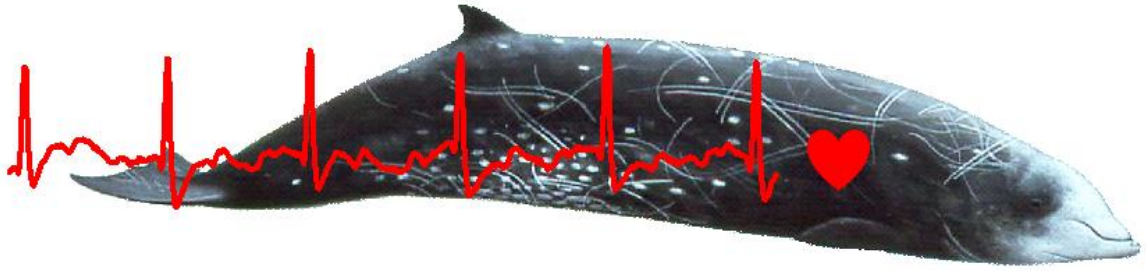
# REPORT ON THE CURRENT STATUS AND FUTURE DIRECTIONS OF MARINE MAMMAL DIVING PHYSIOLOGY:

## CONSIDERATIONS FOR THE EFFECT OF MILITARY SONAR ON DEEP-DIVING CETACEANS



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**Report Prepared by:**
Terrie M. Williams
Paul Ponganis
Andreas Fahlman

**Workshop Reviewers:**
Gerald Kooyman
Tobias Wang
Sam H. Ridgway

**Workshop Attendees and Additional Report Authors/Editors:**
Daniel Costa, Alexander Costidis, Daniel Crocker, Daniel Garcia-Párraga, Petter Kvadsheim, Peter Madsen, Birgitte McDonald, Michael Moore, Shawn Noren, Laura Thompson, with Beau Richter, Jason John, Anthony Pagano, Nicole Thometz, and Jessica Kendall-Bar



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## I. Executive Summary

A review of the status and future of research regarding the physiological responses of marine mammals to diving and naval sonar exposure was undertaken to, 1) assess the return on investment of current US Navy funded programs, 2) identify the data needs and the contributions of current research programs to meeting data needs, 3) determine the ability to meet critical data needs given the current state of understanding and technology, and with this 4) identify new research directions.

A workshop was held from 11-13 September 2017 in Santa Cruz, California. Workshop attendees were key representatives of Navy-funded marine mammal diving physiology studies, additional experts in diving physiology as well as three external reviewers who were selected because of their expertise in diving physiology and integrative responses to anthropogenic disturbance. Prior to the workshop, select attendees were requested to write short reviews of topics concerning the current status of marine mammal diving physiology. Review topics included a summary of the unique anatomy and vulnerability of beaked whales to noise, cardiovascular, pulmonary and gas dynamics related to the risk of decompression illness and gas emboli formation, metabolism, and muscle dynamics, as well as thermal, neural, and endocrine factors associated with diving. The workshop centered around these topical reviews as well as a review of current projects comprising the ONR Marine Mammal Diving Physiology Program.

All reviewers and participants agreed that excellent progress has been made under the ONR Marine Mammal Program, especially in providing new insights regarding variability in the mammalian dive response as a factor leading to species-specific vulnerability to naval sonar. This report presents a synthesis of this evaluation and recommendations by the workshop attendees on the current and future physiological research needed to identify and mitigate potential impacts of naval sonar on diving animals. Recommendations for future research (Table 2) provide specific guidance on crucial physiological responses and adaptations to diving needed to effectively address how these are modulated during sonar exposure/stress/fear. An integrative approach involving model animals in zoological and wild settings as well as free-ranging beaked whales is recommended to provide control data, and to validate tools and research approaches to directly and indirectly examine why beaked whales appear exceptionally vulnerable to anthropogenic disturbance. **In addition to data on the dive response specific to beaked whales (heart rate, diving lung volume, core body temperature), areas of top research priority include characterizing the effect of fear/escape responses on diving physiology in these and other deep-diving cetaceans. Basic research concerning the factors regulating blood flow distribution, pulmonary shunt capabilities, vascular control, and pathological consequences of emboli formation during diving represents a critical need.** Thus, the group strongly recommended that the ONR Marine Mammal Physiology Program be continued and augmented to facilitate this research to enable the Navy to safely accomplish its missions in an environmentally sound manner in the presence of diving mammals.

## II. Contents

## III. Introduction

Marine mammals have the ability to dive to great depths and return to the surface without developing injuries related to decompression sickness (DCS) including hyperbaric oxygen induced seizures, shallow water blackout, and nitrogen narcosis. The physiological mechanisms that support this capability are poorly known. Although there have been significant advances in the observation and measurement of marine mammal diving behavior, there is comparatively little understanding of the respiratory and cardiovascular adaptations critical for managing hypoxia, hypercapnia, and inert gases during diving. Furthermore, there is limited information regarding the integration of physiological and behavioral mechanisms comprising flight responses in these diving mammals. The disruption of normal marine mammal diving behavior, display of opposing physiological responses, and the associated potential for developing gas emboli or DCS remain the leading hypotheses proposed as a cause–effect relationship between mid-frequency active (MFA) sonar use and mass stranding events. To address this, a major goal of the Marine Mammal Biology (MMB) program at the Office of Naval Research is to investigate the traits (physiology, endocrinology, immunology, and biochemistry) that permit marine mammals to dive to extreme depths for long durations without apparent injury. Furthermore, MMB seeks to provide a better understanding of the potential risk of these animals for developing gas emboli or DCS when exposed to anthropogenic noise including Navy sonar.

To provide the best science to address the potential risk of marine mammals for developing DCS or incurring other short- and long-term costs when exposed to Navy sonar, a workshop was arranged to review and assess the current state of knowledge regarding the mechanisms that enable marine mammals to dive for extended periods to deep depths, and the potential physiological and biochemical risk factors that may cause formation of gas emboli and increase DCS risk (Appendix 1). The workshop specifically addressed the potential risk of beaked whales exposed to MFA sonar, and evaluated the potential for other physiological risks. The workshop consisted of a small number of selected participants who contributed their expertise on the state of research and provide recommendations for future research priorities (Appendix 2).

**Future Naval Relevance**

Limited understanding of the physiological effects of anthropogenic noise on marine mammals has resulted in the cessation of naval exercises as well as stalled the testing of new sonar technologies. Key to avoiding these problems is the ability to identify biologically relevant lethal and sub-lethal impacts of disturbance related to Navy sonar and other marine operations. Such discrimination is federally mandated under the US Marine Mammal Protection Act, which requires distinguishing between permanent “takes” affecting a population and non-lethal “harassment” associated with comparatively benign activities. As part of rule-making under the US Marine Mammal Protection Act, the Navy has committed to an Integrated Comprehensive Monitoring Program with the following objectives, 1) monitor and assess the effects of Navy activities on protected marine species, 2) ensure that data collected at multiple locations is conducted in a manner that allows comparison between and among different geographic

locations, 3) assess the efficacy and practicality of the monitoring and mitigation techniques, and 4) add to the overall knowledge base of protected marine species and the effects of Navy activities on these species (DoN, 2013). As part of its environmental compliance, the Navy is required to attempt to quantify the effect of sonar operations on marine mammals in all its operating areas. This requires research to enhance our understanding of the physiological responses of marine mammals to sonar exposure, and to allow the estimation of the relationship between acoustic dosage and other factors that instigate physiological responses.

Here we held a workshop to review the current state of knowledge in this subject area, identify the data needs and the contributions of current research programs to meeting these data needs, as well as determine the ability of researchers to meet outstanding data needs given the current state of technology. This review also helped identify new research capabilities needed to meet the requirements for future research.

**Goals and Objectives**

The specific objectives of the workshop were to:

1. Assess the current state of knowledge for key topic areas of research on the physiological and biochemical mechanisms that permit marine mammals to dive to deep depths for extended periods, and on the potential risks to these mechanisms when animals are exposed to Navy sonar;

2. Evaluate on-going research efforts and identify strengths and weaknesses of current physiological programs;

3. Formulate recommendations for future research based on current knowledge and its potential for advancement based on available research methods. Key data needs, and new research capabilities and conceptual frameworks needed to meet the requirements for future research were also identified.

The workshop was organized to provide an assessment through a peer-reviewed venue that would support the long-term goal of determining the progress of current research and of identifying the need for future research by providing a comprehensive critique of the state-of-the-art in marine mammal physiological research.

The following report is structured as follows. An Introduction that outlines the objectives, a review of the current status which summarizes the various topics reviewed, recommendations for the future, and concluding remarks. The recommendations have been reviewed by the group, and a consensus has been reached, which is detailed in this report.

## IV. Overview - ONR Marine Mammal Diving Physiology Program

The MMB physiology program spans projects encompassing broad focal areas including anatomy and morphology, cardiorespiratory physiology, metabolism, studies on stress, and theoretical modeling that attempts to integrate some of these topics into a comprehensive framework. Diverse approaches have been supported and each has yielded useful data and valuable insights. The program has funded projects that have made tremendous progress to understanding marine mammal diving physiology and determined the major physiological variables that would cause increased risk of gas emboli during exposure to naval sonar, such as anatomy, gas exchange and perfusion. While it has been difficult to obtain direct physiological information on beaked whales, data from other model species have been obtained. In addition, continued development of biologging tools provides an exciting future avenue to obtain direct physiological data on free-ranging whales.

The panel of experts at this workshop focused on short-term, acute responses to MFA sonar, which are believed to result in behavioral and physiological responses that result in symptomatic gas emboli. Modeling work has highlighted the need for species-specific data to provide better estimates of risk. The group agreed that there is a lack of anatomical and physiological data from key deep-diving cetacean species. A limited number of studies have detailed the muscle morphology and vascular details in beaked whales. Although these data provide important information to enhance our understanding of these animals, there is still considerable information about function that is lacking. It was suggested that anatomical data could be obtained from stranded specimens and the group discussed the possibility of a SWAT response team approach to obtain such data. We also discussed a variety of alternative animal models that could be used, where it is possible to obtain detailed physiological data on lung function, cardiovascular responses during diving and during sound exposure/playback. Model species include dolphins and porpoises, sea lions and elephant seals, beluga whales, pilot whales, and narwhals (Table 1). A combination of studies on these species would provide information about normal responses during diving and how these may be modulated during exposure to sonar and under periods of stress. Such improved information would enhance the ability to integrate how changes in behavior during sound exposure or behavioral response studies disrupt normal physiological function to increase the risk of gas emboli formation and DCS.

In summary, the group agrees that it is crucial that the normal physiological responses during diving be better understood to effectively address how these are modulated during sonar exposure/stress/fear. Ultimately, this will vastly improve our ability to identify and mitigate the risk to animals exposed to unanticipated noise. An integrative, comparative approach involving animals in zoological and wild settings will provide control data as well as validate tools and research approaches for studies on poorly accessible pelagic, free-ranging marine mammals such as beaked whales.

## V. Review – Current Status of Marine Mammal Diving Physiology

**Relation to the Impact of Military Sonar on Beaked Whales**

The role of diving physiology on the impact of military sonar on beaked whales includes both acute, potentially catastrophic effects (beaching/death) and chronic, species-survival effects (most notably, disruptions in stress/immune function, foraging behavior and success, energetics, and animal distribution). This workshop primarily focused on acute physiological responses which, of course, underlie potential long-term effects. These were summarized in short topical reviews (see Appendix 3) and addressed both the current understanding of diving physiology and acute physiological responses of marine mammals.

The following section highlights the findings and topics in those summaries that were considered most relevant to evaluating the possible physiological effects of sonar exposure on diving mammals. Readers are also referred to a previous workshop that has already reviewed behavioral responses and passive acoustics leading to changes in dive patterns, foraging behaviors, and distribution of beaked whales (see Harris and Thomas, 2015). Topics reviewed here include: 1) Beaked whales, 2) Diving physiology, and 3) Metabolic stress and general stress responses during diving.

### 1. Beaked whales

#### 1a. Unique vulnerability

Beaked whales, appear especially susceptible to disturbance by naval sonar (D'Amico *et al*., 2009). These animals are notable for routine deep, long-duration dives (800-1300 m, 20-70 min) interspersed by shallower, short-duration dives (< 450 m, < 20 min), with 60-120 min between deep dives (Baird *et al*., 2006; Schorr *et al*., 2014; Tyack *et al*., 2006). Baird's beaked whale (*Ziphius cavirostris*) and several species of the genus, *Mesoplodon*, form the majority of dead/stranded whales associated with such exposure (D'Amico *et al*., 2009). Notable findings from necropsies on stranded whales include vascular congestion, microhemorrhages, fat emboli and intravascular bubbles in the brain, kidneys, and ears (Fernandez *et al*., 2005; Jepson *et al*., 2003). Such findings were consistent with but not diagnostic of decompression sickness. Documentation of nitrogen gas in bubbles further supported the role of decompression sickness in the etiology of the strandings (Bernaldo de Quirós *et al*., 2012). Other possible pathological mechanisms include intense stress responses, arrhythmias, metabolic stress, and hyperthermia (Cox, 2006).

Controlled exposure studies have revealed a variety of behavioral responses, ranging from minor extension of dive duration with prolonged ascents, vigorous stroking, and increased inter-deep dive intervals to the performance of the deepest, longest dives documented in some species (DeRuiter *et al*., 2013; Falcone *et al*., 2017; Miller, 2015; Stimpert *et al*., 2014). A primary goal of this workshop was to address how the physiological responses underlying such changes in

dive performance might contribute to decompression sickness or other mechanisms responsible for the pathological findings in the stranded beaked whales.

### 1b. Morphology

Several features of beaked whale anatomy and morphology are relevant to diving physiology. Using the 'body composition technique' of comparing total mass of metabolically distinct tissues, Pabst and co-workers showed that beaked whales (and other deep-diving whales) use large masses of metabolically inexpensive muscles to store increased oxygen (large muscle depots with high myoglobin concentration) and to offset expensive tissues (thus, overall reducing use of expensive tissues and increasing inexpensive tissues) (Pabst *et al*., 2016). Compared to shallow diving cetacean species in which locomotor muscle represents 28-30% of total body mass, mesoplodonts invest as much as 50% of total body mass in muscle, while the pulmonary, hepatic, intestinal and brain investment is reduced ("inexpensive body hypothesis"). In addition, diving lung volumes and lung masses in beaked whales and other deep-diving whales are smaller than in shallow-diving species (Aoki *et al*., 2017; Miller *et al*., 2016; Miller *et al.*, 2004; Piscitelli *et al.*, 2010, 2013; Scholander, 1940).

In beaked whales, muscle fibers are exceptionally large, have extremely low mitochondrial density, and are comprised of 80% Type II (fast twitch, glycolytic) fibers (Velten *et al.*, 2013). These characteristics should all contribute to low muscle oxygen consumption rates. High myoglobin concentrations in Type II fibers are unusual, and it was suggested that these fast twitch fibers serve as an oxygen store for the adjacent slow twitch (Type I) fibers. Immunohistochemical staining has revealed that myoglobin is expressed in both fiber types in beaked whales (Sierra *et al*., 2015).

Vascular morphology is also relevant to cardiovascular and respiratory function during diving. Although there have been few such investigations in beaked whales, findings are notable for periarterial venous retia around peripheral arteries (possibly for thermoregulation), large pterygoid sinus venous lakes (with a possible role in the prevention of barotrauma), increased vascularization of acoustic fat bodies (also noted for hemorrhage in stranded whales and for high nitrogen solubility of acoustic fat), decreased vascularization of the blubber layer, venous plexuses in the lungs and airways (possibly for the prevention of barotrauma), an extensive epidural rete (as a possible nitrogen absorption or bubble trap as suggested in the past), and a well-developed venous rete around the eye (Blix *et al*., 2013; Costidis and Rommel, 2012; Costidis and Rommel, 2016a, b; Cozzi *et al*., 2005; Davenport *et al*., 2013; Fernandez *et al.*, 2005; Lonati *et al*., 2015; Ninomiya *et al.*, 2005; Vogl and Fisher, 1982).

### 1c. Physiological costs

The potential physiological costs of avoidance/escape behaviors of beaked whales in response to sonar exposure were recently modeled, based on the actual dive and kinematic data obtained from behavioral response studies of Cuvier's beaked whale (DeRuiter *et al.*, 2013; Williams *et*

*al.*, 2017a). Calculated post-exposure dive metabolic rate was approximately 30% greater than the pre-exposure value with a marked shift in the proportion of total dive cost used for locomotion versus maintenance costs. This change was still apparent 1.7 hours post exposure.

## 2. Diving physiology

### 2a. Cardiovascular responses

The regulation of heart rate, cardiac output and blood flow distribution underlies the management of oxygen stores during dives, and also have profound effects on the uptake and distribution of nitrogen during dives (Fahlman, 2017; Ponganis, 2015). The dive response (including a decrease in heart rate and associated changes in peripheral perfusion during a dive) has long been known to be dependent on the nature and circumstances of a given dive. Recent research has emphasized that depth, exercise, multiple reflexes, and voluntary control can all affect heart rate during a dive (Davis and Williams, 2012; Elmegaard *et al.*, 2016; McDonald *et al.*, 2017; Noren *et al.*, 2012b; Ponganis *et al*., 2017; Williams *et al.*, 2015a, b). The potential generation of serious cardiac arrhythmias secondary to autonomic conflict (sympathetic-parasympathetic nervous system conflict) during escape dives of beaked whales has also been raised (Williams *et al.*, 2015a, b). Variability in heart rate profiles and irregular heartbeats are common in marine mammals and even deep-diving penguins (Andrews *et al.*, 1997; McDonald and Ponganis, 2014; Noren *et al.*, 2012b; Williams *et al.*, 2015b; Wright *et al*., 2014). A key question is the nature of the heart rate response during sound exposure and/or during periods of increased underwater activity as demonstrated in the DeRuiter *et al.* study (2013).

Heart rates recorded during various sound exposures in marine mammals have demonstrated a range of responses. Under restrained or restricted conditions, heart rates increased slightly in beluga whales and bottlenose dolphins; in contrast, apneic heart rates in hooded seals did not change, although eupneic heart rates did increase (Bakhchina *et al.*, 2017; Kvadsheim *et al.*, 2010; Miksis *et al*., 2001). In unrestrained dolphins and porpoises, apneic heart rate was unchanged or decreased (Houser *et al.*, 2012; Teilmann *et al*., 2006).

Because of the increased locomotory activity in post-exposure dives of beaked whales (DeRuiter *et al.*, 2013), the effect of exercise on heart rate regulation during dives may play a role in the pathology of post-exposure strandings. In short-duration, "aerobic' dives of dolphins, seals, sea lions, and porpoises, the correlation of heart rate with stroke rate supports the concept that exercise in these dives is associated with an increase in heart rate, i.e., an exercise response (Davis and Williams, 2012; Hindle *et al.*, 2010; Williams *et al.*, 2015b; McDonald *et al.*, 2017). The magnitude of an increase in muscle blood flow under such conditions is unclear. During longer or deeper dives of Steller and California sea lions, further decreases in heart rate, even in the presence of exercise, suggest minimal muscle perfusion and far less of an exercise effect (Hindle *et al.*, 2010; McDonald and Ponganis, 2014). Such variable muscle blood flow patterns and potential arterio-venous shunting (Tift *et al*., 2017a, b) affect not only oxygen store

management but also the volume of distribution of nitrogen during diving (Fahlman, *et al.*, 2006, 2009; Hooker *et al.*, 2009; Kvadsheim *et al.*, 2012).

Post-exposure heart rate profiles of beaked whales remain undocumented. However, recent post-release dive heart rate profiles of narwhals (Williams *et al.*, 2017b) and previous reports of heart rates during extended dives or alarm reactions of diving animals (Ponganis *et al*., 2017) support the concept of a bradycardic response during alarm reactions in marine mammals. In these situations, the parasympathetic nervous system appears to dominate the control of heart rate.

**2b. Gas exchange/lung collapse/pulmonary shunts**

As recently reviewed (Fahlman *et al.*, 2017; Ponganis, 2015), marine mammals have respiratory adaptations that prevent barotrauma and limit gas absorption at depth, in addition to facilitating rapid gas exchange at the surface. Passive alveolar collapse has long been considered the primary protective pulmonary mechanism in minimization of the risk of decompression sickness in marine mammals. The collapse of alveoli with movement of alveolar air into the more rigid tracheo-bronchial tree decreases gas exchange through the development of a pulmonary shunt that allows venous blood to bypass gas exchange surfaces in the lungs and return to the arterial system. Importantly, the development of the pulmonary shunt is gradual and has been considered to increase with depth until a complete cessation of gas exchange occurs (Fahlman *et al.*, 2009; Kooyman and Sinnett, 1982). The depth of complete alveolar collapse is dependent on many factors, including chest wall compliance, lung compliance (both alveolar and tracheo-bronchial tree compliances), total lung capacity and start-of-dive inspired air volume (see above reviews). Measurements of pulmonary shunt development and determinations of depth of complete alveolar collapse have been difficult and few (Falke *et al.*, 1985; Kooyman *et al*., 1973; Kooyman and Sinnett, 1982; McDonald and Ponganis, 2012; Ridgway and Howard, 1979).

Importantly, animal behavior may also contribute to the depth at which gas exchange completely ceases. Available evidence in free-diving sea lions and penguins suggests that these animals inhale greater air volumes (i.e., closer to maximal respiratory air volume) prior to deeper dives compared to shallow dives (McDonald and Ponganis, 2012; Sato *et al.*, 2002; Sato *et al.*, 2011). Estimates of diving lung volumes, based on swim speed – buoyancy calculations, have now been reported for three species of deep-diving cetaceans (Aoki *et al.*, 2017; Miller *et al.*, 2004, 2016). It has also been suggested that active compression of the lung via activation of skeletal or smooth muscle, rather than just hydrostatic pressure, may contribute to earlier alveolar collapse and decreased risk of decompression sickness (Fahlman *et al.*, 2017). A synthesis of available data in marine mammals and sea turtles have resulted in a new hypothesis that suggests that volitional control of heart rate (and pulmonary blood flow), hypoxic pulmonary vasodilatation, and collateral ventilation allows cetaceans to create a ventilation-perfusion ratio that favors exchange of $O_2$ and $CO_2$ while minimizing $N_2$ exchange (García-Párraga *et al*., in review)

Many aspects of pulmonary function in cetaceans and other marine mammals remain unresolved but are potentially relevant to the risk of decompression sickness. These include, 1) the presence of bronchiolar sphincters in many odontocetes, including beaked whales, 2) the presence of intrapulmonary arteriovenous shunts in some deep-diving species, 3) possible collateral ventilation via the pores of Kohn in cetacean lungs, and 4) the unique occurrence of hypoxic pulmonary vasodilatation in the pulmonary arteries of sea lions. Equally important in gas exchange at depth and the risk of decompression sickness is the nature of concurrent bradycardia and cardiac output (Fahlman, 2017; Fahlman *et al.*, 2017; Ponganis, 2015). The magnitude of lung perfusion during a dive, especially above the depth of complete alveolar collapse, is critical to the kinetics of nitrogen entry and exit from blood during a dive.

### 2c. Modeling blood and tissue gas dynamics, risk of decompression sickness

Although theoretical models cannot replace empirical studies, they do provide useful insights and allow us to create hypotheses that can be tested. For example, modeling data from the Weddell seal (Falke *et al*., 1985) suggested that the observed arterial $N_2$ tensions were not evidence of alveolar collapse, but likely evidence of a pulmonary shunt that develops as depth increases (Bostrom *et al*., 2008; Kooyman and Sinnett, 1982; Scholander, 1940). The theoretical calculations suggested that there should be a large decrease in the arterial gas tension as the alveoli collapse, which empirical data later validated (McDonald and Ponganis, 2012). Consequently, the models provide a framework where hypotheses can be made. This is evident from studies of beaked whales. It has been suggested that sonar exposure may alter diving behavior in deep diving whales (DeRuiter *et al.*, 2013), causing increased activity and elevated $CO_2$ levels. Past research suggested that elevated $CO_2$ may increase the risk for gas emboli due to its higher diffusion rate (Behnke, 1951; Bernaldo de Quirós *et al.*, 2012 Bernaldo de Quirós *et al*., 2013; Bernaldo de Quirós *et al.*, 2011; Fahlman *et al.*, 2014; Harris *et al.*, 1945). Models are particularly useful for sensitivity analyses that can help identify variables that are most likely to lead to emboli formation for this hard to study species. These models identified blood flow/cardiac output, blood flow distribution, and pulmonary shunts as factors with the greatest influence on blood and tissue gas tensions (Fahlman *et al*., 2009; Hooker *et al*., 2009), and indicted that deeper dives, larger body masses and slowly equilibrating tissues may contributes to the risk (Fahlman, 2017; Fahlman *et al.*, 2014; Kvadsheim *et al.*, 2012). In summary, combining theoretical models with empirical data can be useful to investigate how changes in behavior and physiology following sonar exposure alter the risk of gas emboli or decompression sickness.

## 3. Metabolic stress and general stress responses during diving

### 3a. Blood oxygen, carbon dioxide, lactate, and acidosis

Metabolic stress during and after breath holds is manifested by hypoxemia (low blood O2), hypercarbia (high carbon dioxide), acidosis and lactate accumulation (Scholander, 1940). Diving bradycardia conserves blood oxygen, and limits the increase in blood carbon dioxide, lactate, and acidosis. At the same time, decreased peripheral perfusion isolates muscle from the circulation,

promoting the depletion of myoglobin-bound oxygen and eventually the onset of glycolysis and accumulation of lactate in muscle (Scholander, 1940), and helps extend the aerobic dive limit (Davis and Kanatous, 1999). An increase in heart rate during prolonged dives would worsen hypoxemia and increase blood carbon dioxide, lactate, and acidosis.

