($DRAV_{pre}$) for the short/shallow, long/shallow, short/deep and long/deep dives, respectively (Fig. 1 shows the results for the short/shallow dive). Using previously published data for a ~43 tonne sperm whale (*Physeter macrocephalus*) (animal SW09_160a in table 1 in Kvadsheim et al., 2012), the $DRAV_{post}$ values for a short and shallow (74 s, 15 m), medium-long and deep (37 min, 404 m) or long and deep (72 min, 1629 m) dive were 5%, 55% and 55% lower, respectively, as compared with the $DRAV_{pre}$ (Fig. 2). If these models of gas exchange are correct, there are
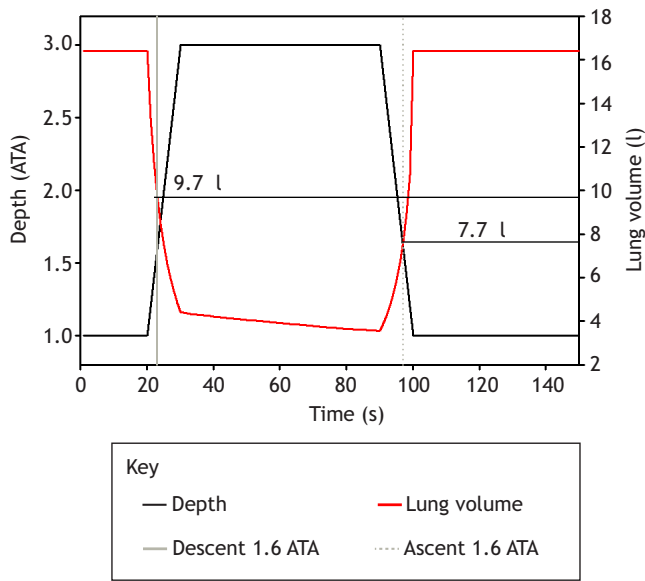
Fig. 1. Estimated lung volume for a 200 kg bottlenose dolphin diving to 20 m (3 ATA) for a total dive duration of 80 s, with 60 s at depth. The black solid line shows dive depth and the red line shows the estimated lung volume from a previously published gas dynamics model (Fahlman et al., 2018a). The gray vertical lines are at 1.6 ATA for ascent and descent, and the horizontal black lines show the lung volume in these two cases. This shows how the differences in gas solubility between O_2 and CO_2 alter lung volume during the dive. Once the animal reaches the surface, respiration resumes and the lung volumes are again based on tidal volumes, which, in this model, are assumed to be constant during the surface interval.
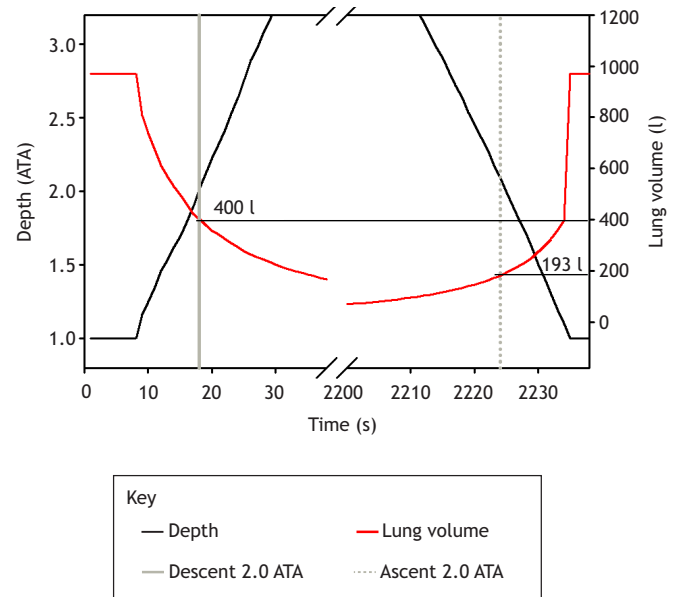
Fig. 2. Estimated lung volume for a 43 tonne sperm whale diving to 404 m (41 ATA) for a total dive duration of 37 min (animal SW09_160 a in Kvadsheim et al., 2012). The whale spent 33 min at a depth exceeding 100 m. The black solid line shows dive depth, and the red line shows the estimated lung volume from a previously published gas dynamics model (Kvadsheim et al., 2012). The gray vertical lines are at 2.0 ATA for ascent and descent, and the horizontal black lines show the lung volume in these two cases. This shows how the differences in gas solubility between O_2 and CO_2 alter lung volume during the dive.

therefore considerable differences in $DRAV_{pre}$ and $DRAV_{post}$, with the differences in respiratory mass balance depending on the metabolic rate, the cardiac output, the level of pulmonary shunt throughout the dive and, consequently, the dive depth and duration.

Estimating $DRAV_{pre}$ from $DRAV_{post}$ based on modeling results

The theoretical modeling estimates presented in this Commentary suggest that the DRAV varies throughout the dive, and suggest that calculations performed using measurements made during gliding primarily during the ascent phase may underestimate $DRAV_{pre}$ by between 12% and 50% (Miller et al., 2004). The estimated mass-specific $DRAV_{post}$ for the sperm whale from changes in gliding speed ranged from 21.9 to 32.6 ml kg^{-1}, with an average value of 26.4 ml kg^{-1} (Miller et al., 2004). This average estimated $DRAV_{post}$ for a 43 tonne sperm whale is roughly 43% of the TLC estimated by Eqn 1 (Fahlman et al., 2011; Kooyman, 1973). However, these $DRAV_{post}$ estimates match the predicted TLC based upon the measurements made by Scholander (1940) in the northern bottlenose whale, a species that has a mass-specific lung mass similar to that of the sperm whale (Clarke, 1978; Miller et al., 2004). These data suggest that either sperm whales have a much lower TLC as compared with other cetacean species, or that they dive with a DRAV that is only 50% of TLC. If we accept that $DRAV_{pre}$ was underestimated by 50%, the mass-specific $DRAV_{pre}$ would be 52.8 ml kg^{-1}, and for a 43 tonne sperm whale the $DRAV_{pre}$ would therefore be 2270 liters (Miller et al., 2004), or 92% of the TLC_{est}.

The mean $DRAV_{post}$ value from changes in gliding speed for the deep-diving northern bottlenose whale (27.4 ml kg^{-1}) matches well with the TLC estimated from excised lungs (Miller et al., 2004; Scholander, 1940), although Miller et al. (2016) noted that this value is low compared with that of other cetaceans, and is only

approximately 50% of TLC_{est}. Other studies have also suggested that deep-diving cetaceans may have relatively lower TLC (Piscitelli et al., 2010) as compared with the TLC_{est} prediction equation (despite the fact that this equation was developed using a large number of both shallow- and deep-diving marine mammals; Fahlman et al., 2011; Kooyman, 1973). Similarly, the measured TLC in the short-finned pilot whale (*Globicephala macrorhynchus/scammoni*) was 100 ml kg^{-1} (Olsen et al., 1969), which agrees with that estimated from TLC_{est} (Eqn 1), but is approximately twice that of $DRAV_{post}$ estimated from changes in gliding speed in the long-finned pilot whale (*Globicephala melas*) (Aoki et al., 2017). As both of these are deep-diving species, we might expect the DRAV to be similar. Accepting that the $DRAV_{pre}$ in the bottlenose whale was actually 50% larger than $DRAV_{post}$, which was based primarily on ascent glides, the $DRAV_{pre}$ would be approximately 62–86% of the predicted TLC_{est}, but greater than expected from their excised lungs.

Whether TLC is actually lower than predicted based on measurements of excised lungs in the sperm whale and the bottlenose whale or whether they dive with a $DRAV_{pre}$ that is a portion of TLC remain to be determined; addressing these issues may help to resolve the uncertainties around whether corrections from $DRAV_{post}$ to $DRAV_{pre}$ are warranted. In the current Commentary, we remain unable to conclude whether the TLC of deep-diving sperm and beaked whales matches that predicted by M_b (Kooyman, 1973). However, the correction from $DRAV_{post}$ to $DRAV_{pre}$ provides a plausible explanation as to why the estimated DRAV values in past studies were lower than expected based on previous work.