In general, however, marine mammals are extremely tolerant of low blood oxygen levels with partial pressures of oxygen ($P_{O2}$) as low as 10 mm Hg (1.33 kPa) in seals (Elsner *et al.*, 1970; Kerem and Elsner, 1973; Meir *et al*., 2009). Blood and end tidal $P_{O2}$ values at the end of dives and spontaneous breath holds of cetaceans are typically 20 to 30 mm Hg (2.66 to 4.0 kPa) (Noren *et al.,* 2012a; Ridgway *et al.*, 1969; Shaffer *et al.*, 1997). Blood oxygen content at lower $P_{O2}$s in cetaceans may be higher than expected because hemoglobin oxygen affinities are higher in some cetacean species relative to terrestrial counterparts (Dhindsa *et al.*, 1974; Horvath *et al.*, 1968; Vedvick and Itano, 1976).

Limited data during dives in pinnipeds indicate that blood pH is well buffered, that $P_{CO2}$ ranges between 40 and 70 mm Hg (5.33 – 9.33 kPa), and that lactate levels do not increase significantly (Qvist *et al.*, 1986; Stockard *et al*., 2007). The greatest changes in blood pH, $P_{CO2}$, and lactate occur during the surface interval after long dives and breath holds (Kooyman *et al.*, 1980; Noren *et al*., 2012a; Ponganis *et al.*, 1997a; Ponganis *et al*., 1997b; Shaffer *et al*., 1997).

### 3b. Muscle metabolic rate, lactate accumulation, and temperature

The high myoglobin concentrations and presence of intra/intercellular lipids in skeletal muscle of beaked whales are consistent with primary utilization of aerobic pathways for muscle energy generation. Pabst *et al.* (2016) have postulated that FT fibers (80% of total fiber area) are not utilized in routine swimming during dives, and that the myoglobin-bound oxygen in both fiber types is utilized by the ST fibers, which are assumed to be more active during routine swimming. The differential use of the two fiber types is consistent with the stroke patterns of beaked whales, which include the more frequent, low amplitude strokes during most of the dive (employing ST fibers), and the abrupt, high amplitude, shorter duration strokes (FT fibers) that are followed by a glide pattern during the latter parts of deep dives of beaked whales (Martín López *et al.*, 2015).

With available data and a series of basic assumptions on muscle fiber recruitment, dive metabolic rate, and behavioral responses to sonar exposure (DeRuiter *et al.*, 2013; Pabst *et al.*, 2016; Williams *et al.*, 2017a), estimated muscle metabolic rates are low, both during routine dives and post-sonar exposure dives (see summary in Appendix 4). Under these conditions, the potential for significant intramuscular lactate accumulation, glycogen depletion, and acidosis is low. Similarly, the potential for muscle hyperthermia also appears minimal. The calculated whole muscle diving metabolic rates are about 1.6 ml $O_2$ $kg^{-1}$ muscle $min^{-1}$ at both preferred and maximum stroke costs pre-exposure, and 1.1 and 5.6 ml $O_2$ $kg^{-1}$ muscle $min^{-1}$ at preferred and maximum stroke costs post-exposure, respectively. All these values are remarkably low, given

that human muscle maximum oxygen consumption rates are near 520 ml $O_2$ $kg^{-1}$ muscle $min^{-1}$ (Richardson *et al.*, 1995). For further details on calculations, see Appendix 4 summary review. Lactate accumulation under anaerobic conditions at maximum swim cost after sonar exposure can also be calculated (Williams *et al.*, 2011; again, see details in Appendix 4). For 10 min of glycolytic energy production under such conditions, the resulting lactate increase over 10 min would be 6.5 mmol lactate kg $muscle^{-1}$. This value is relatively low. In comparison, muscle lactate concentration was 42 mmol lactate $kg^{-1}$ muscle after 15-min forced submersion of a seal (Scholander *et al*., 1942), and 50 mmol lactate $kg^{-1}$ muscle after full gallop in a thoroughbred horse (Snow *et al*., 1985). However, given the 163-min dive performed by a Cuvier's beaked whale in response to sonar exposure (Falcone *et al*., 2017), note that for two hours of anaerobic metabolism at the above metabolic rate, the final muscle lactate concentration would be high - 78.7 mmol lactate $kg^{-1}$muscle.

The change in muscle temperature under anaerobic conditions at maximum swim cost after sonar exposure can also be calculated (Ponganis *et al.*, 1993; see details in Appendix 4.) If heat diffused throughout the entire muscle mass (50% of body mass) after muscle exercise under those conditions for 30 minutes, there would be a 0.4 $^{o}$C temperature rise in the entire muscle mass. The risk of muscle hyperthermia appears minimal under these conditions. Two hours of anaerobic metabolism (as in the long dive reported by Falcone *et al.*, 2017) would result in a 1.6 $^{o}$C rise in in all muscle (i.e., 50% of body mass).

### 3c. General stress response

The generalized stress response is highly conserved among mammals and the basic features are identical in marine mammals except for several important differences that have emerged in recent studies. There are several features of these endocrine responses that might be relevant to impacts of acute and chronic stress on diving, including the form of the stress response.

Nervous control of regulatory adjustments to diving have been demonstrated in both forced and natural dives, yet hormonal changes during dives have been measured in only a few species (Hance *et al.*, 1982; Hochachka *et al.*, ,1995; Hurford *et al.*, 1996; Suzuki *et al.*, 2017). Understanding endocrine regulation during free diving has been logistically difficult due to the need to acquire repeated blood samples during breath holds and exercise. To date, this has been achieved in only a few experimental paradigms and the majority of sampling has occurred in the context of sleep apneas and forced or free submergences in captive phocid seals, lacking the exercise and pressure components of diving. Most of what is known about endocrine regulation during free diving comes from ice-hole experiments with Weddell seals with sampling biased to surface intervals between dives.

Diving is associated with increases in circulating catecholamines (norepinephrine and epinephrine) that likely contribute to peripheral vasoconstriction, and splenic contraction (Hochachka *et al.*, 1995; Hurford *et al.*, 1996). The specific tissue effects of increased circulating

catecholamines will depend on the presence and density of receptor isoforms (α and β) and their unique signal transduction pathways, which can interact with other receptor activated pathways. Stimulation of β-receptors typically results in vasodilation of vascular smooth muscle, and activation of G-Protein /cAMP signaling pathways, increasing heart rate and contractility (Brodde *et al.*, 1992).Thus, diving bradycardia that occurs despite high circulating catecholamine concentrations likely reflects strong counter-regulation and cAMP inhibition by vagally-mediated ACh release or as yet undescribed differences in receptor function. Attenuation of vagal cardiac impacts by endocrine release of catecholamines may partially underlie the modulation of the dive response by exercise shown in some marine mammals (Davis and Williams, 2012; Williams *et al.,* 2015a, b). Splenic contraction and peripheral vasoconstriction are mediated by binding of α-receptors; with circulating epinephrine exerting a stronger effect of peripheral vasoconstriction than norepinephrine.

Catecholamine release during diving may also play important roles in the regulation of fuel substrates during diving and surfacing by inhibiting pancreatic insulin release. This inhibition results in a reduced insulin:glucagon ratio and helps maintain glucose availability during vasoconstriction. Insulin inhibition in concert with glucagon elevation during surfacing likely modulates a post-dive hyperglycemia (Robin *et al.*, 1981). In freely submerging but not exercising elephant seals pups, circulating glucagon levels decreased late in breath holds, but increased markedly during surfacing in association with an increase in plasma glucose (Crocker and Tift, unpublished data). Elevated catecholamines during surfacing and reperfusion also likely promote lipolysis from adipose tissue stores to resupply muscle fatty acids depleted in vasoconstricted muscles (Castellini *et al.,* 1985; Kanatous *et al.*, 2002).

Beside the wide variation in circulating concentrations described during diving, catecholamines are also released as part of the generalized 'fight-or-flight' response in marine mammals. Increased catecholamines have been measured in belugas following transport (Spoon and Romano, 2012); in live captured bottlenose dolphins, with an immediate increase in epinephrine, but highest levels found in norepinephrine (Fair *et al.,* 2014), as well as during cetacean strandings where intense catecholamine exposure has been linked to myocardial damage, muscle damage and acute death (Cowan and Curry, 2008; Herráez *et al*., 2013). Physical restraint of weaned elephant seals resulted in a near-instantaneous 3-fold increase in epinephrine levels when compared to sedated pups (Champagne *et al.,* 2012). However, peak epinephrine concentrations during restraint were still significantly lower than those exhibited during free submergences in the same age class (Crocker and Tift, unpublished data). Small but significant increases in epinephrine, norepinephrine, and dopamine were observed in a beluga exposed to high levels of impulsive noise even after extensive time for clearance (Romano *et al.*, 2004). However no significant changes in catecholamines were reported in belugas exposed to recordings of oil drilling noise (Thomas *et al.*, 1990). Such conflicting reports highlight the variability in stress responses due to characteristics of the exposure, as well as individual perception and experience.

The hormone most closely associated with the stress response in mammals, cortisol, has not been examined in the context of diving physiology. The half-life of released cortisol was 109 minutes in dolphins (Champagne *et al*., 2017), suggesting effects of acute stress-induced cortisol release over multiple subsequent breath-holds. In humans, muscle sympathetic vasoconstrictor activity was reduced by cortisol, and pressor responsiveness to subsequent administration of catecholamines was increased (Whitworth *et al.,* 2005). These potent cardiovascular effects of cortisol in humans suggest at least the potential for interactions with the complex cardiovascular regulation of diving in marine mammals. As expected given the slow half-life of released cortisol, blood samples from freely submerged elephant seals demonstrated no changes across dive cycles (Crocker and Tift, unpublished data). However, plasma cortisol levels declined across several hours of free-submergence. Similarly, grey seals exhibited lower cortisol values while diving in comparison to when hauled out (Takei *et al.*, 2016).

Diving potentially has strong effects on renal perfusion and glomerular filtration, which could alter the release of vasoactive hormones. Voluntary bouts of sleep apnea in Weddell and elephant seal pups resulted in decreased circulating vasoconstrictors Angiotensin II (ANG II) and arginine vasopressin (AVP), and an increase in atrial natriuretic peptide (ANP, a vasoconstrictor inhibitor) (Zenteno-Savin and Castellini, 1998). This increase in ANP was attributed to an increase in cardiac pressure, which is a known stimulus of ANP release.

Numerous studies have shown that the adrenal osmoregulatory hormone, aldosterone, is under an unusual degree of hypothalamic-pituitary-adrenal (HPA) axis control in marine mammals and is thus a highly stress-responsive hormone. There is now evidence for this feature in most marine mammal taxa, including strong aldosterone release in response to a stress test in bottlenose dolphins (Champagne *et al.*, 2017), handling stress in belugas (Schmitt *et al.*, 2010), adrencorticotropin (ACTH ) challenge in phocids (Champagne *et al*., 2015; Ensminger *et al.*, 2014; Gulland *et al.*, 1999), and capture stress in otariids (DeRango *et al.*, submitted).

One health concern regarding activation of the neuro-endocrine responses is modulation of immune responses through direct innervation of immune tissues (Romano *et al*., 2002) or binding of adrenergic or adrenal steroid receptors, particularly Type II, expressed on immune cells (Padgett and Glaser, 2003; Madden and Felten, 1995; Romero, 2004). Suppressed immune activity is associated with increased susceptibility to infection and disease. However, augmented activity, particularly inflammatory responses, may alter the susceptibility of marine mammals to dive related injuries. Immune cell function of beluga whales was found to decrease in response to *in vitro* pressure exposures (simulated dives) during baseline conditions, while increases in activity observed under stressor conditions (increased cortisol and catecholamines) resembled the response of human cells, which may suggest an increased risk of inflammatory damage during diving (Thompson, 2014).

## VI. Assessment and Recommendations for Future Research

Following the review, participants of the workshop actively discussed general data gaps in the context of ongoing research efforts in marine mammal diving physiology both within and outside of current ONR projects. These discussions were subsequently used to formulate recommendations for future research that would help address the potential effect of naval operations on marine mammals, particularly deep-diving cetaceans. The following sections synthesize the major data gaps identified by the reviewers and presenters at the workshop and ranks the recommendations in terms of critical need for providing the most relevant science for evaluating the risk of marine mammals for developing gas emboli or incurring other short- and long-term costs when exposed to anthropogenic noise. In this context, gas emboli/bubble formation refers to conditions resulting from excess nitrogen absorption at depth or inadequate nitrogen washout during ascent (Ponganis, 2015). Each section is divided into key anatomical/morphological factors and physiological systems including cardiovascular, pulmonary, stressor/endocrine, thermal, and neural responses.

### General Data Gaps

1. **Cardiovascular Responses**

   Building on Scholander's model of diving in marine mammals (1940), the workshop attendees recognized that cardiovascular responses during diving can vary by numerous intrinsic and extrinsic factors. Most of these have not been adequately examined for most marine mammals. Factors include the effects of species type (odontocetes, mysticetes, otariids, phocids, etc.), morphological and physiological adaptation for deep diving, age and development, reproductive status, and environmental temperature on diving bradycardia and blood distribution. Importantly, an understanding of the relationship between heart rate, stroke volume, and the perfusion of tissues is currently lacking, despite being critical for development of gas emboli and DCS symptoms that can affect how oceanic noise impacts diving mammals. Additional issues related to perfusion and blood flow distribution that need to be investigated include ischemia/reperfusion injury, thermoregulation, the prevention of hypoxia in oxygen sensitive organs (i.e., brain), the importance of globin proteins (hemoglobin, neuroglobins, cytoglobins), and changes associated with respiration.

   Basic information that is needed to understand the relationship between diving capabilities and response to oceanic noise incudes, 1) species-specific blood volume measurements, 2) assessment of variations in cardiac output and stroke volume, and 3) evaluation of tissue perfusion while breathing, during apnea and while diving. Given these data gaps, key questions are, what do changes in heart rate show, and can they be translated into blood flow to specific organs? To answer these questions, we need to refine current models of blood flow distribution during diving (Zapol *et al.* 1979; Davis

and Kanatous, 1999). This will be facilitated by incorporating and developing new technologies involving mobile NIR spectroscopy, medical imaging, as well as methods for assessing thermal distribution and heat flux related to blood flow. The use of trained-captive and stranded-wild marine mammals will provide opportunities for determining the effects of exposure to stress on heart rate, cardiac output, and consequent blood distribution to central organs and peripheral body sites. (For example, see McDonald and Madsen review in Appendix 4). Additionally, an understanding of cardiovascular variability related to cognitive control, and environmental temperature is currently lacking. Model species range from large and small odontocetes (bottlenose dolphins, beluga whales, harbor porpoises, killer whales, Risso's dolphins,) deep-diving odontocetes (beluga whales, false killer whales, pilot whales, narwhals) and pinnipeds (sea lions, and elephant seals) because of their accessibility either in aquaria, the wild (through ongoing capture-release programs) or both. Obviously, data concerning cardiovascular responses in wild, deep diving cetaceans (e.g. beaked whales, sperm whales etc.), even just heart rate and temperature, would be useful to compare with similar responses in model species.

Because blood distribution is dependent on the species-specific vasculature, a better appreciation of blood vessel anatomy and its mechanical properties and receptor responses that dictate vasodilation and constriction is warranted. Specifically, gross and microscopic anatomy are both valuable. The group discussed the importance of understanding major arteriovenous pathways as well as the roles of finer vasculature (e.g. microvascular densities, presence/absence of arteriovenous anastomoses/shunts, etc.) during diving. Vascular research thus far suggests that countercurrent heat exchangers dominate ALL peripheral structures, implying an overarching heat conserving ability. Conversely, CNS related vasculature especially surrounding spinal cord and base of brain are suggestive of potential juxtaposition of cooled venous blood to neural tissues. This goes toward neuroprotective effects such as ischemia and reperfusion tolerance. This is in addition to a robust body of literature suggesting that regional CNS heterothermy may elicit some of the cardinal dive responses (bradycardia, peripheral vasoconstriction). Lastly, the capacity of the blood to buffer blood carbonates and changes in pH should be assessed for comparative species, in particular deep-diving cetaceans. Many of these studies may be conducted on tissues available during stranding events and opportunistically from annual indigenous hunts. Globally, tissues are routinely available for a wide variety of shallow and deep-diving cetaceans and pinnipeds but will require improved coordination with stranding and hunting organizations.

**2. Respiratory Responses - Gas Dynamics and depth**

Major gaps in knowledge related to the respiratory/pulmonary responses of diving mammals center on quantifying inhalation lung volume both during and after diving.

Correlating the timing, frequency, volume, and duration of breathing with metabolic rate, stroke volume, and heart rate can provide new metrics for determining the cost of a dive but have rarely been conducted. Flow/volume measurements by ultrasound, strain gauge, and CT scanning provide important methods for assessing changes in lung volumes during normal breathing and apnea for smaller marine mammals. When combined with a hyperbaric chamber (Moore *et al.*, 2011) such scanning could provide new insights concerning changes in lung volumes and ventilation/perfusion relationships related to pressure as occurs during a dive. The use of radioactive isotopes during such tests will provide additional insights regarding related blood flow. Because these techniques preclude the use of large and inaccessible marine mammals, validation of the relationship between structure and pulmonary function from carcasses, and in live animal models are important (Fahlman *et al*., 2011; Hodanbosi *et al*., 2016; Kooyman and Sinnett, 1979; Kooyman and Sinnett, 1982). Critical for these studies is assessing appropriate animal models, especially if the goal is to relate the findings to deep-diving cetaceans. Major factors that need to be examined include, 1) discerning volitional, automatic, and extrinsic control of gas exchange and pulmonary shunts, 2) determining factors that disrupt this control, and 3) identifying extreme conditions that result in elevated inert gas uptake and increases the risk of gas emboli formation and DCS symptoms. Importantly, current and future evidence for volitional control needs to be compiled and published.

**3. Stress and Endocrine Effects**

One of the major areas of need is a basic understanding of species-specific catecholamine (epinephrine, norepinephrine) balance in marine mammals. Currently, differences in this balance, even for the more commonly accessible marine mammals such as smaller odontocetes and pinnipeds, is unknown. For example, does cortisol in marine mammals have the same effect as in humans?

The physiological impact of stress hormones, including the effect on immune function, reproduction, and diving physiology is especially critical. A key question specific to the stress response of marine mammals is, what is the HPA axis, and specifically cortisol's effect on diving physiology and associated susceptibility to long-term injury and disease processes? Animals, such as elephant seals and bottlenose dolphins are ideal for these studies because of the ability to sample multiple tissues/fluids (i.e., blubber, blow, blood, and urine). Evaluation of these tissues/fluids will aid in development of sampling protocols for wild animals. Post-mortem sampling, as would likely occur in beaked whales, would address Navy concerns about this vulnerable species and should include assessments of adrenal histomorphology to assess HPA axis health. New techniques for profiling longitudinal stress in marine mammals should be investigated, including the use of gene expression markers in blubber. An understanding of receptor types and

distribution will aid in identifying the response of critical target organs such as the vasculature controlling blood flow to the heart, brain, and other oxygen-sensitive tissues.

Studies that evaluate metabolic stress (i.e., how animals are using oxygen) are needed. This includes differentiating acute vs chronic energetic challenges, short-term effects on gas dynamics, and understanding both relative and absolute energetic costs associated with escape responses. Furthermore, in view of the variability in biochemical profiles of skeletal muscle for deep and shallow divers (Ponganis and Pierce, 1978; Williams *et al*., 2011; Pabst *et al.,* 2016), key questions are, 1) what is the variability in oxygen consumption during normal and escape dives, 2) is there flexibility in aerobic dive limits (ADLs), 3) how often and under what circumstances do deep-diving marine mammals, especially the family of beaked whales, exceed predicted ADLs?, and 4) are deep-diving marine mammal species more vulnerable to metabolic stress than shallow diving species?

Overall better profiling of potential stressors on marine mammals is needed. This includes identifying the frequency of stressors and the metrics that determine acute vs. chronic exposures. For example, movement patterns have been proposed to provide information about behavioral states of free-ranging marine mammals (Clegg *et al.,* 2017; Guesgen and Bench, 2017; Manteca *et al*., 2016; Shorter *et al*., 2017); such metrics can be validated with animals under human care and used to study responses in wild species during sonar playback exposures. The roles of acclimation and sub-lethal effects is especially needed, with data previously collected by researchers at NOAA- Southwest Fisheries Science Center (SWFSC) and Cascadia Research Collective (Greg Schorr) providing initial insights.

**4. Morphology and Anatomy**

One of the greatest needs for this area is consistent necropsies and pathological analyses for marine mammals for standing events in the presence and absence of presumed exposure to anthropogenic noise. Such comparative studies of "exposed" animals to "regular" strandings are critical for differentiating sublethal and lethal pathologies and for comparing the prevalence, severity, and manifestation of embolic disease. A question from the workshop participants was, are all previous cases identifying DCS/gas bubble disease/fat emboli indicative of a barotrauma accident? A better foundation for understanding if and when such injuries might occur in marine mammals is still needed before it can be attributed to extrinsic causative factors. Importantly, distinctions between DCS events and other pathologies related to gas emboli formation and other gas bubble diseases need to be assessed. This will be facilitated by either consistent training and sampling protocols or by a SWAT-team approach for specialized teams to examine stranded individuals.

Basic pulmonary morphology, including intrapulmonary vasculature, is needed to understand ventilation-perfusion relationships in marine mammals in general and beaked whales in particular. Information on lung mass, lung blood volume (blood perfusion volume) and architecture and the general vascular system for beaked whales is currently unknown (see Pabst *et al*., 2016 for a recent summary.

Remarkably, data on basic organ size including the brain and heart is missing across marine mammal lineages. A comparative analysis that includes an investigation of adaptations for deep diving is needed and important for beaked whales. For example, are the arterial retia observed in several species of cetaceans or the expansive aortic bulb observed in numerous cetacean and pinniped species a necessary feature for deep diving (Drabek & Burns, 2002; Shadwick & Gosline, 1994; Rhode *et al.*, 1986)? Despite being a dominant and elaborate structure within the thorax of cetaceans, there have been several conflicting reports about the anatomy of the thoracic rete and considerable speculation about its function. For diving species, the anatomical and functional basis of pulmonary shunts, and evidence for and location of a potential "dive computer/ $N_2$ Sensor" should be investigated. As mentioned above under **Stress and Endocrine Effects** immunohistochemistry tools paired with anatomical studies will help identify the relation between morphology and vasoconstriction and vasodilation functions that control blood flow during a dive. Anatomical exploration of vascular structures (arteriovenous connections, angiosomes, microvascular densities, etc.) and other tissues may be most instructive when performed using multiple complimentary methods. Traditional gross dissections coupled with modern imaging modalities (DICE CT, confocal microscopy, contrast-enhanced CT angiography, MRI, etc.) offer unparalleled opportunities for detailed description and quantification of structures that establish a framework for species-level comparisons.

5. **Thermoregulation (hyper/hypothermia)**

With few exceptions (Williams *et al.*, 1999; Noren *et al*. 1999), there is comparatively little information about thermoregulation during diving in cetaceans. Thus, the relationship between thermal balance, regional heterothermy, and environmental temperature is poorly known during active diving and surface swimming, especially in deep-diving species. For many cetaceans, anatomical structures for heat dissipation including peripheral countercurrent heat exchange (CCHE) remain to be determined. This compares with robust CCHE structures that have been described in the extremities and heads of shallow diving cetaceans (Costidis and Rommel, 2012, 2016; Pabst *et al*. 1995). The paucity of such information is especially apparent for cetaceans with small or absent dorsal fins (a critical thermal window for many dolphins), and those species that reside in comparatively warm waters. Recognizing that many marine mammal stranding events involving anthropogenic noise occurred in waters where surface temperatures

exceeded 20°C, an evaluation of the impacts of increased seasonal and climatic ocean temperature on physiological homeostasis is needed. Currently, there are many questions regarding the relationship between the capability for regulating heat dissipation and ambient temperature. At depth deep divers experience low temperature even in tropical waters, but if prevented from diving and forced to escape close to the surface, will warmer water temperatures present a liability by reducing the gradient for heat flow? Furthermore, are there unique anatomical features in beaked whales (e.g., small dorsal and pectoral fin surface areas) that preclude marked heat exchange through blubber and peripheral sites? Additionally, are deleterious effects of elevated ambient temperatures accentuated by different lipid classes contained within blubber (e.g. Bagge *et al*., 2012)?

Physiological mechanisms related to thermoregulation need to be matched to avoidance/escape behaviors that may alter heat production and dissipation. Species-specific characteristics of hypothermia and hyperthermia in relative and absolute terms must be identified and used to determine what constitutes normal and abnormal thermal responses during swimming and diving. For example, emperor penguins and elephant seals typically maintain or elevate core temperature during a dive, whereas some seals selectively reduce body temperature (Blix *et al*., 2010) to help reduce oxygen consumption. Heat stress as associated with increased exercise in warm water can override the dive response (Hammel *et al.*, 1977; Williams *et al*., 1999; Noren *et al.*, 1999,), whereas for cold-stressed animals the dive response overrides thermoregulatory shivering (Kvadsheim *et al.*, 2005)

**6. CNS Responses and Pressure Effects**

Of all of the physiological processes, neural mechanisms in marine mammals remains the least studied. Specifically, little is known about the potential for high pressure nervous syndrome (HPNS), or the maintenance of neural/sensory functions in marine mammals during diving (see Ridgway *et al*., 2001) despite routine exposure to extraordinary hydrostatic pressure at depth. Areas of research needed to understand physiological homeostasis of marine mammals while at depth include assessing the effects of high end-dive $CO_2$ and $N_2$ narcosis on performance tasks. Interestingly, echolocation, navigation, and mating/courtship behaviors of marine mammals may occur at depth with little impact by $CO_2$ and $N_2$ buildup in the blood. How marine mammals accomplish this is not known. A question remains regarding the effect of intrinsic and extrinsic stresses on this balance. Circumstantial observations indicate anomalous behaviors (disorientation, loss of righting response, impaired navigation, etc.) in live stranded whales, suggesting possible disruption of CNS function. Basic information about the ability of deep diving species to buffer gasses in the blood, navigate, and avoid HPNS is also needed.

**Recommendations and Research Priorities**

Below we provide a list of the recommendations for future research in marine mammal diving physiology along with the rationale for application to addressing Navy concerns regarding the effects of sonar on cetaceans. These recommendations primarily result from listening to, and participating in, discussions during the workshop. The recommendations have been synthesized and summarized by the report authors, and reviewed and prioritized by all participants in the workshop. Underlying assumptions for prioritization of these recommendations are that the research directions will, 1) enhance our understanding of individual-level physiological responses to naval sonar exposure, 2) will lead to explanations for the vulnerability of deep-divers in general and beaked whales in particular to the effects of anthropogenic noise, and 3) are reasonably achievable given current technologies, methods, and accessibility of animals. Where appropriate, cautionary information is provided regarding feasibility. We begin by listing species priorities for future research (Table 1) and then move to key topics in cardiovascular, pulmonary, and metabolic-neural related physiology (Tables 2 a, b, and c).

The group agreed that data related to the dive response of beaked whales (heart rate, diving lung volume, core body temperature) collected on wild animals represented the top priority. In addition, measurements on tissues from stranded beaked whales was considered important. Recognizing the poor accessibility to these whales, similar measurements on other deep-diving cetaceans including narwhals, beluga whales, pilot whales and pelagic dolphins represented the next tier of research priorities followed by other model species (Table 1). For these animals, the top five research priorities according to the workshop leaders and a survey completed by the participants as detailed in Table 2 were:

- Assessment of the relationship between heart rate, systemic and pulmonary blood flow, and blood flow distribution during rest, exercise, diving, and during sound exposure in marine mammals

- Determination of the effect of fear responses in the control of cardiovascular dive responses

- Improvement in technology, attachment/release, saltwater performance for long-term monitoring of ECG in freely swimming and diving marine mammals

- Assessment of lung perfusion and shunting, including an investigation of the interrelationship between pulmonary shunting/gas exchange and lung volume

- Evaluation of chronic pathological consequences including sublethal damage due to bubble formation, and susceptibility to perfusion/reperfusion injuries

Lastly, model species and tissues as well as analytical models to elucidate the integration of physiological function during normal diving and during extreme behaviors related to reactions to noise is still needed. This provides the necessary foundation for understanding the vulnerability of marine mammals to emboli formation as well as the adaptations for preventing tissue injury in individual species.

1. **Species priorities for future work. (Table 1)**
   As found in previous workshops (Department of the Navy, 2013; Harris and Thomas, 2015), beaked whales represent the primary species of interest due to the documented number of strandings and potential vulnerability for developing DCS when exposed to military sonar and other anthropogenic noise (e.g., Jepson *et al*., 2003; Fernandez *et al.*, 2005; Cox *et al.,* 2006). Although physiological studies may be limited, there is enormous value in examining live strandings and tissues collected on beaked whale carcasses. However, such endeavors require advanced coordination with and involvement by stranding networks.

   Because there is comparatively poor accessibility to beaked whales for detailed physiological study, the group reviewed the benefits of using model research species. Key factors were accessibility, phylogenetic relatedness to beaked whales, body mass, and deep-diving behaviors. Model species for deep-diving odontocetes available in aquaria include beluga and pilot whales; free-ranging species available for capture-release studies are the narwhal, beluga whale, and pelagic bottlenose dolphin (Bermuda). Because of its coastal accessibility during annual native hunts, narwhals represent both a unique opportunity for examining anatomical adaptations for deep diving as well as physiological responses to anthropogenic stressors. Pelagic bottlenose dolphins have been successfully studied during a number of health assessments, providing opportunities to study basic physiology (lung function and metabolism) and to tag animals. Wild elephant seals due to their accessibility, continuous diving behavior, and extensive database also provide an important model for assessing marine mammal adaptations for deep diving. The accessibility and trainability of smaller odontocetes and pinnipeds in aquaria and research facilities provide important opportunities to examine anatomical adaptations, and discrete aspects and control of diving physiology for marine mammals as related to beaked whales. Bottlenose dolphins, harbor porpoises, and California and Steller sea lions are available for complex physiological studies in captive and wild conditions, as well as in open ocean release research programs. Additional species of interest include Risso's dolphins, killer whales, false killer whales, and pygmy sperm whales with variable accessibility. Overall, in view of the limited information currently known for species-specific diving physiology, each species provides insight into the anatomical and physiological adaptations for accomplishing deep dives and for responding to

anthropogenic stressors. Thus, many recommendations for research by workshop participant suggest a comparative, multi-species approach.

2. **Recommended research directions in cardiovascular diving physiology (Table 2a)**
Due to the importance of the cardiovascular system in mobilizing gases associated with energetics, gas emboli formation, and the transfer of heat and hormones, a large proportion of recommendations are focused on the relationship between heart rate, cardiac output, and blood flow distribution. Key areas of research need include, 1) the relationships between heart rate and systemic and pulmonary blood flow as well as the distribution of blood during and after a dive, 2) measurements of stroke volume, and 3) neurological control of diving bradycardia and blood flow that also involves volitional control of heart rate in deep-diving marine mammals. How various intrinsic (thermoregulation, metabolic makeup, exercise performance) and extrinsic (depth, water temperature) factors affect the dive response and potential for developing gas emboli were considered priorities for investigation. The relatively "slow lifestyle" of beaked whales involving low cost movements and diving needed to be considered. As a result, in some cases phocid seals rather than otariids or even delphinids may represent a better model for beaked whale physiology. Critical for these studies was the continued monitoring of ECGs over long time scales (days to weeks) to provide longitudinal assessments of impacts. This will require improved methods for tag attachments and ECG recording, and was considered a high priority by the group.

Because changes in blood flow can lead to changes in blood gas distribution and compromised organ function, further studies on the sublethal/chronic effects of cardiovascular responses to noise are warranted. Currently, many methods are limited to live animal studies in controlled settings. As a subset of this, it is important to define how habituation and chronic versus acute effects impact the cardiovascular dive response in animals exposed to noise.

The group recognized that an important but poorly studied area has been characterizing the anatomy of the peripheral and central circulation of deep-diving cetaceans. Areas of particular need are assessments of sympathetic nerve distribution, receptor types and function within the vasculature, the effects of vasoactive compounds, as well as the effects of hypoxia and various vasoactive substances on key vessels including the pulmonary artery. Furthermore, studies of heart anatomy should determine the autonomic nervous system control points, and function and location of coronary artery receptors. To evaluate the susceptibility of organs to functional disruption due to changes in blood flow during diving, comparative studies of capillary density in oxygen-sensitive tissue such as the brain are needed. These should include measurements on beaked whales, with

stranded or hunted whales providing excellent opportunities for determining blood volume, heart rate during stress, blood flow distribution via NIRS and deep muscle temperature methodologies. Tissue samples from post-mortem examinations of beaked whales, narwhals, beluga whales, pilot whales, pelagic dolphins, and elephant seals should be evaluated for vessel anatomy and vasoactive function, as well as vessel responses to vasoactive compounds.

3. **Recommended research directions in pulmonary diving physiology (Table 2b)**
As found for the cardiovascular system, an integrative research approach incorporating physiological studies on live animals (either in the laboratory, under managed care or in field settings), comparative anatomical work on tissues, and determination of gas dynamics as related to beaked whales were recommended. Studies concerning the nuances of variability in diving lung volume were determined to be essential. Such studies could involve measurements on live animals or be inferred from proxies using biologging tags on free-ranging marine mammals including beaked whales. Although anatomical data for the lungs and trachea have been reported for some species, comparative studies focusing on description and function of the sinuses, plexuses, and response of the smooth muscle to vasoactive substances are needed. Two important areas of study that were recommended included an evaluation of the distribution and movement of gases, and lung perfusion and shunting. The relationship between pulmonary shunting, lung volume, and pulmonary blood flow is needed to understand the movements of nitrogen, oxygen, and carbon dioxide throughout the dive. This applies to animals performing normal dives and during exposure to a variety of stressors. The relationship between lung inflation and collateral ventilation would aid in this evaluation of pulmonary function.

Information regarding gas dynamics in beaked whales is exceptionally limited due to poor accessibility. Modeling nitrogen uptake from ascent and descent patterns from depth recorders deployed on wild beaked whales could provide a first estimate of nitrogen uptake and should be conducted. Previous modeling studies have identified blood flow, blood flow distribution, and pulmonary shunt/exchange as the most important variables that alter blood and tissue gas dynamics and thereby the risk of gas emboli. The physiological studies mentioned above will fill critical data gaps to help improve the theoretical framework and mechanistic understanding of physiological capacities in beaked whales. Thus, future modeling studies could be used to provide improved estimate of gas dynamics in diving species in a variety of scenarios, e.g. normal diving vs. escape/stress. Opportunistic availability of beaked whales during live strandings should be used to collect the following data,

- An assessment of the effects of pressure and behaviors on lung volume
- Measurements of lung volume and overall size
- Photogrammetry calibration
- Tissue sampling for decomposition-sensitive imaging methodologies (e.g. confocal fluorescence microscopy, immunohistochemistry)

4. **Recommended research directions in metabolic, endocrine, thermal and neural diving physiology (Table 2c)**

Shorter discussions occurred for several other areas of physiological research but were nevertheless considered important research directions for understanding the factors leading to injury following exposure to anthropogenic noise. Metabolic stress could occur on many time scales and levels in wild beaked whales. Two major areas of study were identified:

- *Physiological consequences of chronic behavioral disruption.* This would involve measuring the metabolic cost of response, the energetic cost of lost opportunity, and time budgets for energy and nitrogen distribution.
- *Chronic pathological consequences*. Here sub-lethal damage due to bubble formation, and the susceptibility to perfusion/reperfusion injuries would be assessed.

It is likely that chronic hormonal effects, particularly via corticosterone, and catecholamine surges could impact reproduction, and immune responses; these should be investigated. Cascading effects may include gene changes, long-term energetic consequences, or suppressed disease resistance, that are currently unknown. Data mining could provide a first look at the adaptations required to perform shallow versus deep foraging dives by marine mammals.

To date there have been limited studies concerning the central nervous system response to deep diving or noise disruption. Areas of specific need are investigations focusing on HPNS, nitrogen narcosis, and the effects of $CO_2$ narcosis during diving. The group considered that the potential for tissue injury may be related in part to species-specific differences in brain structure from neuron density in the cerebellum to on-board globin neuro-protection by neuroglobins, hemoglobin and cytoglobins that should be explored. In addition, the neuroprotective effects and physiological consequences (e.g. bradycardia, vasoconstriction) of CNS cooling demonstrated for pinniped should be investigated for cetaceans.

Lastly, thermoregulation and the need to transfer heat can compete with the cardiovascular responses to diving. To that end the participants of the workshop

recommended further research concerning the anatomical structures required for heat regulation in deep-diving cetaceans, the prevalence and conditions eliciting regional heterothermy, and factors leading to hyperthermia during and after diving.

## VII. Concluding Comments

In summary, we find that the suite of physiological responses to diving represents a unique and fundamental characteristic of marine mammals. The complex integration of cardiovascular, pulmonary, and metabolic events comprising the dive response that enables animals to attain great depths also makes them susceptible to injury when it is disrupted.

However, specific elements of this response remain poorly understood for many species, particularly the deep-diving cetaceans. The newest data for both captive and free-ranging marine mammals indicate that the diving response is highly variable. This is further complicated by specific intrinsic responses by cetaceans and pinnipeds, as well as extrinsic factors including water temperature and diving depth.

Together, the workshop participants agreed that the studies recommended in this report represent a comprehensive list of recommendations that will enable the Navy to predict, avoid and potentially mitigate the impacts of sonar exposure on the physiology of deep-diving marine mammals including beaked whales.

## VIII. Acknowledgments

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**Table 1. Species priorities for future research on the effects of military sonar on the diving physiology of deep-diving cetaceans.**

| Species/species group |
|---|
| Primary Species |
| Beaked whales |
| |
| Deep-Diving Odontocete Models |
| Large delphinids |
| Beluga whales |
| Pilot whales |
| Narwhals |
| Pelagic bottlenose dolphin |
| Large whales (sperm whale, kogia, etc.) |
| |
| Deep-Diving Pinniped Models |
| Elephant seals |
| |
| Accessible Model Species- Odontocetes |
| Bottlenose dolphins |
| Harbor porpoises |
| Other small delphinids |
| |
| Accessible Model Species - Pinnipeds |
| Sea lions |
| Harbor seals |
| Other pinnipeds |

**Table 2. Recommended research directions.** The order of recommendations approximately relates to the order of appearance in report text. The priority score is made up of two metrics, three independent scores provided by the workshop leaders with the average ranking from the remaining workshop participants shown in parentheses below. Reviewers gave each recommendation a score of 1, 2 or 3 with a score of 1 indicating the highest priority level. The independent scoring is ordered with the highest priority score first. Note that, 1) even those topics that scored a 3 are considered important. Topics that were not a priority are not listed, 2) in the assignment of priority, reviewers largely focused on scientific reward with feasibility added as a modifier, and 3) all reviewers tried to follow an advised distribution of scores (20% rank 1, 70% rank 2, 10% rank 3).

**Table 2a. Recommended Research Directions in Cardiovascular Diving Physiology and Theoretical Modeling.**

| ID | Recommendation | Approach | Relative Priority | Feasibility/Cost/Notes |
|---|---|---|---|---|
| 1 | Studies to assess the relationship between heart rate, systemic and pulmonary blood flow, and blood flow distribution during rest, exercise, diving, and during sound exposure in marine mammals | Captive, wild | 1-1-1 (1.0) | Feasible with captive or trained animals; possibly translocations of seals<br><br>Requires a comparative approach with accessible marine mammal models in a variety of settings |
| 1a | Explore measurements of tissue blood flow using near-infrared technologies | Captive, stranded, wild | 1-2-2 (1.9) | Muscle NIR reflects myoglobin saturation primarily, a product of muscle work and blood flow and blood oxygen content; not an ideal index of muscle blood flow<br><br>Same as A |
| 1b | Examine peripheral versus central blood flow using heat flux sensors | Captive, wild | 2-2-2 (2.3) | Feasible for heat flux: requires interpretation for central blood flow; need heart rate<br><br>Same as A |
| 2 | Determine the relationship between heart rate and stroke volume of the heart | Captive, stranded, wild | 1-2-2 (2.0) | Currently feasible in captive animals<br><br>Same as A |

| | | | | |
|---|---|---|---|---|
| 2a | Investigate the use of trans-thoracic echocardiography at depth | Captive, wild | 1-2-2 (2.3) | Currently feasible in captive animals |
| 3 | Determine the role of volitional control in cardiovascular dive responses | Captive | 1-2-2 (2.1) | Feasible in trained animals |
| 4 | Determine the effect of fear responses in the control of cardiovascular dive responses | Captive, wild | 1-1-2 (1.1) | Feasible; To be evaluated in eseals in current NSF project by Hindle, C. Williams, McDonald; needed for cetaceans |
| 5 | Integrative studies to discern the relative role of depth, thermoregulation and exercise level on cardiovascular responses when submerged | Captive, wild | 1-1-2 (1.6) | Feasible |
| 6 | Improve technology, attachment/release, saltwater performance for long-term monitoring of ECG in freely swimming and diving marine mammals | Techno, captive | 1-1-1 (1.1) | Feasible; needs engineering development; improved ECG electrodes /filtering of artifacts. Long term feasibility and effect of electrode placement (either SQ or long-term suction) required. Includes improvements in ECG storage capacity, electrode quality, and longevity of attachment |
| 6a | Consider tests of deep muscle temperature as a proxy for blood flow | Techno, captive, wild | 1-1-1 (2.3) | Feasible in captive, translocated animals, and narwhal |
| 7 | Conduct studies to develop models to differentiate between sub-lethal/chronic and lethal impacts of noise on cardiovascular responses | Models, Stranded | 3-3-3 (2.3) | Feasible, but criteria for such distinction of chronic and acute effects is needed<br><br>Low priority due to the limited methods available for live animal studies |
| 8 | Determine the relationship between cardiovascular stress and potential habituation to noise | Captive, wild | 2-2-3 (2.1) | Criteria needed to measure; especially long-term assessment |
| 9 | Detail the anatomical architecture of peripheral circulation and heart in deep diving marine mammals | Tissue | 1-2-2 (2.0) | Feasible; needs organization for response to strandings and protocols |

| | | | | |
|---|---|---|---|---|
| | | | | Including sympathetic nerve distribution, receptor types, autonomic nervous system and coronary artery receptors in the heart |
| 9a | Evaluate the effects of vasoactive compounds on vasculature of marine mammals | Tissue | 1-2-2 (2.0) | Feasible; needs organization for response to strandings and protocols<br><br>Comparative studies that include shallow and deep divers such as beaked whales are needed |
| 9b | Determine the effects of hypoxia on key blood vessels including the pulmonary artery | Tissue | 1-2-2 (2.3) | Feasible; needs organization for response to strandings and protocols |
| 9c | Measure microvasculature in key oxygen-sensitive organs (e.g. brain, myocardium) | Tissue | 1-2-3 (2.3) | Feasible; needs organization for response to strandings and protocols |
| 10 | Detail the unique cardiovascular adaptations of beaked whales and other deep-diving cetaceans | Stranded, hunted, tissue | 1-1-2 (1.6) | Accuracy of blood volume measurement in stranded animals may be difficult (i.e., required time and adequacy of mixing of markers). Includes blood volume, and heart rate during stress |
| 10a | Conduct comparative studies on blood flow distribution (vasoactive responses, vessel anatomy, NIRS) on beaked whales | Stranded, tissues | 1-1-2 (1.6) | Tissue studies require organization and protocol for stranding; assess relevance of stranded blood flow distribution to wild animals<br><br>Other deep-diving species may serve as models |
| 11 | Theoretical modeling using updated physiological or structural information | Using available dive data | 1-3-3 (1.9) | Feasible; needs documentation of multiple physiological variables<br><br>Other deep diving cetaceans/pinnipeds may be used as models to investigate the effect on certain responses |

**Table 2b. Recommended Research Directions in Pulmonary Diving Physiology**

| ID | Recommendation | Approach | Relative Priority | Note |
|---|---|---|---|---|
| 1 | Studies to assess variability in lung volume during rest, exercise, and diving | Captive, wild, stranded | 1-1-2 (2.0) | Feasible<br><br>Will involve a comparative approach with accessible marine mammal models in a variety of settings |
| 2 | Continue comparative anatomical examinations in deep-diving cetaceans, particularly beaked whales | Stranded, hunted, captive | 1-1-1 (1.5) | Feasible; needs organization for response to strandings and protocols<br><br>Same as 1 |
| 3 | Evaluate the distribution and movement of gases in the lungs and blood | Captive, wild | 1-1-2 (1.7) | Feasible; specific model species needed<br><br>Same as 1 |
| 3a | Assess lung perfusion and shunting, including an assessment of the interrelationship between pulmonary shunting/gas exchange and lung volume | Captive, stranded | 1-2-2 (1.2) | Feasible, captive, selected species<br><br>Same as 1 |
| 4 | Measure acoustic reflection and target strength on pulmonary function | Captive | 3-3-3 (2.2) | Feasible; question regarding accuracy of reflection as index of alveolar collapse |
| 5 | Determine the response of smooth muscle within pulmonary structures to vasoactive substances | Captive | 1-3-3 (2.2) | Feasible; needs organization for response to strandings and protocols |
| 6 | Determine the effects of stress on diving lung volume | Captive, wild | 1-3-3 (2.0) | Criteria needed for identifying stress factors and how to measure diving lung volume |
| 7 | Integrative studies to discern the relationship between lung inflation and collateral ventilation | Captive | 1-2-2 (2.3) | Needs development; applicable to select captive species and stranded animals. |
| 8 | Conduct comparative studies on gas dynamics in beaked whales including assessments of volume versus depth, changes in volume during gliding | Wild, captive, stranded | 2-2-3 (1.8) | Beaked whale studies not feasible; possible with trained animals and/or translocations of seals to some degree; imaging |

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| | ascents/descents to estimate nitrogen uptake. | | | feasible on small, captive species under anesthesia, requires much development. Despite being critical measurements, these are considered difficult due to the inaccessibility of the animals. Medical imaging on captive animals will facilitate. |

**Table 2c. Recommended Research Directions in Metabolic, Endocrine, Thermal and Neural Diving Physiology**

| ID | Recommendation | Approach | Relative Priority | Note |
|---|---|---|---|---|
| 1 | Determine the metabolic cost, energetic cost of lost opportunities, and time budgets for energy and $N_2$ exchange associated with chronic behavioral disruption due to noise | Captive modeling, wild | 2-2-3 (1.5) | Needs input of yet undocumented physiological variables<br><br>Ranking depends on species (1 for beaked whales, 2 for deep diving cetaceans, 3 for other model species |
| 2 | Evaluate chronic pathological consequences including sublethal damage due to bubble formation, and susceptibility to perfusion/reperfusion injuries | Wild, stranded | 1-2-3 (1.3) | Feasible; needs organization for response to strandings and protocols |
| 3 | Determine the impacts of chronic hormonal disruption on reproduction and immunosuppression in marine mammals | Stranded, wild | 2-3-3 (2.2) | Needs documentation of hormonal disruption (measurements feasible in captive models with blood sampling); need to document chronic hormonal responses, and immune responses - how to study chronic effects<br><br>Most notably corticosterones, and chronic catecholamine surges |
| 4 | Continue evaluation of neuro-protection by neuroglobins/cytoglobins | Tissue | 2-3-3 (2.2) | Feasible; needs organization for response to strandings and protocols.<br><br>Dependent on availability of beaked whale tissues |
| 5 | Evaluate metabolic stress and work in beaked whales using an integrative approach involving gene changes, tissue receptors, | Tissue, wild | 2-2-3 (2.2) | Approach is undeveloped and untested; especially with chronic effects; |

| | | | | |
|---|---|---|---|---|
| | oxygen dynamics and utilization, and modeling to determine the chronic effects of noise on metabolic demands | | | lack of basic knowledge of physiological responses |
| 6 | Conduct an evaluation of the metabolic responses to shallow versus deep diving in beaked whales (or model species) using respiratory/cardiovascular parameters calibrated to energetics. | Wild, captive | 1-1-2 (1.5) | Requires basic knowledge of physiological responses in beaked whales; selected models might be feasible. ODBA or MSA might be feasible in selected species. |
| 7 | Determine the anatomical structures for temperature regulation in deep diving cetaceans, particularly beaked whales | Stranding, tissues | 2-2-2 (2.3) | Strand response organization and protocols required |
| 8 | Determine the incidence of hyperthermia following elevated performance by beaked whales | Wild, stranded | 1-1-2 (2.3) | Undeveloped technology apart from trying to measure deep muscle temperature in selected situations; narwhal most feasible<br><br>Requires new technology for free-ranging whales. Deep divers such as narwhals may provide a model |
| 9 | Evaluate the CNS responses to diving and exposure to noise by beaked whales and other deep-diving cetaceans. | wild | 1-2-3 (1.8) | Currently not feasible; technology not yet developed |
| 10 | Conduct anatomical studies specific to the adaptations of CNS structures in beaked whales and other deep diving cetaceans. | Tissues, stranded, wild | 1-1-3 (1.8) | Organization and protocols for strandings required. Includes data for brain size and perfusion, neuron density in different brain regions, and sensory/navigation changes with response to acute noise exposure. |

## APPENDIX 1 - WORKSHOP AGENDA

### ITINERARY

**Monday September 11**

**8:30 Introduction to Review and Workshop–** Michael Weise, Program Officer, ONR

**8:45 Logistics and Goals –** Terrie Williams, Beau Richter, UCSC

**9:00 – 12:00 ONR Program Review**

**9:00 Paul Ponganis** - Blood oxygen conservation in diving sea lions: How low does oxygen really go?

**9:30 Andreas Fahlman -** Modeling gas dynamics in California sea lions

**10:00 Michael Moore -** Measuring compartment size and gas solubility in marine mammals

**10:30 BREAK**

**10:45 Laura Thompson -** Evaluation of non-lethal effects of N2 bubbles on marine mammal health and the potential role of immune activity in facilitating the development of dive related injury

**11:15 Peter Madsen -** Tag-based heart rate measurements of harbor porpoises during normal and noise-exposed dives to study stress responses

**11:45 Terrie Williams -** High risk behaviors in marine mammals: Linking behavioral responses to anthropogenic disturbance to biological consequences

**12:15 – 13:30 LUNCH BREAK – in house**

**13:30 – 17:00 Topical Reviews – Current Status of Marine Mammal Diving Physiology as Related to the Impact of Military Sonar on Beaked Whales**

**13:30 Paul Ponganis -** Introduction to Review Topics

**13:40 Terrie Williams –** Why beaked whales? Predicting physiological costs of escape responses

**14:00 Alex Costidis -** Morphology of beaked whales

**14:20 Discussion regarding the unique vulnerability of beaked whales**

**14:30 McDonald and Madsen - Cardiovascular responses**

**14:50 Garcia-Párraga, Moore, and Fahlman - Gas Exchange/Lung Collapse/Pulmonary Shunts**

**15:10 Fahlman and Garcia-Párraga - Modeling blood and tissue gas dynamics, risk of DCS**

**15:20 Discussion regarding cardio-respiratory responses and DCS**

**15:40 BREAK**

**15:50 Crocker and Thompson - Metabolic stress and general stress responses during diving**

**16:10 Discussion to identify data gaps in Marine Mammal Diving Physiology and future research directions. Identification of breakout groups for Tuesday.**

**16:50 HOMEWORK ASSIGNMENT: Developing a template for a co-authored review article regarding physiological responses of marine mammal to anthropogenic noise**

**17:00 Facility closes**

**18:30 Group Dinner - TBD**

**Tuesday September 12**

**8:30 – 12:00 TOPICAL SESSIONS**

**8:30 Andreas Fahlman – Introduction to sessions - logistics and goals**

**8:50 Session I. Identifying Data Gaps in Marine Mammal Physiology**

**10:15 Summary Discussion**

**10:30 BREAK**

**10:50 Session II. Critical Physiological Research Needed to Address Cetacean Reponses to Sonar**

**12:00 LUNCH BREAK – Field trip to Long Marine Lab pools**

**13:30 Continuation of Session II Critical Research Needed**

**14:30 Summary Discussion from Session II**

**15:00 Group discussion for workshop recommendations, writing assignments**

**16:00 Group discussion on focus of the co-authored review article**

**17:00 Facility closes**

**18:30 Group Dinner - TBD**

**Wednesday September 13**

**8:30 – 12:00 Outlining the workshop reports, and workshop review article**

**Writing assignments and timelines.**

## APPENDIX 2 – WORKSHOP PARTICIPANTS

**Steering Committee**

- Terrie Williams (University of California, Santa Cruz)
- Paul Ponganis (University of California, San Diego)
- Andreas Fahlman (Oceanografic Foundation, Valencia, Spain)

**External Reviewers**

- Gerald Kooyman (University of California, San Diego)
- Sam Ridgeway (National Marine Mammal Foundation, San Diego)
- Tobias Wang (Aarhus University, Denmark)

**Participant List**

- Terrie Williams (University of California, Santa Cruz)
- Paul Ponganis (University of California, San Diego)
- Andreas Fahlman (Oceanografic Foundation, Valencia, Spain)
- Alexander Costidis (Virginia Aquarium and Marine Science Center, Virginia Beach)
- Peter Madsen (Aarhus University, Denmark)
- Michael Moore (Woods Hole Oceanographic Institute)
- Laura Thompson (Brookhaven National Laboratory, Upton)
- Daniel Crocker (Sonoma State University)
- Birgitte McDonald (Moss Landing Marine Lab)
- Shawn Noren (University of California, Santa Cruz)
- Daniel Costa (University of California, Santa Cruz)
- Daniel Garcia-Parraga (Oceanografic Foundation, Valencia, Spain)
- Petter Kvadsheim (Norwegian Defence Research Establishment, Horten, Norway)

## APPENDIX 3 – SUBMITTED TOPICAL REVIEWS

### ONR Diving Physiology Workshop September 11-12, 2017

### Summary Reviews – Beaked Whales and Diving Physiology

### Table of Contents

## Vulnerability of Beaked Whales to Anthropogenic Noise

Terrie M. Williams

### Why beaked whales?

Due to the number of strandings purportedly associated with exposure to military sonar and to pathology reports from those strandings, the family of beaked whales is considered uniquely vulnerable to the effects of anthropogenic noise. Twelve of 126 strandings of beaked whales occurring between 1950 to 2004 are believed to have coincided in space and time with naval activities that may have included active sonar use (D'Amico *et al.*, 2009; Table 1). Causal mechanisms for these strandings are currently lacking, although behavioral flight responses and physiological consequences to acoustic exposure (i.e., inert bubble formation) have been suggested (DoN, 2013). Importantly, not all beaked whales in areas of known sonar deployment strand. This has led to the suggestion that other contextual, physiological, or behavioral factors may contribute to the necessary conditions for stranding to occur (D'Amico *et al*., 2009; Filadelfo *et al*., 2009; Tyack *et al.*, 2011). Potential factors include steep bathymetry, narrow channels, and the number of sonar sources. In addition to these abiotic factors, the biochemical, physiological, morphological, and behavioral biology of beaked whales may conspire to make this cetacean group more susceptible to disturbance by noise than other dolphins or whales. Exceptional diving capacity (see following section) and associated biochemical (Pabst *et al.*, 2016; Williams *et al*., 2008) and morphological specialization (see review by A. Costidis for this workshop) may be challenged in deep-diving cetaceans during escape responses and stranding (Williams *et al.*, 2017b).