If corrections up to 50% (as indicated by the simulations run here) are indeed valid, they would contradict reports that have suggested that TLC is lower in deeper-diving species in order to reduce the risk

of decompression sickness (Piscitelli et al., 2010). If TLC is actually smaller in the deep-diving sperm and bottlenose whales than in species that make shallower dives (as has been proposed in past studies; Piscitelli et al., 2010; Scholander, 1940), and the correction suggested here is correct, our results suggest that the $DRAV_{pre}$ of deep-diving species would be an even greater portion of TLC. Assuming that the pulmonary shunt that develops during diving is entirely driven by passive pulmonary collapse, the results presented here suggest that alveolar collapse may occur at greater depths than formerly thought (Hooker et al., 2009; Kvadsheim et al., 2012). However, if cetaceans are able to voluntarily alter heart rate (Elmegaard et al., 2016; Elmegaard et al., 2019; Elsner et al., 1966; Fahlman et al., 2019b; Ridgway et al., 1975), they may be able to manage gas exchange during natural dives to minimize N_2 uptake while still exchanging O_2 or CO_2 (García-Párraga et al., 2018). Our simulations suggest that the $DRAV_{pre}$ in deep-diving species may be greater than previously estimated, and that deep-diving whales may therefore utilize pulmonary O_2 over deeper portions of their dive than previously estimated. If that were true, the benefit of an active mechanism to prevent excessive uptake of N_2 – to lower the risk of gas emboli during the ascent/decompression – would be even more apparent.

Conclusions and future prospects

Here, we have proposed that the large differences in gas solubility between O_2, CO_2 and N_2 in blood, plasma and tissues result in temporal changes in the mass balance of the respiratory gases during diving. These changes produce temporal changes in DRAV; therefore, the $DRAV_{pre}$ will be greater than $DRAV_{post}$. The gas-exchange model presented here indicates that the difference could be as much as 50%, but it is important to note that the gas-exchange models themselves are based upon many uncertain parameters and processes. Further work on the cardiorespiratory physiology of air-breathing marine vertebrates, and increased confidence in the accuracy of gas-exchange models, will help us to better validate the temporal changes in pulmonary gas volume during diving.

Crucially, future studies that can estimate or compare $DRAV_{pre}$ and $DRAV_{post}$ will allow us to empirically evaluate how changes in RQ, gas solubility, blood flow, metabolic rate and dive behavior affect DRAV in nature. This is important for our understanding of diving physiology, as variation in DRAV affects the respiratory O_2 store and gas dynamics during a dive; studies that help resolve the question of whether animals inhale or exhale before a dive would allow a better understanding of the gas dynamics during diving (Fahlman et al., 2009; Ponganis, 2015). The glide method discussed herein produces an estimate of DRAV based on the glides performed late in the ascent phase when the respiratory gases are expanding owing to reductions in hydrostatic pressure (Aoki et al., 2017; Miller et al., 2016, 2004; Narazaki et al., 2018; Sato et al., 2002). Glides performed during descent generally start to occur only after the gas has been highly compressed (as divers need to produce thrust during the initial phase of descent to counter the positive buoyancy of the DRAV); thus, these glides cannot be used as effectively to parameterize estimates of $DRAV_{pre}$. One approach to circumvent this could be to use more accurate measurements of the thrusting acceleration produced during initial descent stroking periods (e.g. see fig. 2 in Martín López et al., 2016) to more directly estimate $DRAV_{pre}$, which could then be related to $DRAV_{post}$ estimated from glide analyses.

Further development of these or any other methods to more accurately determine $DRAV_{pre}$ will not only provide a better understanding of how to mitigate harm caused to deep-diving whales by anthropogenic stressors, but will also help to improve estimates of the pulmonary O_2 store, a factor that is critical to our ability to estimate the calculated aerobic dive limit (Butler and Jones, 1997) and our understanding of the aerobic limitations to breath-hold diving. In addition, the $DRAV_{pre}$ alters an animal's buoyancy, which, in turn, alters the energetic requirements of underwater swimming, which is the effect measured by the glide method. Thus, understanding the $DRAV_{pre}$, along with improved understanding of cardiorespiratory coupling in air-breathing dive-adapted vertebrates, is crucial to allow us to understand the limitations to diving (Cauture et al., 2019; Elmegaard et al., 2016, 2019; Elsner, 1965; Fahlman et al., 2019b) and for advancing our knowledge of the ecophysiology of air-breathing marine vertebrates.

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