**Table 1. Beaked whale stranding events associated with military sonar activities (DoN, 2013) in which carcasses were recovered.**

| Date | Location | Number of Animals, evidence, and pathology |
|---|---|---|
| 1996 May | Greece | 12 Cuvier's; no pathology, circumstantial location |
| 2000 March | Bahamas | 9 Cuvier's, 3 Blainville's, 2 other beaked whales, plus 2 minke and spotted dolphin; hemorrhage of brain, fats, intracochlear areas, kidney, and other organs |
| 2000 May | Portugal, Madeira Islands | 3 Cuvier's; renal congestion, subarachnoid and peribulbar hemorrhage |
| 2002 September | Spain, Canary Islands | 14 beaked whales; possible DCS fat emboli and macroscopic intravascular bubbles, head and lymph node congestion and hemorrhage in multiple tissues (kidney, brain, ears, jaws) |
| 2006 January | Spain, Mediterranean Sea | 4 beaked whales; no pathology, circumstantial location |

**Normal dive behavior.** Beaked whales are considered elite divers in terms of the duration and depths attained. Compared with many other cetaceans, the family of beaked whales spend extraordinary amounts of time submerged and routinely perform sequential dives of long duration. For example, routine dives for Blainville's (*Mesoplodon densirostris*) and Cuvier's

(*Ziphius cavirostris*) beaked whales in Hawaiian waters ranged from 19-24 min, while prolonged dives lasted 48 – 68 min (Baird *et al.,* 2006). Maximum depths exceeded 800-1330 m for individuals. Numerous other studies have confirmed the extraordinary diving capabilities of beaked whales with average foraging dives of 58 min to 1070 m for the Cuvier's beaked whale and 47 min to 835 m for the Blainville's beaked whale in deep waters off Italy and the Canary Islands, respectively. The average ascent rate is typically less than 50% of the descent rate for both species (Tyack *et al.,* 2006). Long-term records from Cuvier's beaked whales tagged off the coast of California revealed one of the deepest dives for any cetacean, 2992 m occurring over 137.5 min (Schorr *et al.,* 2014). Usually, a series of short recovery dives follow these long foraging dives; inter-dive intervals of >60 min are common and presumably are associated with the recovery of oxygen stores (Tyack *et al.,* 2006). Gait switching may promote energy and oxygen conservation during prolonged dives (Martin Lopez *et al*., 2015). Using extrapolations from Weddell seals, Tyack *et al.* (2006) suggested that beaked whales markedly exceed aerobic dive limits during long foraging dives. However, repetitive shallow dives rather than these prolonged, deep dives may pose a greater risk for the generation of supersaturated tissue and subsequent vulnerability to decompression stress (Tyack *et al.,* 2006; Cox *et al.,* 2006; Zimmer and Tyack, 2007). Consequently, behavioral disruption of the typical dive patterns of beaked whales in response to exposure to sonar may present a plausible underlying mechanism for inducing stranding and/or damage to tissues.

**Stranding pathology**. As might be expected, the number of fresh specimens from stranded beaked whales has been limited. Consequently, much of what is known for these species has been based on pathology reports from Jepson *et al*. (2003, 2005), Fernandez *et al. (*2005), and Ketten *et al.* (2004). Several consistent pathologies across the beaked whales and with other stranded cetaceans purportedly exposed to anthropogenic noise are noted. Of the beaked whale strandings listed in Table 1, three incidents reported vascular congestion or hemorrhaging in multiple organs including the kidney, ears, and brain. During the 2002 Canary Islands incident, eight Cuvier's beaked whales, a Blainville's beaked whale, and a Gervais' beaked whale (*Mesoplodon europaeus*) showed severe, diffuse vascular congestion and marked, disseminated microvascular hemorrhages, and fat emboli within vital organs (acoustic jaw fat, ears, brain, and kidneys). Intravascular bubbles were also present in several organs including the liver (Jepson *et al*., 2003). When fresh carcasses are available, gas and fat emboli are often noted (Fernández *et al.,* 2005; Fahlman *et al*, 2014). Ketten *et al*. (2004) reported damage to the ears and brain in addition to general cardiovascular collapse incidental to standing for beaked whales and other cetaceans following the 2000 stranding incident in the Bahamas. Intra-cochlear and temporal region subarachnoid hemorrhages with lateral ventricular clots (blood within inner ear chambers and hemorrhaging in fluid spaces surrounding the brain) were found. The cranial trauma observed for these animals was not always immediately lethal, and therefore was considered an important contributory, if not causal, factor for the strandings.

The formation of nitrogen bubbles consistent with decompression sickness has been a subject of intense debate for stranded beaked whales and will be reviewed elsewhere in the workshop. Some hypothesize that bubble formation is directly related to activation by rectified diffusion via sonar signals (Crum and Mao, 1996); others suggest that it is initiated by startle responses resulting in rapid ascents by fleeing beaked whales following sonar exposure (Fernández *et al.,* 2005; Jepson, *et al.,*2003). Regardless of etiology, tissue injury due to bubble formation by both nitrogen and carbon dioxide gases have been hypothesized (Fahlman *et al.,* 2014).

**Controlled exposure responses.** Numerous studies have examined the response of instrumented beaked whales experimentally exposed to acoustic disturbance including ship noise and low frequency sonar. In 2013 DeRuiter *et al.* monitored the movements, stroke frequency, and diving behavior of two Cuvier's beaked whales exposed to sonar. The animals showed a shift in swimming gait from primarily burst and glide to nearly constant stroking following exposure to noise (Fig. 1a). Both animals responded strongly to playbacks at low received levels (RLs; 89–127 dB re 1 mPa), initially ceasing normal fluking and echolocation. This was followed by a rapid swimming escape with dives increasing in duration. Further analyses by Williams *et al.* (2017) for two matched dives to approximately 440 m demonstrated that the most obvious behavioral effect was an 18.3% decrease in dive time and an increase in the range and mean stroke frequency (pre-exposure 437 m dive = 7.2 $\pm$ 1.6 strokes.min$^{-1}$ during an 1-min descent and 13.6 $\pm$ 0.8 strokes.min$^{-1}$ during a 19-min ascent; post-exposure 444 m dive = 10.2 $\pm$ 1.0 strokes.min$^{-1}$ during a 9-min descent and 16.9 $\pm$ 0.9 strokes.min$^{-1}$ during a 15-min ascent). One whale spent over four minutes gliding on descent during the pre-exposure dive but did not use this critical energy saving gait during post-exposure diving.

Similar behavioral responses by the largest of the beaked whales, the Baird's beaked whale (*Berardius bairdii*), were reported by Stimpert *et al.* (2014) during controlled exposure to simulated mid-frequency active sonar (3.5–4 kHz). Within 3 minutes of exposure onset, the tagged whale increased swim speed from approximately 1.5 m.s$^{-1}$ to 3.0 m.s$^{-1}$, increased overall dynamic body acceleration movements (ODBA, a metric of energetics), and continued to show unusual dive behavior for three subsequent dives. Bottlenose whales, a deep diving odontocete, similarly respond to naval sonar signals by altering movements and swimming away from the sound source. Altered diving behaviors were recorded for this species for more than seven hours post exposure (Miller *et al*., 2016). Often vocalizations cease or are disrupted during these escape behaviors. Together, these studies indicate that the escape response of beaked whales to anthropogenic noise generally consists of alterations in the spatial movements and diving duration (both increases and decreases from normal), as well as reductions in both ascent rates and vocal behaviors that may last across dives or days (see McCarthy *et al.*, 2011; Tyack *et al*., 2011).

**Potential energetic costs.** To examine the potential energetic cost of flight behaviors by beaked whales, Williams *et al.* (2017) calculated oxygen utilization rates for a 2500 kg Cuvier's beaked whale (*Ziphius cavirostris*) exposed to naval sonar (Fig. 1C). Total energetic costs and oxygen stores utilized for normal and post-exposure dives were determined from accelerometer-depth data from DeRuiter *et al.* (2013) and locomotor costs for stroking by odontocetes. The behavioral changes in swimming gaits described in the previous section resulted in a concomitant increase in energetic cost of a dive. Calculated metabolic rate during the post-exposure dive was 30.5% higher than before exposure, with a marked shift in the proportion of the dive cost used to support maintenance or stroking demands. Regardless of depth, dives occurring before or partially during noise exposure were supported by metabolic rates averaging 3.16 $\pm$ 0.15 $mlO_2.kg^{-1}.min^{-1}$ (n = 6 dives) that were partitioned as 45% for maintenance costs with the remainder divided nearly equally into preferred and maximum aerobic stroking costs. For post-

exposure dives, metabolic rate increased to 4.76 $\pm$ 0.24 $mlO_2.kg^{-1}.min^{-1}$ (n = 3 dives) with maintenance and preferred stroking costs dropping to 29.7% and 11.8% of the diving cost, respectively, and maximum stroking costs increasing to 58.5%. This shift in energetic partitioning was apparent even 1.7 hours following exposure to a single sonar event.

The beaked whale tended to defend its theoretical dive limit, and hence remain aerobic, even during the post-exposure escape period. This was accomplished by reducing the depth and duration of post-exposure dives while increasing the use of energetically costly high-speed stroking. In this way, the proportion of the oxygen store expended remained below the total calculated store of 295 $LO_2$ that would lead to prolonged anaerobiosis and lactate accumulation.

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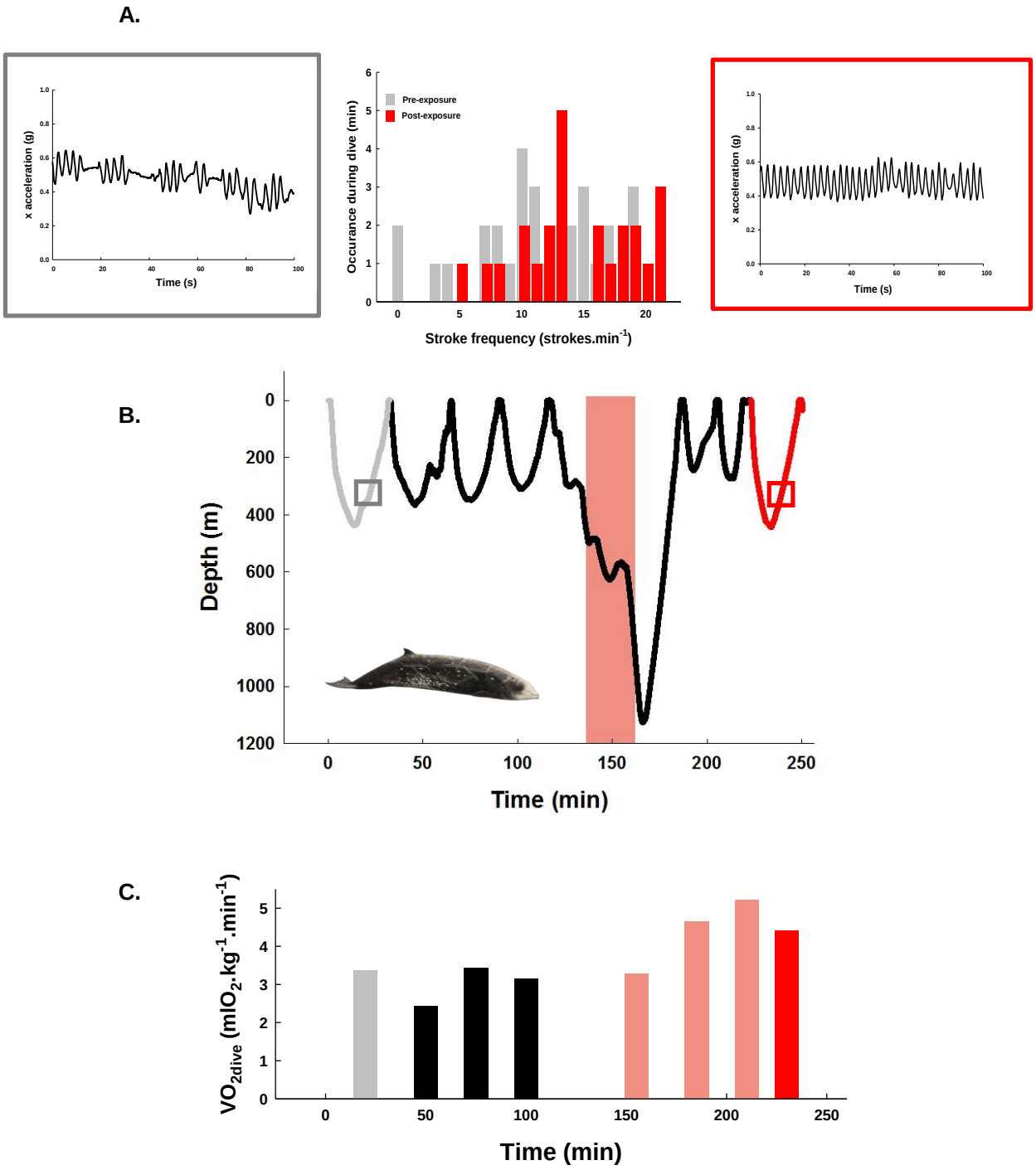


**Figure 1. Kinematic (A), behavioral (B), and energetic (C) responses to anthropogenic noise by a diving Cuvier's beaked whale.** Panel B shows the four-hour sequential dive pattern of an adult beaked whale before, during and after exposure to mid-frequency sonar, the timing of which is denoted by the pink background. Matched dives to approximately 440 m are drawn in grey (pre-exposure) and red (post-exposure) (data from DeRuiter *et al.*, 2013). Small boxes on the ascent portion of two matched dives in B correspond to the colored boxes in panel A showing

the prevalent stroking gait pattern from the accelerometer during each period. The middle graph in panel A illustrates the shift in the range of stroke frequencies for these two matched pre- (grey) and post- (red) exposure dives. Here the height of the bars corresponds to the occurrence of each level of stroke frequency (calculated per minute) during the entire dive. In panel C, the calculated rate of oxygen consumption in relation to sequential dive time as shown in panel B is compared for pre-exposure dives (grey and black bars), and dives taking place during and immediately after exposure (pink bars) and nearly two hours post-exposure (red bar).

## Morphology of Beaked Whales - Body Composition and Anatomical Features

Alexander M. Costidis

The current state of knowledge regarding beaked whale morphology is exceedingly lacking. While cetacean morphological knowledge is generally lacking, knowledge of beaked whale morphology is woefully inadequate and generally limited to the gross morphology of the viscera, skeleton and vocal structures (e.g. Mead, 1975). Critical aspects of beaked whale morphology have been minimally explored and are generally limited to morphology of muscular (Sierra et al., 2015; Velten et al. 2013), fatty (Lonati et al. 2015; Koopman et al., 2006), acoustic (Cranford et al, 2008a; 2008b; Soldevilla et al., 2005) and vascular structures (Costidis and Rommel, 2016a; 2016b). Therefore, physiological modeling by necessity uses data from other cetaceans, marine mammals, and even terrestrial mammals to extrapolate values for beaked whales.

### *Gross and microscopic visceral morphology relevant to diving*

An immunohistochemical and histomorphometric examination of beaked whale muscles showed some of the highest myoglobin concentrations of any mammal measured to date, lowest mitochondrial content in Type II myofibers—representing ~80% of muscle fiber type composition--of any mammal yet reported and extremely large Type II myofiber sizes (Velten et al., 2013). These findings, in conjunction with the fact that deep diving cetaceans like sperm whales, pygmy sperm whales and beaked whales have markedly smaller lung masses and volumes than shallower divers, suggest that beaked whale are designed to operate at low cost (reviewed in Pabst et al., 2016). Using the 'body composition technique' of comparing total mass of metabolically distinct tissues, Pabst et al. (2016) showed that beaked whales (and other deep divers) use large depots of metabolically inexpensive muscles to store increased oxygen (large muscle depots with high myoglobin concentration) and offset expensive tissues (reduced expensive tissues and increased inexpensive tissues) (Pabst et al., 2016). Compared to shallow diving cetacean species in which locomotor muscle represents 28-30% of total body mass, mesoplodonts invest as much as 50% of TBM in muscle, while the pulmonary, hepatic, intestinal and brain investment is notably reduced ("inexpensive body hypothesis") (Pabst et al., 2016).

### *Vascular morphology*

Our knowledge of the gross vascular morphology of beaked whales is at its infancy. With the exception of two manuscripts on the cephalic vascular morphology (Costidis and Rommel, 2016a; 2016b), there are no other published studies of beaked whale vascular anatomy. What little is known about the topic is focused on the head and neck of beaked whales and is only focused on Mesoplodont species, leaving Cuvier's beaked whales, arguably the most affected by ensonification and decompressions-like sickness, completely unknown. Costidis & Rommel showed that most peripheral arteries are surrounded by periarterial venous retia. Such retia may simply be responsible for conserving body heat, but could also aid in maintaining lower tissue temperatures in the blubber or reducing metabolic costs of tissues. Such a mechanism could result in greater nitrogen absorption capacity in fatty tissues and

reduce offloading into the blood, except when stressful conditions alter heat production and vascular tone. Could heating of blubber due to increased blood flow result in greater nitrogen offloading during stressful conditions?

Unlike delphinids and phocoenids that possess intricate pterygoid venous networks embedded in considerable connective tissue (fibrovenous plexuses) (Boenninghaus, 1904; Fraser & Purves, 1960, Costidis & Rommel, 2012), beaked whales possess large pterygoid venous lakes loosely subdivided by very thin, delicate membranes (trabeculae) containing considerable arteriovenous investment. It is unclear what degree of gas (e.g. nitrogen) diffusion may occur across the trabecular membranes, if any, however the valveless nature of the venous system and proximity of the aforementioned structures to critical sensory and neural structures may warrant further study. The link between this arteriovenous lake and trabecular system and the immediately adjacent pterygoid air sac is unclear, however the frequently discovered thick froth in the air sac may be indication of off-gassing from the adjacent vascular system.

Costidis & Rommel (2016a; 2016b) demonstrated considerable vascularization of the acoustic fat bodies in mesoplodont species. While capillary densities have not been studied, arterioles and thin-walled venous sinusoids abound in these tissues and stranded beaked whales have been found with hemorrhages in their intramandibular fat bodies (Fernandez et al., 2005). Lonati et al. (2015) showed that mandibular fat bodies of deep divers had significantly higher nitrogen solubility coefficients than blubber. It seems reasonable to suggest that significant vascular perfusion of tissues with high nitrogen saturation coefficients may result in relatively high nitrogen saturation within the mandibular fat bodies. There is evidence that even shallow diving marine mammals (delphinids and phocids) entangled in fishing nets can off gas postmortem and are therefore likely to have certain degrees of nitrogen saturation (Moore et al., 2013). Similarly, live stranded delphinids were also found to have gas bubbles in the hepatic-portal circulation and kidneys, while live-caught, shallow water delphinids did not show gas bubbles (Dennison et al., 2011). It is therefore possible that ensonified beaked whales strand due to decompression-related acoustic debilitation followed by pronounced intravascular and interstitial nitrogen bubble growth due to off-gassing during a prolonged surface interval. Such a mechanism may help explain the disseminated fat emboli observed in some stranded beaked whales.

The vascularization of beaked whale blubber has also received little attention. McLellan et al. (2012) quantified microvascular densities in the bottlenose dolphin, pygmy sperm whale and ziphiids, finding the blubber to be well-vascularized in dolphins, less so in pygmy sperm whales, and even more sparse in beaked whales. As the largest adipose depot in their body, beaked whale blubber may have the potential to act as a substantial nitrogen sink that can offload nitrogen at inopportune moments, as during a decompression event. This could explain why deeper divers appear to have lower microvascular densities in their blubber.

A potentially critical structure, the epidural rete has not been described in any beaked whale. While researchers like Blix, Wallie and Messelt (2013) and Reidenberg and Laitman (2015) have attributed roles such as epidural fat nitrogen absorption from supersaturated blood and

nitrogen bubble trapping to the epidural rete, respectively, the evidence for such functions is both minimal and based on notably different species. Is the volume of epidural fat enough to mitigate nitrogen gas from arriving at the brain? Even if it is, the main arterial flow would presumably remain within the major arteries, not the capillaries supplying the epidural fat, so the nitrogen buffering effect could likely be negligible. Are the main epidural retial arteries small enough to act as traps? My experience suggests they are not, as such morphology would significantly limit blood flow to the brain. Morphological and histopathological studies could answer such questions relatively easily.

Interestingly, Cuvier's beaked whales have been implicated in a striking vascular disease (verminous arteritis) (Diaz-Delgado et al., 2016). It has been reported that numerous (n=13) *Z. cavirostris* have displayed severe deformities (e.g. mineralization, aneurisms, etc.) of arteries due to larval migration of parasitic nematodes (*Crassicauda* sp.). Similarly, severe dystrophic mineralization of major arteries has been observed in numerous (n=5) pregnant and/or recently pregnant female Cuvier's beaked whales stranded along the Gulf and Atlantic coasts (pers. obs.). Such findings may suggest a certain degree of vascular-induced predisposition to cardiovascular pathologies (e.g. hypertension, etc.) that may increase vulnerabilities to decompression-like sickness or compromise their innate coping mechanisms against such conditions.

The vascularization within the lungs of beaked whales is very poorly described. Intrapulmonary capillaries can act as traps for gas and fat emboli (Neuman, 1999), yet nothing is known about such a function in beaked whales. It is currently unknown whether or not intrapulmonary anatomic arteriovenous shunts exist in cetacean lungs, and such shunts could act as pulmonary bypasses to allow arteriolization of venous gas emboli. Ninomiya et al. (2005) studied the vasculature of the terminal air spaces in Baird's beaked whale. Although they make no mention of arteriovenous shunts in pulmonary circulation, they state that a robust submucosal venous plexus is connected to the pulmonary veins and contains oxygenated blood. Unfortunately, they do not provide proof (e.g. gross dissection or corrosion cast images) of the connections between the submucosal plexus and pulmonary veins or describe how this was determined, so it is not possible to assess whether this was based on an assumption from serial microscopic sections or from visual confirmation of the connections. Cursory reports of a submucosal plexus within the airways of some cetaceans indeed suggest the presence of a robust venous network occupying the lumen of the major airways (Costidis & Rommel, 2012; Cozzi et al., 2005; Davenport et al., 2013), however, no other information currently exists on the volume, extent, or vascular connections of that network. Preliminary postmortem contrast angiographic studies and gross dissections conducted on a common dolphin (*Delphinus delphis)* suggest that the submucosal plexus is in fact connected to systemic venous circulation at the jugular and thoracic caval levels (pers. obs.) rather than the pulmonary venous level as stated by Ninomiya et al. (2005). Such a difference could be significant with respect to the level of oxygenation, transmural pressures, and dynamics of engorgement experienced by this plexus. Either way, such a network could have significant impacts on the physics of pulmonary compression and alveolar collapse (Bostrom et al., 2008), as it would likely engorge during a dive and accommodate volume diminution without

airway compression. Such a mechanism could affect the depth at which alveolar gas exchange ceases and therefore influence the amount of nitrogen absorbed across the lungs. A preliminary study has been funded by ONR to investigate the structure of this submucosal plexus (Award #: N000141713146).

Finally, there has been little research into the vascularization of the eyes of cetaceans (Costidis and Rommel, 2012; 2016b; Ninomiya and Yoshida, 2007), however such morphology may be relevant. Costidis and Rommel (2012; 2016b) showed a robust venous plexus surrounding much of the eye and especially extensive and robust along the ventral aspect and the lateral canthi. Evidence exists in humans that elevated pressure in and around the eye can elicit profound physiological cascades (dysrhythmia up to asystole, arterial hypotension, apnea and gastric hypermotility) through stimulation of the oculo-cardiac and subsequently the trigeminocardiac reflex (Schaller, 2004). It may, therefore, be possible for direct or indirect control of engorgement of the valveless ophthalmic plexus veins to exert differential intraocular pressures and consequently affect physiological processes critical to diving and to management of diving gases.

***Future morphological research needs and/or directions:***

-expert dissection/sampling of stranded beaked whales for cause of death (swat team approach).

-describe whole body angiosomes for narrowing down sources of gas/fat emboli

-epidural rete investigations (morphology, thermal influences, lipid quantification, etc.)

-expensive tissue hypothesis (body compartments BUT with analysis of cost of tissues…traditional doctrine may not be accurate). Elaboration of Pabst et al., (2016) work.

-two-way venous flow examination. Does it happen, and if so, where? What determines flow (e.g. muscular contraction/non-cardiac pumps).

-pterygoid sinus gas exchange potential.

-aerial surveys following sonar deployment (find carcasses for examination; document effects/stranding frequency).

-Understand the lungs. It's where it all starts! Blood flow patterns in live animals. Vascular morphology. Muscular effects on blood flow/perfusion (e.g. Lillie et al., 2013).

-Microvascular/capillary densities in lipid structures (actual density measurements from corrosion casting, immunolabeling or diceCT and confocal microscopy or microCT), presence/absence of A/V anastomoses

-Microvascular associations in pterygoid venous lake and air sac. Capillary investment of structures bathed in blood, of air sac mucosa, etc.

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## Cardiovascular Responses: Heart Rate and Vascular Responses to Depth/Duration, Exercise, and Stress/Disturbance

Birgitte I. McDonald and Peter T. Madsen

The dive response is a key mechanism allowing breath-hold divers to perform deep and long dives via cessation of breathing, bradycardia, and peripheral vasoconstriction (Scholander 1940, Blix and Folkow 1983, Butler and Jones 1997). Heart rate, blood flow distribution, and muscle workload are the primary determinants of the rate and pattern of oxygen store utilization and ultimately breath-hold duration (Scholander 1940, Davis and Kanatous 1999, Ponganis 2015). Additionally, heart rate may also be important in limiting nitrogen uptake from non-collapsed lungs, and to enable critical unloading of super-saturated tissues during ascent and at the surface (Fahlman et al. 2006, Hooker et al. 2009, Hooker et al. 2012, Kvadsheim et al. 2012).

For deep diving cetaceans, primarily beaked whales, which strand in association with naval sonar exercises, it has been proposed that sonar pings trigger behavioral and/or physiological responses that may increase their risk of DeCompression Sickness (DCS) (Hooker et al. 2012). Breath-hold diving can lead to DCS (Paulev 1965) and repeated dives within lung collapse depth in dolphins have been shown to produce nitrogen tensions that may cause DCS in humans (Ridgway and Howard 1979, Houser et al. 2001, Houser et al. 2010). Modeling of $N_2$ management during natural and disturbed diving of beaked whales has produced inconclusive results on whether the behavioral changes would increase risk of DCS; however, they have found that the modeled level of bradycardia and the depth at which these modulations take place do influence end tissue $N_2$ levels, and therefore likely the risk of DCS (Fahlman et al. 2006, Hooker et al. 2009, Kvadsheim et al. 2012). These models assume cardiac output and circulation patterns based on diving Weddell seals (Davis and Kanatous 1999). In order to understand $N_2$ management in deep diving cetaceans, it is critical to obtain data on cardiovascular regulation during both deep and shallow dives in cetaceans in general and for beaked whales in particular. However, before such data are collected, it is relevant to consider the known drivers of changes in diving heart rate in marine mammals in general.

During forced submersion a severe bradycardia results in isolation of muscle and peripheral organs from blood flow, thereby conserving blood oxygen for the heart and brain, initially leading to the concept of a diving reflex (Scholander 1940, Irving et al. 1941, Zapol et al. 1979). Later studies on trained and freely diving animals show that cardiovascular regulation is often more moderate and variable, with declines in heart rate depending on species, dive duration, activity, and expectations (Kooyman and Campbell 1972, Jones et al. 1973, Thompson and Fedak 1993, Jobsis et al. 2001, Davis and Williams 2012, Ponganis 2015, Elmegaard et al. 2016). Such a combination of sensory modulation and volitional control of the diving response has likely been selected for through the evolution of marine mammals by enabling them to fine tune management of their oxygen and nitrogen levels to maximize net energy gains during foraging dives. However, all the virtues of a dynamic dive response that makes it much more

than a reflex may be in turn also be the main problem for beaked whales exposed to sonar if behavioral disruptions lead to mismanagement of tightly controlled cardiovascular responses a function of dive depth, exercise, and duration. This mini-review will therefore focus on modulators of heart rate in freely diving captive and wild animals, particularly cetaceans when possible, in the broader context of how anthropogenic disturbance with physiological consequences may explain mass stranding of beaked whales. For more detailed reviews of the cardiovascular response to diving please see Blix and Folkow (1983), Butler and Jones (1997), and in particular Ponganis (2015).

**Neuroregulation of diving heart rate**

*Autonomic nervous system*. In order to maintain blood pressure during diving, both the parasympathetic and sympathetic nervous system are activated in order to decrease heart rate and to increase vasoconstriction, respectively (Blix and Folkow 1983, Butler and Jones 1997, Elliott et al. 2002). Most research suggests that the parasympathetic system dominates during diving (Signore and Jones 1996, McPhail and Jones 1999, Elliott et al. 2002). It was recently suggested that the activation of both systems may result in autonomic conflict, i.e. the activation of both the vagus nerve (decrease heart rate) and sympathetic cardiac fibers (increase heart rate); possibly resulting in dangerous arrhythmias in cases of anthropogenic disturbance (Williams et al. 2015a, Williams et al. 2015b). Ponganis et al. (2017) reviewed the literature and used sea lions as an example to present an alternative hypothesis. Based on previous studies they propose that the parasympathetic system dominates over the sympathetic system and that any exercise modulation would be due to a decrease in vagal tone. The authors concluded that these adjustments commonly result in benign arrhythmias. The authors suggest that factors such as depth, exercise, and volitional control regulate the parasympathetic system (Ponganis et al. 2017).

**Influence of depth and duration on diving heart rate**

The negative relationship between dive duration and heart rate was noted in some of the earliest studies on freely diving marine mammals, although it is less pronounced in some species (Kooyman and Campbell 1972, Fedak et al. 1988, Thompson and Fedak 1993), and has been supported in many more studies (Andrews et al. 1997, Hindell and Lea 1998, Williams et al. 1999, McDonald and Ponganis 2014, McDonald et al., 2018). Although depth and duration are highly correlated in most species, and can therefore be difficult to tease apart, the degree of bradycardia seems to be driven primarily by duration as extreme bradycardia is observed in long shallow dives (Thompson and Fedak 1993, McDonald and Ponganis 2014, McDonald et al. In prep-a). Although duration may drive the degree of bradycardia, recent studies suggest that depth may impact the influence of exercise on heart rate (Hindle et al. 2010, Williams et al. 2015b, McDonald et al. in prep-b).

**Influence of exercise on diving heart rate**

The study of the heart rate response to exercise in diving cetaceans may provide us with an explanation for strandings' associated with sonar exposure; DeRuiter et al (2013) found that in some contexts sonar exposure resulted in beaked whales swimming rapidly away and extending

dive duration. This could lead to a disruption in the dive response resulting in mismanagement of $O_2$ and $N_2$.

Early studies investigated the relationship between workload and heart rate during surface swimming. Phocid seals typically did not alter surface or submerged heart rate, but instead decreased the time they spent submerged (Fedak et al. 1988, Williams et al. 1991). At the surface stroke volume was approximately twice the submerged volume and was not influenced by workload (Ponganis et al. 1990). In contrast to phocids, sea lions increased dive and surface heart rate with increasing workload, while maintaining time submerged (Williams et al. 1991). Sea lions submerged heart rates were also much higher than observed in phocids and stroke volume exhibited an increase with workload. Bottlenose dolphins exhibited a response more similar to the sea lions with an increase in heart rate, $O_2$ consumption and respiration rate with increased workload (Williams et al. 1993). Additionally, post high-intensity exercise heart rate, stroke volume, and cardiac output were increased 104±43%, 63±11% and 234±84%, respectively, compared to resting values (Miedler et al. 2015). The moderate bradycardia during diving documented in the above studies, in addition to similar decay curves of bolus injections of metabolites between rest and surface swimming (Castellini et al. 1985), indicate some blood muscle blood flow during surface swimming. The differences in response suggest some species exhibit an exercise response similar to terrestrial animals when swimming at the surface (sea lions and dolphins).

With advancing technology, researchers have more recently been able to investigate the relationship between exercise and heart rate/blood flow in freely diving animals. The initial studies often used swim speed as an indicator of workload, or compared free dives to stationary dives, and concluded that the dive response dominates over the exercise response (Thompson and Fedak 1993, Williams et al. 1999). With the advent of accelerometers, stroke rate or activity indexes have been used to investigate the influence of exercise on the dive response (Hindle et al. 2010, Davis and Williams 2012, Williams et al. 2015b, Madsen and McDonald 2016, McDonald et al. In prep-a, McDonald et al. in prep-b). Unfortunately, blood flow and muscle perfusion are difficult to study in freely swimming animals and has only been studied in a non-swimming muscle of Weddell seals and the primary swimming muscle in emperor penguins using the isolated dive hole protocol (Guyton et al. 1995, Williams et al. 2011). Therefore, a positive relationship between exercise and heart rate is often used as an indicator of increased muscle blood flow. These more recent studies proposed that the dive response is exercise modulated (Davis and Williams 2012, Noren et al. 2012, Williams et al. 2015b). For example, in Weddell seals and bottlenose dolphins there is a positive relationship between heart rate and stoke rate (Davis and Williams 2012, Williams et al. 2015b). However, this may not be a simple relationship. In porpoises, when the animals did know how long the dive would be, there was a mild relationship between activity and heart rate, but no relationship was not apparent when freely diving (McDonald et al. In prep-a). In sea lions both depth and exercise influence heart rate throughout dive, but at depth exercise has a much smaller impact (McDonald et al. in prep-b). Thus, it is not clear whether the strong fluking during behavioral responses to sonar in beaked

whales (Deruiter et al., 2013) also involve higher heart rates and hence increased lung and muscle perfusion that would alter nitrogen on and off loading.

**Cognition/Volitional control**

Since the early forced submersion studies in seals the volitional control of heart rate has been noted (Grinnell et al. 1942). Grinnell et al (1942) noted that the seals increased heart rate before dives when the experimenter raised his hand. In the 1960's, Elsner et al. made similar observations in both seals and dolphins (Elsner 1965, Elsner et al. 1966). For example they noted that dolphins seemingly adjusted the intensity of the cardiovascular response depending of the anticipated duration of the dive (Elsner et al. 1966). Since these early studies, researchers have often noted the ability of both pinniped and cetaceans to fine tune heart rate based on expectations in wild and captive freely diving animals (Kooyman and Campbell 1972, Jobsis et al. 2001, Noren et al. 2012, McDonald and Ponganis 2014, McDonald et al. In prep-a). Specifically, both sea lions (Ridgway et al. 1975) and porpoises (Elmegaard et al. 2016) have been shown to be able to plan the level of bradycardia independent of diving stimuli. In addition to the many sensory drivers, cognitive control is very likely also an important modulator of the diving response. Thus, what was previously believed to be a diving reflex is now seen as a dynamic process that is influenced by duration, exercise, and conscious control (Noren et al. 2012, McDonald and Ponganis 2014, Elmegaard et al. 2016). This unfortunately also means that noise-induced disturbances may interfere with the physiological regulation of the cardiovascular and respiratory system, possibly explaining a series of mass strandings of beaked whales with symptoms of DCS (Jepson et al. 2003, Hooker et al. 2012). Unfortunately, little is known about cardiovascular responses to stress or acoustic stimuli in cetaceans, and nothing is known about diving heart rates and their regulation in wild cetaceans.

**Stress/disturbance effects on the diving response**

Results from an acoustic playback experiment found that one of two bottlenose dolphins increased heart rate in response to acoustic stimuli (Miksis et al. 2001). Similarly a study on a recently captured beluga whale found that the whale increased heart rate when first exposed to sonar and displayed heart rate variability similar to terrestrial animals when stressed (Bakhchina et al. 2017). A study exposing hooded seals to sonar found no response during the dive, but heart rate was elevated at the surface (Kvadsheim et al. 2010). These studies suggest cetaceans may exhibit the typical stress response to acoustic stimuli displayed by terrestrial mammals. In contrast, Houser et al. (2011) did not find any physiological indicator (increased heart rate or epinephrine) of an acute stress response in captive bottlenose dolphins exposed to mid-frequency sonar. Similarly, captive harbor porpoises exposed to pingers, sonar, or a startle sound either decreased heart rate or showed no heart rate response, despite a behavioral response (Teilmann et al. 2006, Madsen and McDonald 2016). It has been proposed that in marine mammals, evolutionary pressures in the form of near surface predators may have resulted in a physiological stress response that maintains oxygen management, permitting the diving mammal to stay at depth longer (Houser et al. 2011).

**Concluding remarks**

As outlined above, there is mounting evidence to suggest that the diving response in cetaceans in many ways are modulated by the same sensory, volitional, and cognitive mechanisms demonstrated for pinnipeds. Breath-hold diving animals can get DCS if they perform dives within lung collapse depth in concert with unfavorable perfusion of lungs and tissue leading to nitrogen loading of tissues that may result in supersaturation at or near the surface. The complex interactions of autonomic and cognitive control of the cardiovascular regulation during diving makes it, in our view, plausible that sonar induced behavioral disruptions and fright responses may lead to gas mismanagement and hence the emboli related pathologies associated with beaked whale strandings in conjunction with naval sonar exercises. Critically, to test this working hypothesis, we need to measure the heart rates of beaked whales during natural and disturbed dives in the wild. Although obtaining data for beaked whales is ideal, for some physiological measurements (muscle perfusion, blood flow) it will be necessary to use model species with some similar traits (i.e. deep diving elephant seals that regularly exceed their cADL and deep diving sea lions that dive on inhalation).

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**Gas Exchange/ Lung Compression/ Alveolar Collapse/ Pulmonary Shunts: Lung characteristics – collapse and shunt development; shunts & stress (sea turtles)**

Daniel Garcia Párraga, Michael Moore & Andreas Fahlman

**GOALS: a) relevant cetacean physiology/anatomy, b) the potential physiological responses of beaked whales in relation to the possible underlying pathology of strandings associated with sonar exposure, c) the likelihood that this topic is indeed involved in the pathology of the strandings, and d) the potential value and role of comparative studies in other species (sea turtles).**

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**The Use of Gas Dynamics Models to Understand the Risk of Decompression Sickness and Risk of Gas Emboli**

Andreas Fahlman & Daniel Garcia Párraga

In humans or terrestrial mammals breathing air experience has shown that elevated pressure, longer exposure to elevated pressure, and increasing decompression rate all increase the risk of decompression sickness (DCS) (Weathersby et al., 1984; Weathersby et al., 1992). In addition, there is large variability within and between species (Berghage et al., 1979; Berghage et al., 1974; Weathersby et al., 1984), and the occurrence is seldom either a certainty or zero for any hyperbaric exposure (Weathersby et al., 1984; Weathersby et al., 1992). Consequently, there is no certain threshold that cause DCS, and for this reason, probabilistic models have been used to predict the risk of DCS (Berghage et al., 1974; Weathersby et al., 1984; Weathersby et al., 1992). Work have shown that both individual and allometric variation in cardiac output is most likely the major underlying cause for the large variation in risk (Fahlman, 2017). The risk for DCS in breath-hold diving vertebrates is a bit more complicated as understanding how blood flow and gas exchange is altered while diving is limited. However, a few models based on present knowledge of the physiological changes during diving have been used to make predictions how blood and tissue gas levels may change during diving.

A range of models have been used to estimate blood and tissue gas uptake, removal, and distribution in marine mammals. These range from simple calculations that estimate the maximal tension possible based on available lung gas and body composition (Kooyman et al., 1972), those that include the dynamics of gas uptake and removal , (Fahlman et al., 2006; Houser et al., 2001; Ridgway and Howard, 1979), and more complex models that also include the effect of pressure on gas exchange rate (Fahlman et al., 2009; Fahlman et al., 2007; Hooker et al., 2009; Kvadsheim et al., 2012; Zimmer and Tyack, 2007). Some models have estimated bubble growth to estimate risk (Zimmer and Tyack, 2007), while a more simplified approach has been to estimate the supersaturation; the gas tension above ambient that initiates and drives bubble growth (Fahlman et al., 2009; Fahlman et al., 2007; Hooker et al., 2009; Kvadsheim et al., 2012). The more complex models have estimated how variation in diving behavior, physiology (dive response), body composition, and passive lung compression alters gas exchange (Fahlman et al., 2009; Fahlman et al., 2006; Fahlman et al., 2007; Hooker et al., 2009; Kvadsheim et al., 2012; Zimmer and Tyack, 2007). While these models differ substantially, they all agree that the level of blood flow, blood flow distribution, and level of gas exchange at depth have the biggest influence on model outcome. A recent study showed that using species specific parameters significantly alters the estimated blood and tissue $N_2$ levels (Hodanbosi et al., 2016). Thus, certain parameters (the level of pulmonary shunt and blood flow) significantly impact the model output and should be chosen carefully for future studies. For example, improved understanding about how gas exchange and blood flow are altered while diving and at depth will help improve estimates from these models. The following provide a more in-depth explanation about the mechanics of these models and a summary of some possible avenues for future research

Uptake and distribution of gases are governed by partial pressure gradients from high to low, and may be limited by blood flow (perfusion) or diffusion across the exchange membrane, e.g. alveolar collapse which prevents gas exchange between the lung and pulmonary capillaries or arterial venous shunt which prevents blood to reach and perfuse alveolar capillaries. For an inert gas, like $N_2$, uptake and removal are regulated by the following equation:

$$\frac{dP_{tiss}}{dt} = \frac{(P_{blood} - P_{tiss})}{\tau_{tiss}} \qquad \text{Eq. 1}$$

where $P_{tiss}$ and $P_{blood}$ (or alveolar partial pressure) are inert gas tensions of the tissue and arterial blood, respectively. Inert gas uptake and removal is regulated by the composition (gas solubility), volume of the tissue, and the local perfusion rate. The rate of uptake or removal can be expressed as a tissue time constant ($\tau_{tiss}$) that determines the time it takes to reach a new equilibrium after a change in the external pressure. $\tau_{tiss}$ is made up of several terms regulating uptake and removal:

$$\tau_{tiss} = \frac{\dot{Q}_{tiss}}{V_{tiss}} \bullet \frac{S_{blood}}{S_{tiss}} \qquad \text{Eq. 2}$$

where $\dot{Q}_{tiss}$ is rate of blood flow through the tissue, $V_{tiss}$ is tissue volume and $S_{tiss}$ and $S_{blood}$ are the solubilities of inert gas of the tissue and blood (Berghage et al., 1979; Berghage et al., 1974; Fahlman, 2017; Kety, 1951). Consequently, inert gas uptake and removal from a tissue is governed by the volume of the tissue, the solubility of inert gas (composition and structure), and the local rate of blood flow. Uptake and removal of $O_2$ and $CO_2$, on the other hand, depends on the local rate of blood flow, the consumption of $O_2$, production of $CO_2$ and the solubility of each of these gases. Estimating exchange of $O_2$ and $CO_2$ is therefore more complicated as it requires knowledge of both $\dot{Q}_{tot}$ and the metabolic cost of diving. The former has been measured in diving mammals (Fahlman et al., 2015; Ponganis et al., 1991; Williams et al., 1993; Yazdi et al., 1999). The metabolic cost of diving, on the other hand, is difficult to measure directly in freely diving animals for dives more than a few meters of depth but have been done for a few species (Fahlman et al., 2013; Kooyman et al., 1973).

The time it takes for the whole animal to reach a new steady state, or saturation, after an increase in external pressure is determined by cardiac output ($\dot{Q}_{tot}$), the degree of alveolar collapse (based mainly on depth and total lung volume at the beginning of the dive), the distribution of blood flow (through the lungs and through systemic circulation) and the proportion of tissues with different inert gas solubilities. An animal where gas exchange is continuous, and the supply is continuous/infinite has two ways of altering inert gas uptake and removal: changing $\dot{Q}_{tot}$ or its distribution. Consequently, a realistic model to estimate blood and tissue $P_{N_2}$ must have accurate values for body composition, blood flow and blood flow distribution. In a breath-hold diving animal, on the other hand, the source of gas is limited, and a pulmonary shunt develops that further complicates how to estimate gas uptake. In addition, the dive response alters the blood flow distribution throughout the body and complicates the ability to accurately predict gas uptake and removal.

In most early models it was assumed that $P_{blood}$ followed lung $P_{N_2}$ as long as the alveoli had not collapsed (Falke et al., 1985; Kooyman et al., 1972; Ridgway and Howard, 1979). In a mammal that is breathing and have a continuous supply of air, uptake and removal of gases are either perfusion or diffusion limited or a mixture of both. Fick's law of diffusion tells us that the diffusion rate of a gas across the alveolar membrane is determined by the a) surface area available for diffusion, b) the thickness of the membrane, c) the partial pressure gradient, and d) the diffusion constant. The diffusion constant is affected by the gas solubility and the molecular weight. At rest in an individual with healthy lungs, $O_2$, $CO_2$ and $N_2$ are perfusion limited. For $O_2$ and $CO_2$ with relatively high solubility, diffusion limitation can occur during intense exercise when the transit time in the capillary is reduced, but for $N_2$ gas exchange across the lung is always perfusion limited (Farhi, 1967; West, 2012). For a breath-hold diving mammal, on the other hand, the lung gas is finite and while there is gas exchange, the partial pressure of the gases will change. Experimental work have shown that a pulmonary shunt develops that changes with pressure and diving lung volume (Kooyman and Sinnett, 1982). However, it is not known whether the shunt is anatomical or physiological. In sea turtles, evidence exist for anatomical shunts (sphincter and right to left and left to right shunts, Hicks and Wang, 1996; Lorenzo Bermejo et al., 2016). In the sea lion, unlike other terrestrial mammals studied under hypoxic pulmonary vasoconstriction (Ariyaratnam, 2013; Elliott et al., 1991; MacEachern et al., 2004), pulmonary hypoxic regions may vasodilate causing a ventilation perfusion mismatch (Olson et al., 2010). During diving, the depth of alveolar collapse and cessation of gas exchange is determined by the diving lung volume and the structural properties (compliance) of the respiratory system (chest, alveolar space and conducting airways, Bostrom et al., 2008; Fahlman et al., 2009; Fitz-Clarke, 2009). Initial models used parameters to estimate lung compression and alveolar collapse from terrestrial mammals (Bostrom et al., 2008; Fahlman et al., 2009) but a recent study showed that blood and tissue $N_2$ levels significantly changed when species specific parameters were used (Hodanbosi et al., 2016).

Theoretical models cannot replace empirical studies, they do provide useful insights and allow us to create hypotheses that can be tested. For example, modeling data from the Weddell seal (Falke et al., 1985) suggested that the observed arterial $N_2$ tensions were not evidence of alveolar collapse, but likely evidence of a pulmonary shunt that develops as the depth increases (Bostrom et al., 2008; Kooyman and Sinnett, 1982; Scholander, 1940). The theoretical calculations suggested that there should be a large decrease in the arterial gas tension as the alveoli collapse (see Fig. 2 below from Fahlman et al., 2009) which empirical data later validated (see Fig. 1 below from McDonald and Ponganis, 2012). Consequently, the models provide a framework where hypotheses can be made. They can be used to test new ideas how the system may work. For example, it has been suggested that sonar exposure may alter diving behavior in deep diving whales (DeRuiter et al., 2013), causing increased activity and elevated $CO_2$ levels. Past research have suggested that elevated $CO_2$ may increase the risk for gas emboli due to its higher diffusion rate (Behnke, 1951; Behnke et al., 1935; Bernaldo de Quirós et al., 2012; Bernaldo de Quirós et al., 2013; Bernaldo de Quirós et al., 2011; Fahlman et al., 2014; Harris et al., 1945; Harvey, 1945). Models can also be used to perform sensitivity analyses that help identify variables that are most likely to have the largest influence for a certain outcome, and they have been used to identify blood flow/cardiac output, blood flow distribution, and pulmonary shunt as the variables that have the highest influence to alter blood and tissue gas tensions (Fahlman et al., 2009; Fahlman et al., 2007; Hooker et al., 2009), and shown that deeper dives and larger body mass may cause a higher risk (Fahlman, 2017; Fahlman et al., 2014;

Kvadsheim et al., 2012). Model results have suggested that slowly equilibrating tissues may pose a risk when the diving behavior is disrupted (Fahlman et al., 2014), and this may help explain the prevalence of gas emboli in certain tissues.

In summary, combining theoretical models with empirical data can be useful to investigate how changes in behavior and physiology following sonar exposure alter the risk of gas emboli or DCS. Past studies have suggested that the alveolar collapse depth is deeper than early studies suggested (Bostrom et al., 2008; Fahlman et al., 2009; Fahlman et al., 2006), that mean dive depth and duration is a poor index for estimating DCS risk as subtle behavioral differences exist that may differentiate diving physiology in these species, and deeper dives may cause greater risk (Fahlman et al., 2014). The models may help determine the reason why some species appear to strand more often following sonar exposure (Hooker et al., 2009), and improved knowledge of the physiological changes caused by diving and stress will help improve these estimates (Kvadsheim et al., 2012).

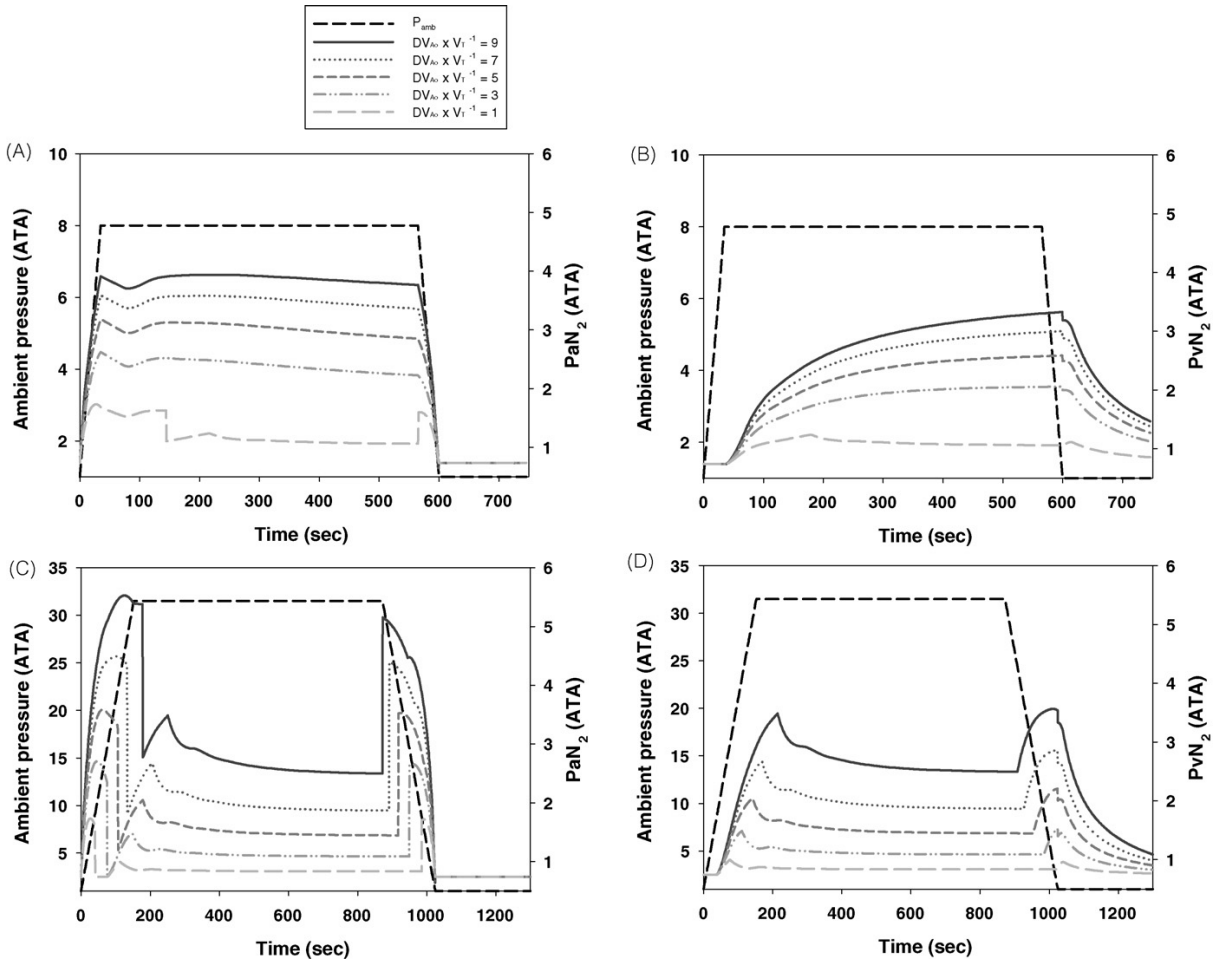


**Fig. 2.** Estimated arterial ($PaN_2$ , panel A and C) and mixed venous $N_2$ ($PvN_2$, panel B and D) tensions (ATA) for a dive to 70 m (panel A and B) or 305 m (panel C and D), assuming a constant dead space volume of 0.5 l ($V_D$) for a 100 kg elephant seal. DVL was varied between 50 and 10 ml $kg^{-1}$ and initial alveolar volume ($DVA_o$ , l) estimated as: $DVA_o = DVL - V_D$.

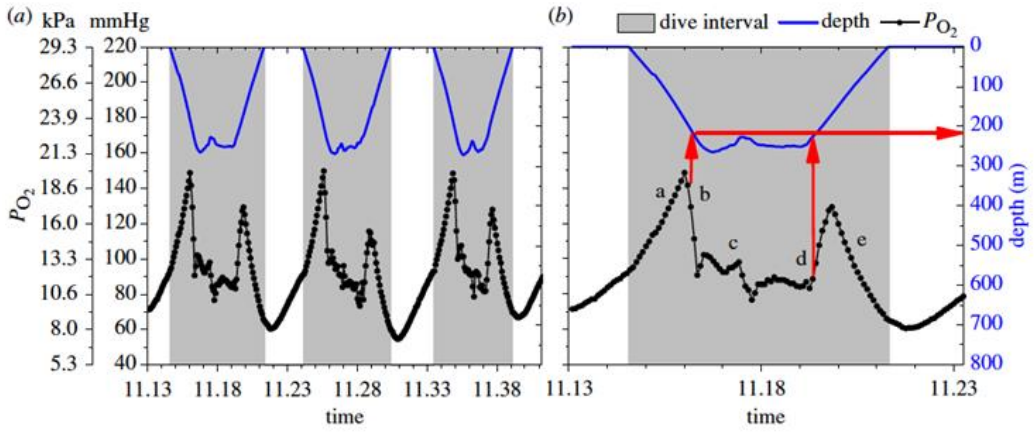


Figure 1. Arterial $P_{O_2}$ (black line, 5 s sampling interval) and depth profiles (blue line, 1 s sampling interval) from serial deep dives of a California sea lion. (*a*) Characteristic $P_{O_2}$ profiles of serial deep dives. (*b*) Typical abrupt changes in $P_{O_2}$ occur in this dive at approximately 200 m depth during descent and ascent (indicated with red arrows), consistent with significant cessation of gas exchange at depth. Profiles were characterized by a, an initial compression hyperoxia; b, an abrupt decline at approximately 200 m depth; c, a gradual decline in $P_{O_2}$ in the bottom segment of the dive; d, a rapid increase in $P_{O_2}$ during early ascent; e, a decline in $P_{O_2}$ during ascent.

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**Calculated Aerobic Dive Limits of Beaked Whales and the Propensity for Acidosis, Hypercarbia, and Hypoxia During Avoidance of Sonar**

Shawn R. Noren

Beaked whales are thought to be among the longest and deepest diving marine mammals. Indeed, Northern bottlenose whale (*Hyperoodon ampullatus*) can dive to a maximum depth of 1453 m (Hooker and Baird 1999), Blainville's (*Mesoplodon densirostris*) beaked whales have been observed to dive to 1,599 m for up to 83.38 minutes (Baird et al. 2008), and maximum dive depths and durations of 1777 m and 64.4 minutes have been recorded for Baird's beaked whale (*Berardius arnuxii*; Minamikawa et al. 2007). However, the record breaker is Cuvier's beaked whale (*Ziphius cavirostris*), with a maximum dive depth of 2992 m for a maximum duration of 137.5 min (Schorr et al. 2014). Under normal circumstances, these exceptionally long and deep dives do not pose a risk for these animals because of anatomical, physiological, and behavioral adaptations.

However, recently there have been multi-species mass strandings of marine mammals that were coincident with naval sonar exercises, and among the stranders were beaked whales (Jepson et al. 2003). It has been shown that when exposed to sonar, Cuvier's alter their behavior by extending dive duration and swimming rapidly (DeRuiter et al 2017). With increased swim speed the level of bradycardia is not as pronounced (Noren et al. 2012), which undoubtedly promotes increased oxygen consumption, increased carbon dioxide production, and a lowering of blood pH if carbon dioxide production overwhelms the buffering capacity of the blood. At the same time, blood pH is lowered by the accumulation of lactic acid when dive durations go beyond aerobic dive limits (Kooyman 1989). Moreover, the alveolar collapse that occurs at depth in marine mammals could exacerbate the buildup of carbon dioxide in the blood since the lung is not available as a sink. Thus, sonar avoidance behavior may increase the propensity of beaked whales to suffer from the consequences of hypoxia, hypercarbia, and acidosis. To evaluate if diving behaviors associated with sonar avoidance cause levels of hypoxia, hypercarbia, and acidosis that could cause morbidity or mortality in beaked whales, I calculated aerobic dive limits (cADLs) and maximum achievable dive depths of beaked whales (Table 1), compared these cADLs to at-sea diving behaviors of beaked whales, estimated respiratory blood gases ($PO_2$ and $PCO_2$) and pH in the blood at the end of submergence and compared these estimates to empirical measurements of blood gas and pH levels at the termination of breath-holding and diving in marine mammals (Table 2).

Aerobic dive limits have been estimated for many diving vertebrates using the calculated aerobic dive limit (cADL; ratio of total body oxygen stores and the rate at which these stores are utilized; Kooyman 1989), and can be used to predict limits to diving. Because little is known about the oxygen utilization rates of deep diving cetaceans (see Williams et al. 2017 for review), I explored a range of metabolic rates for the cADLs of beaked whales. Briefly, I assumed a diving metabolism equivalent to resting metabolism in marine mammals (two times Kleiber metabolism; Kleiber 1975) since the majority of empirically derived aerobic dive limits were best estimated by calculated aerobic dive limits assuming that level of metabolism (for review see Noren et al. 2012). A second calculation assumed a 39% reduction in resting metabolism (hypometabolism) because California sea lions (*Zalophus*

*californianus*) demonstrated this level of oxygen consumption when performing voluntary submergences (Hurley and Costa 2001) and this level of metabolism could potentially account for the hypothetically low diving metabolisms of beaked whales (Pabst et al. 2016). However, when encountered with a sound source beaked whales swim rapidly away (DeRuiter et al 2017), and active muscle is metabolically expensive (Pabst et al. 2016), thus oxygen consumption rates based on cost per stroke that could reflect the flight (escape) metabolic rate of beaked whales (Williams et al. 2017) were also considered.
Assessing aerobic dive limits by exploring several different levels of oxygen consumption rates is similar in approach to that used by Noren et al. (2012). Please see appendix 1 for the assumptions used for determining the cADLs.

As expected, cADLs varied across beaked whale species in accordance with body size, and within species in accordance with assumptions surrounding oxygen depletion rates while diving (Table 1). The cADLs associated with hypometabolism (57-82 minutes) support maximum dive depths of 1919 – 2449 m (assumes a touch and go dive with ascent and descent swim speed of 1.0 m $s^{-1}$), which matched observations of maximum dive depths for free-ranging beaked whales (1,453 - 2992 m; Hooker and Baird 1999, Minamikawa et al. 2007, Baird et al. 2008, Schorr et al. 2014). Hypometabolism is likely to occur in beaked whales during natural diving because Mesoplodonts have an extreme morphology that provides for low diving metabolic rates (Pabst et al. 2016). Compared to the shallow diving species harbor porpoise (*Phocoena phocoena*) and bottlenose dolphin (*Tursiops truncatus*), beaked whales invest a smaller percentage of their total body mass (TBM) in metabolically expensive brain and viscera and a larger percent of their TBM in inexpensive integument, bone, and muscle (Pabst et al 2016). The locomotor muscle of beaked whales also has features that contribute to relatively low tissue metabolic rates, including large muscle fiber diameters and low mitochondrial volume densities (Pabst et al 2016).

Closer examination of the diving behavior of Cuvier's beaked whale indicate that these animals routinely do shallow dives (mean duration and depth of 21 min and 275 m) and deep dives (mean dive duration of 67.4 min and mean dive depth of 1401 m; Schorr et al. 2014), and the reactive dives to experimental sonar exposure (DeRuiter et al. 2013) fall within this range of diving behaviors (Schorr et al. 2014). The shallow, short duration dives easily fall within the calculated dive duration (19 min) and dive depth (574m) limit that assumed a post-exposure oxygen consumption rate (Table 1), but the deep, long duration dives fall outside of this limit. Yet surface interval (SI) did not vary with dive duration across the routine shallow and deep dives suggesting that all of these dives are within aerobic dive limits (Schorr et al. 2014).

Williams et al. (2017) suggested that beaked whales in a "flight response" would have elevated diving metabolisms, and I concur, but the energetic cost per stroke from Williams et al. (2017) that was used in the post-exposure cADL may have overestimated the diving metabolism of beaked whales. Due to the difficult logistics of measuring diving metabolism in cetaceans, Williams et al. (2017) metabolic estimates were based on empirical measurements taken from comparatively shallower and shorter duration diving odontocetes [harbor porpoises, bottlenose dolphins, beluga whales (*Delphinapterus leucas*) and a killer whale (*Orcinus orca*)]. Compared to these alternate odontocete species, Mesoplodonts have

an extreme morphology that provides for low oxygen depletion rates while diving (Pabst et al. 2016). Nonetheless, the inter-deep dive intervals (IDDI; period from the end of one deep dive to the beginning of the next deep dive) of post exposure whales were towards the longest IDDI exhibited by the natural diving whales, such that exposure to sonar may increase IDDI (Schorr et al. 2014), suggesting that some recovery period was necessary. Thus, the oxygen consumption of a diving beaked whale exhibiting escape behavior undoubtedly oscillates among the range of possible oxygen consumption rates explored here (Table 1) as beaked whales switch between gliding, continuous stroking, and burst-and-glide gaits throughout a dive (Martín López et al 2015).

Knowledge of how respiratory gases and pH change in the blood during breath-hold (Table 2) could help us better understand the potential for hypoxia, hypercarbia, and acidosis during extended dives, as might occur for beaked whales perturbed by naval sonar. Theoretically reductions in blood $P_{O2}$ during breath-hold will be accompanied by a concomitant increase in blood $P_{CO2}$ and decrease in blood pH, while large body size should facilitate prolonged dive durations due to the disproportionate scaling of oxygen stores and metabolic rate with body mass (Noren and Williams 2000). What is known about how body size influences changes in blood gas and pH levels in the blood during breath-hold in odontocetes comes from Noren et al. (2012). Changes in blood gas and pH in the blood were measured in three species (mass range: 96 – 3,850 kg; 40-fold difference in body size) performing voluntary upside breath-holds (animals rested on their backs during the trials such that their blowholes were submerged to mimic a dive). The measured ADLs suggested that metabolism during breath-hold approximated two times Kleiber metabolic rate (Noren et al. 2012), and variation in the rate of change for $P_{O2}$ in the blood varied across species according to body size (-0.6 mmHg $O_2$ $min^{-1}$ for the 96±4.2 kg Pacific white-sided dolphins (*Lagenorhynchus obliquidens*) and 189±14.1 kg bottlenose dolphins compared to -0.3 mmHg $O_2$ $min^{-1}$ for the 3,850 kg killer whale). Meanwhile, the rates of change for $P_{CO2}$ (-0.1 mmHg $O_2$ $min^{-1}$) and pH (0.007 to 0.0008 pH units $min^{-1}$) during breath-hold were statistically indistinguishable (Noren et al. 2012). This result was likely because for a given change in the partial pressure of oxygen the corresponding change in the partial pressure of carbon dioxide is much smaller (Boutilier et al. 2001) due to the relatively higher physical solubility of carbon dioxide (25 times higher compared to oxygen) and the chemical binding of carbon dioxide as bicarbonate (Piiper 1990).

The body size of the breath-holding killer whale was similar to that of the smallest beaked whale in this study and there was only a 4-fold difference in body size across the beaked whale species (mass range: 3,600-15,000 kg), thus I used the relationships measured for the killer whale to estimate blood gas and pH levels in beaked whales at the termination of a dive ($P_{O2}$ = 83.0 breath-hold duration$^{-0.3}$, $P_{CO2}$ = 50.1 breath-hold duration$^{0.1}$, and pH = 7.316 – 0.007 breath-hold duration). However, because the relationship for breath-hold duration and blood pH is linear, and the cADLs of beaked whales far exceed that of the killer whale (13.2 min assuming a metabolism of 2 times Kleiber; Noren et al. 2012), I provide an additional estimate for blood pH at the termination of a dive based on the relationship describing how pH changes with changes in $P_{CO2}$ in killer whales (pH = 7.603 – 0.005

$P_{CO_2}$) using the estimated $P_{CO_2}$ as the input. At the termination of 35-50 minute dives (cADLs across beaked whale species based on diving metabolism of two times Kleiber metabolism since that was the estimated metabolism of the breath-holding killer whale), $P_{O_2}$, $P_{CO_2}$, and pH in the blood should approximate 26-29 mmhg, 71-74 mmhg, and 6.787-6.891 (estimated from breath-hold duration) and 7.233-7.246 (estimated from estimated $P_{CO_2}$ endpoint). These levels were within the range of levels measured in other marine mammals (Table 2).

Admittedly, using empirically derived relationships from a killer whale to predict how blood gas and pH changes with breath-hold in beaked whales cannot account for the hypometabolism capabilities of beaked whales because killer whales do not have the same intrinsic abilities. Thus, the estimated $P_{O_2}$, $P_{CO_2}$, and pH levels in the blood at the termination of 35 – 50 min dives are likely to be less extreme than those reported here. Nonetheless, the final oxygen tensions in marine mammals indicate hypoxemic tolerance, as they are often well below mean arterial $P_{O_2}$ (24 mmHg) of climbers on ambient air on Mt Everest and below human thresholds for shallow water black out (about 25 mmHg) (Ferretti et al. 1991, Ferrigno and Lundgren 2003, Groscott et al. 2009). This tolerance may be necessary to allow for more effective utilization of the blood oxygen store (Stockard et al. 2007). Meanwhile $P_{CO_2}$ measured in the blood of diving marine mammals is often less than that associated with $CO_2$ narcosis in humans (Nunn 1977). Marine mammals are also well suited to avoid acidosis, because despite high levels of carbon dioxide (Table 2) and the confounding influence of lactic acid accumulation, blood pH of diving marine mammals only reached a low of 7.05 across the empirical data collected to date (Table 2). Higher capillary densities and high glycogen concentrations in the brain, alterations in neuroglobin/ cytoglobin concentration or function, and scavenging of reactive oxygen species, as well as elevated tissue buffering capacities may all contribute to tissue survival under such conditions (for review see Ponganis 2011).

Please note that the estimates provided in this study are only for physically mature beaked whales. Recent investigations on odontocetes have demonstrated that the buffering capacity of the muscle (Noren 2004, Noren et al. 2014; Noren and Suydam 2016; Noren and West 2017), the muscle oxygen store (Noren et al. 2001, 2014; Noren and Suydam 2016; Noren and West 2017), and the blood oxygen store (Noren et al. 2002, Noren et al. in review) are all underdeveloped at birth. Limited body oxygen stores combined with comparatively high oxygen consumption rates while diving, as suggested by the limited ability for bradycardia in immature bottlenose dolphins (*Tursiops truncatus*; Noren et al. 2004), act synergistically to limit the breath-hold capacity of immature odontocetes (Noren et al. 2002, 2014, Noren and Suydam 2016). The duration required for the maturation of muscle buffering capacity and the body oxygen stores is species specific, and typically varies with the maternal dependency period across pinnipeds and cetaceans (Noren et al. 2005, 2014). A review of the postnatal development of buffering capacity, oxygen stores, and metabolism in marine mammals is warranted to predict the diving capacity of immature beaked whales and to assess the vulnerability of this critical age class to acidosis, hypercarbia, and hypoxia during avoidance responses to sonar.

**Appendix 1. Assumptions used for determining the aerobic dive limits of beaked whale species.**

*Modeling breath-hold limits*

The calculated aerobic dive limit (cADL) was determined by dividing calculated total body oxygen stores by estimates of diving metabolic rate following methods described in Kooyman (1989). The cADL accurately predicts the experimentally determined aerobic dive limit (ADL; Kooyman 1989, Kooyman and Ponganis 1998) when estimates of body oxygen stores and metabolic rate are reliable (Ponganis et al. 1997). I used species-specific oxygen store data and diving metabolic rates typical of odontocetes. Details of the assumptions are provided below.

The calculations for the oxygen storage capacity of the blood in L are as follows:

$$\text{Arterial } O_2 = (0.33*BV*m)\,(Hb*0.00134\ \text{L } O_2 \cdot \text{g } Hb^{-1})\,(0.95 - 0.20 \text{ saturation}) \quad (1)$$

$$\text{Venous } O_2 = (0.67*BV*m)\,(Hb*0.00134\ \text{L } O_2 \cdot \text{g } Hb^{-1})\,[0.95 \text{ saturation} - (0.05*0.95 \text{ saturation})] \quad (2)$$

where 0.33 and 0.67 are the estimated proportions of arterial and venous blood, respectively (Lenfant et al., 1970), BV is blood volume, m is body mass, Hb is hemoglobin, and 0.00134 is the oxygen binding capacity of Hb (l O2 $g^{-1}$ Hb; Kooyman 1989). For consistency across species, body mass values were obtained from Evans (1987). These differed from the body mass values used by Hooker et al (2009), who used 5000 kg, 1000 kg and 2050 kg for Hyperoodon, Mesoplodon, and Ziphius, respectively. Measured Hb in a beaked whale was 22.9 g $dl^{-1}$ (Velten et al. 2013). The proportion of saturation and depletion of arterial and venous oxygen reserves is described in detail in Ponganis (2011).

The blood volume (BV) value used in equations (1) and (2) was estimated according to:

$$BV = 813\ Hb - 38.6 \quad (3)$$

where BV is in ml $kg^{-1}$ and the Hb (g $ml^{-1}$) value is as described for equations (1) and (2). Equation (3) is from Snyder (1983); this equation was derived from the relationship between hemoglobin concentration and blood volume in terrestrial and diving vertebrates, including bottlenose dolphins. Because of the difficult logistics of measuring actual BV in cetaceans, this equation has been used in other studies to calculate BV to ascertain blood oxygen stores for cADLs (Noren et al. 2002, 2104; Williams et al. 2011; Noren and Suydam 2016). Moreover, Noren and Suydam (2016) found that BV estimates from equation (3) based on measured Hb for adult belugas (a deep diving odontocete) in a study by Ridgway et al. (1984) had an average error of < 1% compared to the measured BV in Ridgway et al (1984).

The calculation for the oxygen storage capacity of the muscle in liters is as follows:

$$\text{Muscle } O_2 = (Mb \times 0.00134)(m \times p) \quad (4)$$

where Mb is in g 100 $g^{-1}$ wet muscle mass, 0.00134 is the oxygen binding capacity of Mb (l $O_2$ $g^{-1}$ Mb; Kooyman 1989), m is body mass, and p is the proportion of body mass appropriated to muscle. Mb was assumed to be 7.34 g 100 $g^{-1}$ wet muscle mass, based on the average adult Mb value across 3 beaked whale species (Mesoplodon; Velten et al. 2013). I used a value of 0.48 for p, based on the observation that adult beaked whales have relatively large amounts of muscle (48% of body mass; Velten et al. 2013) compared to other adult cetaceans, such as 15.9% and 36% of body mass for beluga whales (*Delphinapterus leucas*; Sergeant and Brodie 1969) and bottlenose dolphins (*Tursiops truncatus*; Goforth 1986), respectively.

The calculation for the oxygen storage capacity of the lung in liters is as follows:

$$\text{Lung } O_2 = TLV * 0.50 * 0.15 \quad (5)$$

Due to a lack of data on measured lung volumes, total lung volume (TLV) in L was calculated from an allometric regression for marine mammals, where $TLV = 0.1 * m^{0.96}$ (Kooyman 1989) and body mass (m) in kg. This approach has been used for the calculation of the lung oxygen store in other cADL studies for odontocetes (Noren et al. 2002, 2014; Noren and Suydam 2016; Williams et al. 2011). Diving lung volume was assumed to be 50% of TLV (based on data from dolphins; see Ponganis 2011), with 15% representing the oxygen extracted during the dive (Kooyman 1989).

The rate at which oxygen stores are depleted is the metabolic rate of the animal. Because little is known about the oxygen utilization rates of deep diving cetaceans (see Williams et al. 2017 for review), I explored a range of metabolic rates for the cADLs of beaked whales. Assessing aerobic dive limits by exploring several different levels of oxygen consumption rates is similar in approach to that used by Noren et al. (2012). First, I assumed a diving metabolism equivalent to two times Kleiber metabolism (Kleiber metabolism = $0.0101 * mass^{0.75}$ in $LO_2$ $min^{-1}$; Kleiber 1975) because across air-breathing diving vertebrates, including three Odontocetes (Noren et al. 2012), shallow diving emperor penguins (*Aptenodytes forsteri*; Ponganis et al. 2010) and freely diving Weddell seals (*Leptonychotes weddelli*; Castellini *et al*., 1992; Ponganis et al. 1993), cADLs based on this level of metabolism best approximated experimentally determined ADLs (Noren et al. 2012). In addition, this level of metabolism is often used to estimate the cADLs of cetaceans (Noren et al. 2002, 2014; Noren and Suydam 2016). A second calculation assumed a 39% reduction in the two times Kleiber metabolic rate because California sea lions (*Zalophus californianus*) demonstrated this level of oxygen consumption when performing voluntary submergences (Hurley and Costa 2001) and this level of metabolism accounts for the hypothetically low diving metabolisms of beaked whales (Pabst et al. 2016).

Two alternate metabolic rates were also considered that reflect how oxygen stores might be utilized during a submerged, flight (escape) response, as might occur when beaked whales encounter a sound source (Williams et al. 2017). Based on the energetic cost per stroke across odontocetes and the stroking behavior of beaked whales prior to, during, and post noise exposure, Williams et al. (2017) calculated that regardless of depth, diving metabolic rate averaged 3.16± 0.15 ml O2 $kg^{-1}$ $min^{-1}$ before or partially during noise exposure and increased to 4.76±0.24 ml O2 $kg^{-1}$ $min^{-1}$ for post-exposure dives. Maximum achievable dive depth was calculated for each cADL assuming a touch and go dive with no bottom time. A swim speed of 1.0 m $s^{-1}$ was used based on the range reported for mean ascent (0.47 – 1.44 m $s^{-1}$) and descent (0.44 – 1.48 m $s^{-1}$) swim rates across 3 individual beaked whales performing shallow and deep dives (Baird et al. 2006). This speed is about half of the minimum cost of transport speed measured for dolphins (*Tursiops truncatus*), which, by definition, elicits the lowest oxygen consumption rate (Williams et al., 1993).

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**Table 1.** Calculated aerobic dive limits of beaked whales assuming a range of diving metabolisms. Males (M) and females (F) were calculated separately when data was available for both sexes.

| SPECIES | Northern bottlenose whale M (Hyperoodon ampullatus) | Northern bottlenose whale F (Hyperoodon ampullatus) | Blainville's beaked whale (Mesoplodon densirostris) | Baird's beaked whale M (Berardius arnuxii) | Baird's beaked whale F (Berardius arnuxii) | Cuvier's beaked whale M (Ziphius cavirostris) | Cuvier's beaked whale F (Ziphius cavirostris) | Reference |
|---|---|---|---|---|---|---|---|---|
| Body Mass (tonnes) | 7.5 | 5.8 | 3.6 | 13.5 | 15 | 5.6 | 6.5 | Evans (1987) |
| **Body Mass (kg)** | **7500** | **5800** | **3600** | **13500** | **15000** | **5600** | **6500** | |
| Total Lung Capacity (L) | 524.88 | 410.10 | 259.45 | 922.83 | 1021.05 | 396.52 | 457.51 | Kooyman (1989) |
| Diving Lung Capacity (L) | 262.44 | 205.05 | 129.72 | 461.41 | 510.53 | 198.26 | 228.75 | Ponganis (2011) |
| **TOTAL LUNG OXYGEN (L)** | **39.37** | **30.76** | **19.46** | **69.21** | **76.58** | **29.74** | **34.31** | |
| Muscle Mass = 48% Body Mass (kg) | 3600 | 2784 | 1728 | 6480 | 7200 | 2688 | 3120 | Velten et al. (2013) |
| **Myoglobin (Mb; g per kg of muscle)** | **73.4** | **73.4** | **73.4** | **73.4** | **73.4** | **73.4** | **73.4** | Velten et al. (2013) |
| Total Body Myoglobin (g) | 264240.00 | 204345.60 | 126835.20 | 475632.00 | 528480.00 | 197299.20 | 229008.00 | |
| Oxygen Combining Capacity (L $O_2$ per g Mb) | 0.00134 | 0.00134 | 0.00134 | 0.00134 | 0.00134 | 0.00134 | 0.00134 | Kooyman (1989) |
| **TOTAL MUSCLE OXYGEN (L)** | **354.082** | **273.823** | **169.959** | **637.347** | **708.163** | **264.381** | **306.871** | |
| **Hemoglobin (Hb; g per L of blood)** | **229** | **229** | **229** | **229** | **229** | **229** | **229** | Velten et al. (2013) |
| Blood Volume (ml $kg^{-1}$) = 813 Hb (g $ml^{-1}$) - 38.6 | 147.58 | 147.58 | 147.58 | 147.58 | 147.58 | 147.58 | 147.58 | Snyder (1983) |
| **Total Blood Volume (L)** | **1106.83** | **855.95** | **531.28** | **1992.29** | **2213.66** | **826.43** | **959.25** | |
| Arterial Volume (L) | 365.25 | 282.46 | 175.32 | 657.46 | 730.51 | 272.72 | 316.55 | Lenfant (1970) |
| Venous Volume (L) | 741.57 | 573.48 | 355.96 | 1334.83 | 1483.15 | 553.71 | 642.70 | Lenfant (1970) |
| Oxygen Combining Capacity (L $O_2$ per g Hb) | 0.00134 | 0.00134 | 0.00134 | 0.00134 | 0.00134 | 0.00134 | 0.00134 | Kooyman (1989) |
| Oxygen Carrying Capacity (L $O_2$ per L blood) | 0.307 | 0.307 | 0.307 | 0.307 | 0.307 | 0.307 | 0.307 | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Arterial 100% Oxygen Carrying Capacity (L $O_2$) | 112.08 | 86.68 | 53.80 | 201.75 | 224.16 | 83.69 | 97.14 | |
| Venous 100% Oxygen Carrying Capacity (L $O_2$) | 227.56 | 175.98 | 109.23 | 409.61 | 455.12 | 169.91 | 197.22 | |
| Start Dive: Arterial 95% Oxygen Carrying Capacity (L $O_2$) | 106.48 | 82.34 | 51.11 | 191.66 | 212.95 | 79.50 | 92.28 | Ponganis (2011) |
| Start Dive: Adjusted Venous = 95% Oxygen Carrying Capacity ($LO_2$) - 5 ml $O_2$ per dl of blood | 205.37 | 158.82 | 98.58 | 369.67 | 410.74 | 153.34 | 177.99 | Ponganis (2011) |
| End dive: Arterial 20% Oxygen Carrying Capacity Remaining (L $O_2$) | 22.42 | 17.34 | 10.76 | 40.35 | 44.83 | 16.74 | 19.43 | Ponganis (2011) |
| End dive: Arteriral $O_2$ Used (95% Oxygen Carrying Capacity - 20% Oxygen Carrying Capacity) | 84.06 | 65.01 | 40.35 | 151.31 | 168.12 | 62.77 | 72.85 | Ponganis (2011) |
| End dive: Venous $O_2$ Used (All of adjusted 95% Carrying capacity; 0% saturation remaining) | 205.37 | 158.82 | 98.58 | 369.67 | 410.74 | 153.34 | 177.99 | Ponganis (2011) |
| **TOTAL BLOOD OXYGEN (L)** | **289.43** | **223.83** | **138.93** | **520.98** | **578.87** | **216.11** | **250.84** | |
| **TOTAL BODY OXYGEN (L)** | **682.88** | **528.41** | **328.35** | **1227.54** | **1363.61** | **510.23** | **592.03** | |
| **% Oxygen Stored in Lung** | **6** | **6** | **6** | **6** | **6** | **6** | **6** | |
| **% Oxygen Stored in Muscle** | **52** | **52** | **52** | **52** | **52** | **52** | **52** | |
| **% Oxygen Stored in Blood** | **42** | **42** | **42** | **42** | **42** | **42** | **42** | |
| | | | | | | | | |
| **Diving Metabolic Rate (MR)** | | | | | | | | |
| Hypo MR = 39% reduction of RMR ($LO_2$ $min^{-1}$) | 9.93 | 8.19 | 5.73 | 15.43 | 16.70 | 7.98 | 8.92 | Hurley and Costa (2001) |
| Resting MR = 0.202 $mass^{0.75}$ ($LO_2$ $min^{-1}$ ) | 16.28 | 13.43 | 9.39 | 25.30 | 27.38 | 13.08 | 14.62 | Noren et al (2012) |
| Pre-exposure MR = 0.00316 mass ($LO_2$ $min^{-1}$ ) | 23.70 | 18.33 | 11.38 | 42.66 | 47.40 | 17.70 | 20.54 | Williams et a l (2017) |
| Post-exposure MR = 0.00476 mass ($LO_2$ $min^{-1}$) | 35.70 | 27.61 | 17.14 | 64.26 | 71.40 | 26.66 | 30.94 | Williams et al (2017) |
| | | | | | | | | |
| **Calculated Aerobic Dive Limit (min)** | | | | | | | | |
| **HypoMR (min)** | **68.77** | **64.52** | **57.34** | **79.54** | **81.65** | **63.97** | **66.37** | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **RMR (min)** | **41.95** | **39.36** | **34.97** | **48.52** | **49.80** | **39.02** | **40.49** |
| **Pre-exposure MR (min)** | **28.81** | **28.83** | **28.86** | **28.77** | **28.77** | **28.83** | **28.82** |
| **Post-exposure MR (min)** | **19.13** | **19.14** | **19.16** | **19.10** | **19.10** | **19.14** | **19.13** |
| | | | | | | | |
| **Maximum Dive Depth (m; assumes 1.0 $ms^{-1}$ ascent & decent swim speed)** | | | | | | | |
| **Assuming HypoMR cADL (m)** | **2063** | **1936** | **1720** | **2386** | **2449** | **1919** | **1991** |
| **Assuming RMR cADL (m)** | **1258** | **1181** | **1049** | **1456** | **1494** | **1171** | **1215** |
| **Assumig Pre-exposure MR cADL (m)** | **864** | **865** | **866** | **863** | **863** | **865** | **865** |
| **Assuming Post-exposure MR cADL (m)** | **574** | **574** | **575** | **573** | **573** | **574** | **574** |

**Table 2.** Apnea duration and end of apnea blood biochemistry, including partial pressures of oxygen ($P_{O2}$) and carbon dioxide ($P_{CO2}$) and pH for voluntarily breath-holding marine mammals.

| Species | Apnea (min) | $P_{O2}$ (mmHg) | $P_{CO2}$ (mmHg) | pH (units) |
|---|---|---|---|---|
| Sedentary voluntary submerged breath-hold | | | | |
| Pacific white-sided dolphin[a] (*Lagenorhynchus obliquidens*) | 2.4-3.5 (<ADL) | 30-37 | 69.70-70.3 | 7.31-7.33 |
| Bottlenose dolphin[a] (*Tursiops truncatus*) | 3.3-4.5 (<ADL) 6-6.8 (> ADL) | 17-21.7 21.4-22.7 | 57.3-63.1 57.4-63.3 | 7.31-7.40 7.29-7.32 |
| Killer whale[a] (*Orcinus orca*) | 9.6-13.3 (<ADL) | 31-40 | 63-75.2 | 7.21-7.27 |
| Beluga whale[b] (*Delphinapterus leucas*) | 17 | 20-23 | 83 | 7.17 |
| Sedentary breath-hold | | | | |
| California Sea lion[c] (*Zalophus californianus*) | --- | 38-92 | 128 | 7.05 |
| Weddell seal[d] (*Leptonychotes weddellii*) | Max 4.5 & 8 | 25 | 55 | 7.32 |
| Northern elephant seal[e] (*Mirounga angustirostris*) | 3.1-10.9 | 15-31 | 55-72 | 7.26-7.31 |
| Sedentary head submerged breath-hold | | | | |
| Harbor seal[f] (*Phoca vitulina*) | 18.5 | 10 | 100 | 7.1 |
| Weddell seal[g] (*Leptonychotes weddellii*) | 42-54 | 7-15 | 80-108 | 6.89-7.18 |
| Actively Diving | | | | |
| California Sea lion[h] (*Zalophus californianus*) | 6.0± 0.5 | 74± 17 | --- | --- |
| Weddell seal[i] (*Leptonychotes weddellii*) | <17 >17 | 24.5± 2.86 19.9± 2.05 | 48.0± 4.22 50.5± 1.17 | 7.37± 0.027 7.34± 0.025 |
| Northern elephant seal[j] (*Mirounga angustirostris*) | > 10 | venous 2 - 10 arterial 12 -23 | --- | --- |

[a]Noren et al. 2012 (end of breath-hold), [b]Shaffer et al. 1997 (extreme values), [c]Ponganis et al 2017 (venous blood gas and pH during anesthesia), [d]Kooyman et al. 1980 (extreme values), [e]Stockard *et al.* 2007 (extreme values; venous and arterial data combined), [f]Karem and Elsner 1973 (end arterial blood gas and pH at asphyxiation), [g]Elsner et al 1970 (range of blood and pH at EEG "slowing" during asphyxia; trials with nitrogen breathing or immature animals not included), [h]McDonald and Ponganis 2013 (final arterial levels after active diving), [i]Qvist *et al*. 1986 (levels within 2 min of surfacing for dives < 17 min and within 9 min of surfacing for dives > 17min), [j]Meir *et al*. 2009 (range of minimum values over range of dive durations; emphasizes minimum values from routine dives > 10 min).

**Estimated Muscle Oxygen Consumption in Beaked Whales – Metabolism and Heat**

Paul J. Ponganis

This summary of recent anatomical findings and cost of transport models in beaked whales is an attempt to review and assess muscle metabolism in Cuvier's beaked whale (*Ziphius cavirostris*) during routine dives and during post sonar exposure dives. The topics addressed include: a) muscle fiber type recruitment during different fluke stroke patterns, b) muscle metabolic rate, c) the potential magnitude of muscle lactate accumulation, and d) the potential increases in muscle temperature These estimations are largely based on recent papers by Drs. Ann Pabst and Terrie Williams (Pabst et al., 2016; Williams et al., 2017), and a series of assumptions and calculations. The essential conclusions are that muscle metabolic rate is low, and the potential for a significant increase in either muscle lactate or temperature is minimal at the estimated metabolic rates.

## Muscle Composition

Muscle constitutes 50% of body mass in beaked whales (Pabst et al., 2016). In these animals, slow twitch (ST) muscle fibers constitute about 20% of muscle cross sectional area while fast twitch (FT) fibers occupy the remainder (Velten et al., 2013). Muscle myoglobin concentration is elevated at 5-8 g 100 $g^{-1}$muscle in most beaked whales, although myoglobin content is only 4.3 g 100 $g^{-1}$ muscle in Cuvier's beaked whale (Noren and Williams, 2000; Velten et al., 2013). Myoglobin immunolabelling has revealed that myoglobin gene expression occurs in both fiber types (Sierra et al., 2015). Therefore, myoglobin content is presumably distributed in both fiber types. Intracellular lipid droplets and intercellular lipid deposits are more common in these deep-diving species than in shallow-diving cetaceans (Sierra et al., 2015). The large diameters and cross sectional areas of both fiber types have been hypothesized to contribute to a lower baseline muscle metabolic rate (Velten et al., 2013).

## Muscle Metabolism, fiber type recruitment and gait-switching in beaked whales

The high myoglobin concentrations and presence of intra/intercellular lipids in skeletal muscle of beaked whales are consistent with primary utilization of aerobic pathways for muscle energy generation. Pabst et al. (2016) have postulated that FT fibers (80% of total fiber area) are not utilized in routine swimming during dives, and that the myoglobin-bound oxygen in both fiber types is utilized by the ST fibers, which are assumed to be more active during routine swimming (Pabst et al., 2016). The differential use of the two fiber types is consistent with the stroke patterns of beaked whales, which include the more frequent, low amplitude strokes during most of the dive (ST fibers), and the abrupt, high amplitude, shorter duration strokes (FT fibers) that are

followed by a glide pattern during the latter parts of deep dives of beaked whales (Martín López et al., 2015).

Here, here the metabolic rate of ST muscle is calculated under the Pabst hypothesis that ST fibers alone are utilized during routine diving activity. With assumptions of no muscle blood flow during a dive, an estimated muscle oxygen content of 58 ml $O_2$ per kg muscle (based on myoglobin content of whole muscle of Cuvier's beaked whale), and complete muscle oxygen depletion by the end of a 45-min dive, the oxygen depletion rate of the whole muscle would be 1.3 ml $O_2$ $kg^{-1}$ muscle $min^1$. If that oxygen depletion were only due to ST fiber metabolism and ST fibers are 20% of total locomotory muscle mass (per the hypothesis of Pabst et al.), then, under these conditions, the metabolic rate of active muscle fibers would be 6.5 ml $O_2$ $kg^{-1}$ ST fibers $min^{-1}$ during the dive.

One could refine the model and have the oxygen depleted at 38 min at which point in a 45-min dive, the beaked whale begins the high amplitude, short duration strokes (FT fibers) with postulated glycolytic energy production as per Martín López et al. (2015). In that case, the ST muscle would have a metabolic rate of 7.5 ml $O_2$ $kg^{-1}$ ST fibers $min^{-1}$ during the 38-min aerobic phase. That is a ball park, reasonable number. It would be higher if there were some muscle blood flow; it would be lower if the FT fibers consumed some of their oxygen during their contractions and at rest (which they probably do). The active locomotory muscle metabolic rate may also be slightly higher because probably not all muscle (50% of body mass) is utilized in fluke stroking. For example, if only 80% of total muscle mass were used in active stroking, the calculated muscle metabolic rate would be increased by 20%.

None the less, this estimated metabolic rate for ST muscle is not unreasonable values, given typical resting muscle metabolic rates of 1-3 ml $O_2$ $kg^{-1}$ muscle $min^{-1}$ in smaller mammals, and estimated muscle metabolic rates of 12-14 ml $O_2$ $kg^{-1}$ muscle $min^{-1}$ in diving Weddell seals (*Leptonychotes weddellii*) and emperor penguins (*Aptenodytes forsteri*) (Castellini et al., 1992; Williams et al., 2011). Note that if the Pabst hypothesis that the larger fibers of beaked whales contribute to a lower resting muscle metabolic rate is correct, then resting muscle metabolic rate in beaked whales may be less than 1 ml $O_2$ $kg^{-1}$ muscle $min^{-1}$.

The energetic modelling paper of Williams et al. estimated diving metabolic rate and % locomotory cost in beaked whales before and after sonar exposure (Williams et al., 2017). These data provide another source to calculate muscle metabolic rate during diving. These calculations are independent of any muscle blood flow assumptions. Based on Table 1 in that paper, and a muscle mass of 50% total body mass, the calculated whole muscle diving metabolic rates are about 1.6 ml $O_2$ $kg^{-1}$ muscle $min^{-1}$ at both preferred and maximum stroke costs pre-exposure, and 1.1 and 5.6 ml $O_2$ $kg^{-1}$ muscle $min^{-1}$ at preferred and maximum stroke costs post-exposure, respectively.

Given these two distinct approaches to estimate muscle metabolic rate for whole muscle under normal dive conditions, the results (1.6 and 1.3 ml $O_2$ $kg^{-1}$ $min^{-1}$) are rather close. Similarly, if only ST fibers contribute to that normal dive whole muscle metabolic rate of 1.6 ml $O_2$ $kg^{-1}$ $min^{-1}$ (based on the Williams energetic model), then the ST muscle fiber metabolic rate would be 5x

greater or 8 ml $O_2$ $kg^{-1}$ ST fibers $min^{-1}$. Again, this value is close to the 7.5 ml $O_2$ $kg^{-1}$ ST fibers $min^{-1}$ calculated on assumptions of the Pabst model and no muscle blood flow during the dive. All these values are remarkably low, given that human muscle maximum oxygen consumption rates are near 520 ml $O_2$ $kg^{-1}$ muscle $min^{-1}$ (Richardson et al., 1995).

**Lactate accumulation**

As per the gait switch model of Martín López et al., muscle metabolism during the final 7 min of a 45-min dive would rely on anaerobic metabolism. To estimate lactate production during those 7 min, recall the ST muscle metabolic rate of 7.5 ml $O_2$ $kg^{-1}$ ST fibers $min^{-1}$ calculated during the

38-min aerobic phase. Since Martín López et al. estimate that costs of the glycolytic stroke-glide phase are the same as the slow twitch phase, then the theoretical metabolic rate (in terms of oxygen consumption) of FT muscle would be one-fourth of 7.5 since there is four times more FT muscle than ST muscle. This value is 1.9 ml $O_2$ $kg^{-1}$ FT fibers $min^{-1}$ during that final phase of the dive. As per the calculations of Williams et al. for emperor penguins (2011), one can convert 1.9 ml $O_2$ $kg^{-1}$ FT fibers $min^{-1}$ to ATP equivalents, and then calculate the anaerobic metabolic rate and net production of lactate over the final 7 min of the dive. Assuming phosphocreatine breakdown accounts for 1/3 of the anaerobic energy production (Williams et al. 2011), the resulting glycolytic rate would produce 0.24 mmol lactate $kg^{-1}$ FT fibers $min^{-1}$, or a total increase of 1.7 mmol $kg^{-1}$ FT fibers at the end of the 45-min dive. If this lactate also diffused into the adjacent ST fibers, the end-of-dive total muscle lactate concentration would be elevated by 1.36 mmol lactate $kg^{-1}$ muscle.

With or without diffusion of lactate from FT fibers to ST fibers, this is a very small rise in muscle lactate concentration (see comparisons in Williams et al 2011). Note that if even a small amount of FT fiber oxygen was consumed by resting FT fibers during the first 38 min, the locomotory metabolic rate would be slightly less and the accumulated lactate would be less (under the assumptions of the model).

Based on the energetic model of Williams et al., muscle metabolic rate at preferred stroke costs after sonar exposure is low, about 68% the pre-exposure rate. At maximum stroke costs post-exposure, the metabolic rate is 3.4x the pre-exposure rate. The elevated metabolic rate during the maximum stroke costs can be calculated. Based on the 38-min ST fiber calculation above, normal dive ST fiber metabolic rate is 7.5 ml $O_2$ per kg ST muscle per min. A 3.4-fold increase in metabolic rate would result in a metabolic rate of about 26 ml $O_2$ $kg^{-1}$ ST fibers $min^{-1}$.

Lactate accumulation under anaerobic conditions at maximum swim cost after sonar exposure can also be calculated. If baseline lactate production that accounts for anaerobic metabolism during the final 7 min of a normal dive muscle metabolic rate is 0.24 mmol lactate $kg^{-1}$ FT fibers $min^{-1}$, and only glycolysis in FT fibers is used after sonar exposure, then a 3.4-fold increase in metabolic rate would result in lactate production rate of 0.82 mmol lactate $kg^{-1}$ FT fibers $min^{-1}$. For 10 min of such conditions, the FT fiber concentration of lactate would be elevated at 8.20 mmol $kg^{-1}$ FT fibers. If lactate also diffused into ST fibers, the resulting lactate increase over 10

min would be
6.5 mmol lactate kg muscle$^{-1}$. Note that, for longer periods, the lactate production rate would probably increase because phosphocreatine would eventually become exhausted. None the less, these values are low. In comparison, muscle lactate concentration was 42 mmol lactate kg$^{-1}$ muscle after 15-min forced submersion of a seal (Scholander et al., 1942), and 50 mmol lactate kg$^{-1}$ muscle after full gallop in a thoroughbred horse (Snow et al., 1985).

Given the 163-min dive performed by a Cuvier's beaked whale in response to sonar exposure (Falcone et al., 2017), note that for two hours of anaerobic metabolism at the above metabolic rate, there would be an additional 98.4 mmol lactate kg $^{-1}$FT fibers, which, if it diffuses into ST fibers, would result in 78.7 mmol lactate kg$^{-1}$muscle.

**Muscle temperature**

These metabolic rate values can be used to examine heat generation and muscle temperature (Ponganis et al., 1993). A major assumption in this model again is that there is no muscle blood flow during this period.

Heat generation can be calculated per Ponganis et al (1993):

$\Delta T$ = (metabolic rate x 20.1 x 0.8) ÷ heat capacity,

where T is temperature in degrees C, metabolic rate is tissue oxygen consumption in ml $O_2$ kg$^{-1}$ muscle min$^{-1}$, heat capacity of muscle is assumed to be equivalent to that of blood and is 3846 J kg$^{-1}$ muscle degree$^{-1}$ C (Astrand and Rodahl, 1970), and a 20% metabolic energy efficiency is assumed. In this equation, the primary metabolic rate measurement is converted to joules (1ml $O_2$
= 20.1 J, (Schmidt-Nielsen, 1983)).

Based on these assumptions, and a post-exposure 3.4-fold increase in muscle metabolic rate during maximal stroke costs:

If only slow twitch (ST) fibers (20% of muscle mass) are used for 30 min (26 ml $O_2$ kg$^{-1}$ ST fibers min$^{-1}$), there is a 1.9 degree C temperature increase in the ST muscle over 30 min. Heat will also diffuse into adjacent muscle/tissues (minimizing the increase in temperature).

If only fast twitch (FT) fibers (80% of muscle mass or 4x ST fiber mass: = ¼ metabolic rate) are used for 30 min, there would be a 0.5 degree C temperature rise in FT fibers.

If all muscle (100% or 5x ST muscle mass = 1/5 metabolic rate) for 30 min, there would be a 0.4 degree C temperature rise in the entire muscle mass.

Therefore, the temperature rise and muscle mass specific metabolic rate depend on how much of the muscle mass is recruited during this 3.4-fold increase in muscle metabolic rate. And all this is under the assumption of no muscle blood flow during this time period (this emphasizes the importance of the cardiovascular response; if some muscle blood flow occurred, the increase in muscle temperature would be even less). Regardless of the percentage muscle mass utilized, the increase in muscle temperature would not be exceptional. The risk of muscle hyperthermia appears minimal under these conditions.

Two hours of anaerobic metabolism (as in long dive reported by Falcone et al. 2017) would result in a 2 degree C rise in FT fibers (FT fiber activation alone), and in a 1.6 degree C rise in all muscle (if all muscle fibers were activated).

## Conclusions

With available data and a series of basic assumptions, estimated muscle metabolic rates are low, both during routine dives and post sonar exposure dives. The potential for significant intramuscular lactate accumulation, glycogen depletion, and acidosis is low. Similarly, the potential for muscle hyperthermia also appears minimal.

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**Stress Responses and Diving**

Daniel E. Crocker & Laura A. Thompson

The interplay between marine mammal diving physiology and stress responses is still an emerging consideration in marine mammal research and conservation. There has been extensive recent work on the stress physiology of marine mammals with a focus on potential differences to terrestrial animals (reviewed in Atkinson *et al.,* 2015). The generalized stress response is highly conserved among mammals and the basic features are identical in marine mammals except for several important differences that have emerged in recent studies. Here we focus on those endocrine features that might be relevant to impacts of acute and chronic stress on diving, including the form of the stress response.

Nervous control of regulatory adjustments to diving have been demonstrated in both forced and natural dives, yet hormonal changes during dives have been measured in only a few species (e.g. Weddell seal; Hochachka *et al*. 1995, Hurford *et al*., 1996; and Harbor seals; Hance *et al.* 1982; Indo Pacific bottlenose dolphins; Suzuki *et al.,* 2017). Understanding endocrine regulation during free diving has been logistically difficult due to the need to acquire repeated blood samples during breath holds and exercise. To date this has been achieved in only a few experimental paradigms and the bulk of sampling has occurred in the context of sleep apneas and forced or free submergences in captive phocid seals, lacking the exercise and pressure components of diving. Most of what is known about endocrine regulation during free diving comes from ice-hole experiments in Weddell seals with sampling biased to surface intervals between dives. Measurements of perfusion during forced submersion in Weddell seals suggested strong vasoconstriction of the pancreas and thyroid, while the adrenals underwent comparatively moderate reductions in blood flow (Zapol *et al.,* 1979). These differences may facilitate some of the endocrine changes evident during free-diving in this study system. Increased development of instrumentation for blood sampling during free-diving will have important impacts on our understanding of endocrine responses during diving and are critical to increasing the taxonomic breadth of studies in this area.

Diving is associated with increases in circulating catecholamines (norepinephrine and epinephrine) that likely contribute to bradycardia, peripheral vasoconstriction, and splenic contraction (Hochachka *et al.* 1995, Hurford *et al.,* 1996)). In freely diving Weddell seals, catecholamine concentrations during surfacing were associated with the duration of the previous dive, suggesting a role in regulating the magnitude of the dive response and post-dive tachycardia, and were cleared rapidly with a half-life of <10 minutes (Hance *et al.*, 1982). The specific tissue effects of increased circulating catecholamines will depend on the presence and density of receptor isoforms (α and β) and their unique signal transduction pathways which can interact with other receptor activated pathways. Stimulation of β-receptors typically results in vasodilation of vascular smooth muscle, and activation of G-Protein /cAMP signaling pathways, increasing heart rate and contractility (Brodde *et al.,* 1992). Thus, diving bradycardia that occurs despite high circulating catecholamine concentrations likely reflects strong counter-regulation and cAMP inhibition by vagally-mediated ACh release or as yet undescribed differences in

receptor function. Attenuation of vagal cardiac impacts by endocrine release of catecholamines may partially underlie the modulation of the dive response by exercise shown in some marine mammals (Davis & Williams 2012, Williams *et al.,* 2015). Splenic contraction and peripheral vasoconstriction are mediated by binding of α-receptors; with circulating epinephrine exerting a stronger effect of peripheral vasoconstriction than norepinephrine.

Catecholamine release during diving may also play important roles in the regulation of fuel substrates during diving and surfacing by inhibiting pancreatic insulin release. This inhibition results in a reduced insulin:glucagon ratio and helps maintain glucose availability during vasoconstriction. Insulin inhibition in concert with glucagon elevation during surfacing likely modulates a post-dive hyperglycemia (Robin *et al.,* 1981). In freely submerging but not exercising elephant seals pups, circulating glucagon levels decreased late in breath holds, but increased dramatically during surfacing in association with an increase in plasma glucose (Crocker and Tift, unpublished data). Elevated catecholamines during surfacing and reperfusion also likely promote lipolysis from adipose tissue stores to resupply muscle fatty acids depleted in vasoconstricted muscles (Castellini *et al.,* 1985, Kanatous *et al.,* 2002).

Despite the wide variation in circulating concentrations described during diving, catecholamines are also released as part of the generalized 'fight-or-flight' response in marine mammals. Increased catecholamines have been measured in belugas following transport (Spoon & Romano 2012); in live captured bottlenose dolphins, with an immediate increase in epinephrine, but highest levels found in norepinephrine (Fair *et al.,* 2014); as well as during cetacean strandings where intense catecholamine exposure has been linked to myocardial damage, muscle damage and acute death (Cowan & Curry 2008, Herráez *et al.,* 2013). Similar signs of catecholamine damage have been reported in South American Fur seal pups which died following handling and sampling procedures (Seguel *et al*., 2013). Physical restraint of weaned elephant seals resulted in a near-instantaneous 3-fold increase in epinephrine levels when compared to sedated pups (Champagne *et al.,* 2012). However, peak epinephrine concentrations during restraint were still significantly lower than those exhibited during free submergences in the same age class (Crocker and Tift, unpublished data). Small but significant increases in epinephrine, norepinephrine, and dopamine were observed in a beluga exposed to high levels of impulsive noise even after extensive time for clearance (Romano *et al.,* 2004). However, no significant changes in catecholamines were reported in belugas exposure to recordings of oil drilling noise (Thomas *et al.,* 1990). Such conflicting reports highlight the variability in stress responses due to characteristics of the exposure, as well as individual perception and experience. The emerging technology available for acquiring blood samples during diving will allow better contextualization of how stress-induced catecholamine release compares to natural variation during diving and the potential for impacts on cardiovascular regulation during diving. For example, catecholamine release associated with flight behaviors have been proposed as a source of cardiac arrhythmias in diving marine mammals (Williams *et al.,* 2015). While the potential for cardiac damage due to stress responses during diving may be unlikely because of dominating vagal control of the heart (Ponganis *et al.,* 2017), other sub-lethal impacts have received less consideration.

The hormone most closely associated with the stress response in mammals, cortisol, has not been examined in the context of diving physiology. The half-life of released cortisol was 109 minutes in dolphins (Champagne *et al.,* 2017), suggesting effects of acute stress-induced cortisol release over multiple subsequent breath-holds. In humans, muscle sympathetic vasoconstrictor activity was reduced by cortisol, and pressor responsiveness to subsequent administration of cathecholamines was increased (Whitworth *et al.,* 2005). These potent cardiovascular effects of cortisol in humans suggest at least the potential for interactions with the complex cardiovascular regulation of diving in marine mammals. As expected given the slow half-life of released cortisol, blood samples from freely submerged elephant seals demonstrated no changes across dive cycles (Crocker and Tift, unpublished data). However, plasma cortisol levels declined across several hours of free-submergence. Similarly, grey seals exhibited lower cortisol values while diving in comparison to when hauled out (Takei, 2016).

Following exogenous ACTH stimulation, measured changes in circulating glucocorticoids tend to be greater in pinnipeds as compared with cetaceans (Atkinson *et al*., 2015). Several taxa of marine mammals (dolphins, otariids, sea otters) have been shown to release biologically significant amounts of corticosterone during a stress response as well as cortisol, the typical dominant glucocorticoid in most mammals (Ortiz & Worthy 2000; Larson *et al.,* 2009; Derango, submitted). Both glucocorticoids compete for binding globulins, and the dominant glucocorticoid likely binds with greater affinity, allowing for differences in free bioavailable concentrations of either hormone. The two glucocorticoids can result in differences in second messenger system activation during acute stress that can perform different physiological or behavioral functions (Koren *et al.,* 2012).

Diving potentially has strong effects on renal perfusion and glomerular filtration, which could alter the release of vasoactive hormones. Voluntary bouts of sleep apnea in Weddell and elephant seal pups resulted in decreased circulating vasoconstrictors Angiotensin II (ANG II) and arginine vasopressin (AVP), and an increase in atrial natriuretic peptide (ANP, vasoconstrictor inhibitor) (Zenteno-Savin & Castellini 1998). This increase in ANP was attributed to an increase in cardiac pressure, which is a known stimulus of ANP release. In terrestrial mammals, reductions in renal blood flow would activate the release of renin and the renin-angiotensin system (RAS), increasing concentrations of Ang II and promoting increased peripheral vasoconstriction. The reductions in AngII reported in weddell and elephant seals may reflect inhibition of renin release into circulation due to vasoconstriction of the kidney and unopposed suppression of the RAS by ANP. However, a recent application of a system that acquired blood samples during free diving in captive grey seals revealed only small, marginally significant changes in vasoactive peptides during diving (Takei *et al.,* 2016). Thus, the role of vasoactive peptides in freely diving and exercising animals has yet to be determined. In the typical mammalian stress response, both AVP and ANG II are increased and function as important synergistic factors in stimulating secretion of other stress hormones (Saavedra & Benicky 2007). These changes oppose those reported during apneas in phocids, suggesting potential for interactions with this component of diving regulation.

Numerous studies have shown that the adrenal osmoregulatory hormone, aldosterone, is under an unusual degree of hypothalamic-pituitary-adrenal (HPA) axis control in marine mammals and is thus a highly stress responsive hormone. There is now evidence for this feature in most marine mammal taxa, including strong aldosterone release in response to a stress test in bottlenose dolphins (Champagne *et al.*, 2017), handling stress in belugas (Schmitt *et al.*, 2010), adrencorticotropin (ACTH) challenge in phocids (Gulland *et al.*, 1999; Ensminger *et al.*, 2014; Champagne *et al.*, 2015) and capture stress in otariids (DeRango *et al*., submitted). Similarly, analysis of fecal adrenocorticoid metabolites in right whales suggest strong associations between cortisol and aldosterone (Burgess *et al.*, 2017). This important difference in the form of the stress response is likely a result of changes in endocrine regulation required for diving adaptation.

Typically, the release of aldosterone is regulated by the RAS system. Reductions in renal tubular flow lead to the release of renin which converts angiotensinogen to angiotensin I (ANG I), a prohormone with little biological activity. ANG I is converted by a converting enzyme found predominantly in the pulmonary vasculature to ANG II, which is a potent systemic vasoconstrictor that raises blood pressure and renal blood flow. ANG II binds to receptors on the adrenal gland to stimulate the release of aldosterone which promotes $Na^+$ retention by the kidney and resultant increases in blood volume and pressure. Alterations in renal and pulmonary blood flow during repetitive diving may preclude the use of typical RAS regulation of aldosterone in marine mammals. Interestingly, acute induced and chronic high-altitude hypoxemia blunted the release of aldosterone but not cortisol in response to ACTH challenge in humans (Ramirez *et al.* 1988). A recent study in grey seals found that the dominant circulating forms of angiotensin was not ANG II but ANG III, a further cleaved form of the peptide that exhibits reduced pressor activity but is a potent secretogogue for aldosterone (Takei et al. 2016) and is typically enhanced in disease states like atherosclerosis (Olkowicz *et al.* 2015). Together, these findings suggest important alterations in marine mammals to the RAS, one of the primary systems regulating salt balance, blood volume and blood pressure. The enhanced release of aldosterone in response to acute stress in marine mammals may result in increased salt retention in feeding animals with potential cardiovascular impacts through increased blood volume and pressure. This may represent an important mechanism by which stress interacts with the cardiovascular system of marine mammals. However, studies on the effects of aldosterone in feeding animals are lacking.

One health concern regarding activation of the neuro-endocrine responses is modulation of immune responses through direct innervation of immune tissues (Romano *et al.*, 2002) or binding of adrenergic or adrenal steroid receptors, particularly Type II, expressed on immune cells (Romero, 2004; Madden & Felten, 1995; Padgett & Glaser, 2003). Suppressed immune activity is associated with increased susceptibility to infection and disease, however augmented activity, particularly inflammatory responses, may alter susceptibility of marine mammals to dive related injuries. Beluga immune cell function was found to decrease in response to *in vitro* pressure exposures (simulated dives) during baseline conditions, while increases in activity observed under stressor conditions (increased cortisol and catecholamines) resembled the response of human cells, which may suggest an increased risk of inflammatory damage during diving (Thompson, 2014). Deficiencies in coagulation and the development of disseminated intravascular coagulation, driven by a stress response to sound, have also been suggested to play

a role in the stranding of beaked whales in response to sonar (Rommel *et al*., 2006) though no direct studies have been carried out.

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# Thermoregulation

Petter H. Kvadsheim

## The basic challenge

The most important physiological adaptations seen in marine mammals compared to their terrestrial ancestors are related to some very fundamental physical differences between air and water. Compared to air, water contains only 1/30 the amount of oxygen, the viscosity is more than 70 times higher, the thermal conductivity is 23 times higher and the heat capacity is 3000 times higher. The result of the latter two is that heat is transferred from the body of an animal in water much faster than in air, even if the body and ambient temperature are the same. Marine mammals are homeothermic, and it is a great physiological challenge to maintain body temperature in cold polar waters. Species living in more tropical waters, were the surface water temperature is much higher, might not have the same problem, but deep divers like beaked whales dive regularly to depths >1000m, and since tropical oceans are typically characterized by a rapid decrease in temperature with depth (thermocline), waters temperature at those depths are not any higher than in the polar oceans.

Humans are fascinated by animals that can do things we can't do, and to us nothing gives the impression of extreme cold as the ice water of the polar oceans. The thermoregulatory challenges of marine mammals, in particular polar species, were therefore the subject for some very early but classical studies of marine mammal physiology (e.g. Scholander et al. 1950, Irving et al. 1969). The main adaptation found in marine mammals is that the insulating fur has been replaced by a subcutaneous, layer of fat or blubber. The thermal resistance of fur drops to almost 0 when submerged in water whereas in blubber it remains unchanged (Scholander et al. 1950, Kvadsheim & Aarseth 2002). Herman Melville dedicated a whole chapter of Moby Dick to blubber, and called it "the blanket" of the whale. In uninsulated extremities like fins (e.g., Scholander & Schevill 1955) and flippers (Tarasoff and Fisher 1970, Blix et al. 2010), and to reduce respiratory heat loss (e.g., Folkow and Blix 1989), vascular arterial-venous countercurrent systems are developed. Such countercurrent vascular arrangements have also been demonstrated in blubber of whales (Parry 1949). Despite these adaptations, it was the common belief among marine mammal scientist for many years that both seals and whales had elevated heat production rate compared to similar sized terrestrial animals in order to maintain body temperature (e.g. Irving et al. 1935). More recent studied have shown that this is not the case (reviewed by Lavigne et al. 1986). Another fundamental difference between fur and blubber as insulation is that blubber is vascularized live tissue, and by regulating blood perfusion through the blubber the animal can regulating the insulation. When an animal need to conserve heat to prevent body temperature from dropping blood perfusion of the blubber and skin is reduced to a minimum, and blood to extremities (flippers, flukes and fin) is directed through vascular countercurrent heat exchangers. In this situation heat is transferred through the blubber by passive conduction only (Kanwisher & Sundnes 1965, Kvadsheim & Folkow 1997). However, if the animal needs to increase heat dissipation to prevent temperature from increasing, the blubber and skin are perfused by blood which carries the heat through the blubber by convection thereby decreasing the insulating effect of the blubber to almost nil (Kvadsheim & Folkow 1997, McGinnis et al. 1972). Furthermore, extremities such as flippers and flukes and the dorsal fin and tongue (*Corpus Cavernossum*

*Maxillaris)* in cetaceans are used as special organs for heat dissipation. These are highly exposed to sea water and are well vascularized, often also containing arteriovenous anastomoses (e.g. Elsner et al 1974, Blix et al. 2010). An interesting consequence of this adaption is that, contrary to well insulated terrestrial animals, maximum and minimum heat loss are basically independent in marine mammals. The high insulating property offered by a thick layer of unperfused blubber can be almost completely bypassed and will not prevent heat dissipation achieved through blood perfusion of blubber and skin.

**The inherent conflict between diving and thermoregulation**

Marine mammals are endothermic and regulate their deep body temperature to a fairly constant level which is optimal for metabolic processes. Many marine mammals are big and have a very low surface to volume ratio which gives them an advantage in terms of heat conservation, but even seal pups seem to have adaptations which enable them to maintain body temperature in ice water without much energetic cost (e.g. Kvadsheim & Folkow 1997). Given the thermal properties of water, it's hard to imagine that heat dissipation of excess heat would ever be a problem to aquatic mammals (Folkow 1992), not even in dolphins swimming in tropical waters at temperatures close to their body temperature (Heath & Ridgway 1999).
Thus, marine mammals have an amazing ability to both conserve heat and dissipate heat as needed. They do this mainly through vascular mechanism and metabolic adjustments. However, marine mammals are also surface breathing divers, and adaptions to rationalize with oxygen stores during diving also involve vascular and metabolic mechanism. A very interesting aspect of marine mammal physiology is that thermoregulatory needs and physiological adjustments during diving are not always compatible. Like terrestrial animals, seals that are cold stressed will shiver to increase metabolic heat production and maintain body temperature when they are at the surface (Hammel et al. 1977, Kvadsheim et al. 2005). However, if they dive, the dive response seems to override the thermoregulatory requirement by inhibition of shivering with the consequence that body temperature drops (Kvadsheim et al. 2005). This might be seen as apposing physiological requirement, but Blix et al. (2010) showed that body temperature is actively downregulated during diving potentially to reduce oxygen requirement of the brain. This is achieved by active perfusion of the flippers through arteriovenous shunts and return by way of large superficial veins (Blix et al. 2010). Contrary to the observation that the vascular dive response overrides thermoregulatory induced metabolic responses (shivering) (Kvadsheim et al. 2005), Hammel et al. (1977) found that in heat stressed animals (experimental increase of hypothalamic temperature) the thermoregulatory induced peripheral vasodilatation was maintained during diving, thus in this case the dive response did not override thermoregulatory responses. In terms of trade-offs between diving and thermoregulation, this might indicate that animals are more sensitive to hyperthermia than hypothermia, or that there is a difference between metabolic and vascular thermoregulatory mechanisms. Peripheral vasodilatation can be maintained without compromising oxygen saving much during diving, if skin and flippers or flukes are perfused through arteriovenous shunts, whereas shivering increases oxygen consumption directly and would therefore compromise the diving capacity.

**Thermoregulation and responses to naval sonar**

Naval sonars have been associated with atypical mass strandings of cetaceans (D'Amico et al.,

2009). These events have mostly involved beaked whales (family Ziphiidae), but some other species have also been involved. The cause and effect relationship between sonar and these strandings is not clear, and the question is if it's possible that thermoregulatory issues are involved. Cetaceans have been shown to change their behavior in response to naval sonar (recent reviews by Southall et al. 2016, Harris et al. 2017). E.g. minke whales (Kvadsheim et al. 2017) and killer whales (Miller et al. 2014) have been shown to display high speed avoidance of the sonar source near the surface, which might lead to up to three fold increase in heat production (Blix & Folkow 1995). Beaked whales on the other hand, seem to perform very deep dives to escape from the sonar (Tyack et al 2011, DeRuiter et al 2013, Miller et al 2015). Such avoidance dives are associated with increased fluke stroke rate which has been estimated to increase heat production by 30% (Williams et al. 2017). In the case of the minke whale and killer whales, the increase in heat production is quite dramatic, but as long as the animals are close to the surface their ability to dissipate heat through vascular adjustments should also be very high. In the case of the beaked whales, the increase in heat production is not very dramatic, and given the very long avoidance dives observed (90 min, Miller et al. 2015) the animal can probably not increase it any further. Even though vascular adjustments to save oxygen (peripheral vasoconstriction) might impact the animals ability to dissipate heat effectively, it's does not seem very likely that such a moderate increase in heat production would be enough to create serious problems with hyperthermia. More research is needed on thermoregulation in deep diving marine mammals, but it's not expected from current knowledge that thermal stress is a factor contributing to sonar related strandings. However, once the animals are on the beach for whatever reason, and out of the water, hyperthermia could very easily become a serious problem, and it is possible that this is what eventually kills stranded animals (e.g. NOAA & US Navy 2001, Cox et al. 2006).

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# Potential Physiological Effects of Deep Diving on the Central Nervous Systems of Beaked Whales

Paul J. Ponganis

In diving animals, the function of the central nervous system may be affected by a) increased ambient pressure due to changes in depth (high pressure nervous syndrome – HPNS), b) increased partial pressures of nitrogen ($P_{N2}$) due to nitrogen absorption ($N_2$ narcosis), and c) elevations in the partial pressure of carbon dioxide ($P_{CO2}$) due to continued metabolism during a breath hold ($CO_2$ narcosis). This summary reviews these three syndromes, and the potential for their occurrence during dives of the "flight" responses of beaked whales after sonar exposure.

## High pressure nervous syndrome

HPNS in humans is typically associated with depths greater than 190 m (20 atmospheres absolute (ATA)) and is characterized by hyperexcitability of the central nervous system (Bennett and Rostain, 2003a). Symptoms begin with the onset of tremors and may advance to convulsions, seizures and death. HPNS has occurred as shallow as 130 m, and may also be precipitated by changes in depth of greater than 30 m $min^{-1}$ (Bennett and Rostain, 2003a; Halsey, 1982). The presence of $N_2$ in breathing gas mixtures has a suppressive effect on HPNS (Bennett and Rostain, 2003a).

In tissue studies, increased pressure disrupts gamma aminobutyric acid and aspartate neurotransmission in the brain (Bennett and Rostain, 2003a). Increased pressure has mixed effects on various ion channels, including voltage gated sodium channels, voltage dependent potassium channels and voltage dependent calcium channels (Aviner et al., 2010). Changes in ion flow across these membrane channels can affect action potential amplitude as well as release of neurotransmitters. The exact mechanisms underlying HPNS are still unknown.

## Nitrogen narcosis

Symptoms of nitrogen narcosis begin to occur at about 30 m depth in humans breathing air (Bennett and Rostain, 2003b; Dean et al., 2003). The $P_{N2}$ in air at this depth corresponds to 2400 mm Hg, 320 kPa, or 3.2 ATA. Symptoms and signs include euphoria, cognitive impairment, and loss of consciousness. At the cellular level, increased $P_{N2}$ is associated with release of at least two neurotransmitters, dopamine and glutamate (Rostain et al., 2011). The underlying mechanism probably involves the binding of $N_2$ to protein ion channels in the membranes of neurons.

**Carbon dioxide narcosis**

Carbon dioxide narcosis is manifested by decreased mentation, confusion, short term memory loss, drowsiness, and eventual loss of consciousness (Dean et al., 2003). Symptoms can begin to occur at 50 to 70 mm Hg $P_{CO2}$ (6.66-9.33 kPa) in humans. Loss of consciousness has occurred at end tidal $P_{CO2}$ values greater than 90 mm Hg (12.0 kPa) in human divers. The mechanism underlying $CO_2$ narcosis is thought to involve neuronal intracellular acidosis secondary to the elevation in $P_{CO2}$.

**Potential for physiological effects on the central nervous system in beaked whales exposed to sonar**

The routine dive depths and successful foraging of deep-diving marine mammals suggest that HPNS does not occur in deep divers. The absence of HPNS is especially remarkable, however, given the maximum depths and descent rates of beaked whales (Baird et al., 2006; Baird et al., 2008; Minamikawa et al., 2007; Schorr et al., 2014; Tyack et al., 2006). Those values are much greater than the HPNS thresholds in human divers. HPNS also appears unlikely in the dives of beaked whales after sonar exposure because those responses do not involve dives with greater maximum depths or more rapid descents (DeRuiter et al., 2013; Stimpert et al., 2014; Tyack et al., 2011). Although sonar exposure of a northern bottlenose whale (*Hyperoodon ampullatus*) resulted in the deepest dive reported for that species, the descent rate did not appear exceptional, and the maximum depth was within the range reported for other beaked whales (Miller, 2015; Schorr et al., 2014).

Actual measurements of $P_{N2}$ in seals and dolphins are generally less than the threshold for symptoms of nitrogen narcosis in humans (Falke et al., 1985; Houser et al., 2010; Kooyman et al., 1973; Ridgway and Howard, 1979). Numerical models of nitrogen uptake and distribution during normal dive behaviors in marine mammals, including beaked whales, have resulted in estimations of brain and central circulation $P_{N2}$ values in the 4-5 ATA range (Fahlman et al., 2009; Hooker et al., 2009). Numerical models of several cetacean species, including beaked whales, during sonar exposure resulted in estimated nitrogen values in the same range in the central circulation and brain (Kvadsheim et al., 2012). Although such values are above the human threshold for early symptoms of nitrogen narcosis, the risk for nitrogen narcosis after sonar exposure does not appear different from that during normal dives. It appears unlikely that nitrogen narcosis occurs in these deep-diving animals. In general, moderate elevation of $P_{N2}$ may also contribute to suppression of HPNS in these animals.

The potential for elevated carbon dioxide and carbon dioxide narcosis in beaked whales after sonar exposure is unknown, dependent on many variables, and probably an unlikely etiology for the stranding and deaths of these whales after sonar exposure. The dives after sonar exposure have been highly variable in terms of dive durations. Stroke effort is often elevated to varying degrees in beaked whales after sonar exposure. However, any rise in blood $P_{CO2}$ would be dependent on the intensity of the dive response and the amount of muscle blood flow during that exercise. Although the heart rate response during dives may be affected by exercise (Davis and Williams,

2012; Hindle et al., 2010; Noren et al., 2012b; Signore and Jones, 1996; Williams et al., 2015), heart rates are still low during dives and even lower during longer dives (Andrews et al., 1997; McDonald and Ponganis, 2014; Thompson and Fedak, 1993). Heart rate responses, the magnitudes of muscle blood flow and other tissue flows, and the potential rise in blood $P_{CO2}$ remain unknown during routine dives and post sonar exposure dives of beaked whales. $P_{CO2}$ values near 55 to 75 mmHg (7.33-9.99 kPa) routinely occur without problem during and after dives of pinnipeds, during sleep apnea of seals, and after trained breath holds in cetaceans (Kooyman et al., 1980; Noren et al., 2012a; Qvist et al., 1986; Stockard et al., 2007). During forced submersions of seals, arterial $P_{CO2}$ values as high as 100-110 mm Hg (13.33-14.67 kPa) are also tolerated without apparent complication (Elsner et al., 1970; Kerem and Elsner, 1973). Therefore, carbon dioxide narcosis during post sonar exposure dives of beaked whales seems unlikely.

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