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Abstract Book

Modeling gas dynamics in California sea lions

A. Fahlman
Fundacion Oceanografic, Valencia Spain, email: afahlman@oceanografic.org, Phone: +34-654-492-427

Background

Theoretical models are useful in providing a framework to understand complex processes. Previous studies using these models have estimated the potential risk of bubble emboli/decompression sickness in deep diving whales exposed to Navy sonar (Fahlman et al., 2014b; Hooker et al., 2009; Kvadsheim et al., 2012). However, model results are significantly affected by their input parameters. A sensitivity analysis of the model identified the perfusion rate, blood-flow distribution and pulmonary shunt to be variables having the greatest impact on inert gas uptake and removal (Fahlman et al., 2009). Due to insufficient data from marine mammals, many of the input parameters used in previous modeling efforts have been from terrestrial animals. Until recently, only limited data exist describing the pressure-volume (P-V) relationship for the respiratory system of marine mammals, past models have used compliance data from dog lungs and stress strain curves from dolphin tracheas (Bostrom et al., 2008). Variation in respiratory compliance significantly affects the model output because the alveolar collapse depth, and pulmonary shunt will change.

This work was aimed at assessing how variation in the input parameters that alter lung compression, and alveolar collapse depth would affect estimated tissue and blood N_2 (PN_2), O_2 (PO_2) and CO_2 (PCO_2) tensions in breath-hold diving California sea lions. We used dive data from previously ONR funded studies (ONR award N00014-10-1-0514, McDonald and Ponganis, 2012; McDonald and Ponganis, 2013; McDonald and Ponganis, 2014), and respiratory lung compliance estimates from California sea lions (ONR award N00014-12-1-0269, Fahlman et al., 2011; Fahlman et al., 2014a; Fahlman and Madigan, 2016) to assess the overall importance of species-specific estimates for lung compliance. In addition, we used the updated model parameters to determine how well the model could predict measured venous and arterial PO_2 levels during dives in the sea lions. These recent studies allowed us to revise the existing model's input parameters and calibrate the model against empirical data. In addition, this work highlighted the importance to better understand the anatomy and physiology of marine mammals to improve our understanding how man-made changes in behavior and/or physiology may alter the survival.

Objectives

*Objective 1***:** Revise the existing model with new species-specific parameter estimates for California sea lions.

Objective 2: Compare estimated and measured arterial and venous P_{O_2} for diving California sea lions.

Methods

Objective 1:

Pressure-volume (compliance) loops from excised California sea lions lungs and upper airways (trachea) were used to update the parameters that estimate the pulmonary shunt during diving (Bostrom et al., 2008). The pulmonary shunt equations, which are an integral component to estimate gas dynamics during diving, were revised with species-specific parameters for the upper and lower airways. This provided updated estimates of how blood and tissue gases are managed during diving.

In addition, the model was revised to include the species-specific relationship between P_{O_2} and O_2 affinity (O_2 dissociation curve) and Bohr effect, both of which affect available O_2 for tissue specific O_2 consumption (McDonald and Ponganis, 2013).

Objective 2:

Dive data, and concurrent venous and arterial P_{O_2} measurements, for California sea lions were obtained from Drs. Paul Ponganis and Gitte McDonald. These data were used to compare estimated and observed blood P_{O_2} levels during diving. A sensitivity analysis was performed to assess the new and current parameter estimates and error of the model output. The future aim is to test the hypothesis that a peripheral a-v shunt cause a ventilation/perfusion mismatch which explains the measured arterial and venous P_{O_2} profiles, as previously suggested (McDonald and Ponganis, 2013).

Results

Objective 1:

Updated Model Parameters

Alveolar compliance increased and tracheal compliance decreased using species-specific parameter estimates (Fig 1). Pulmonary shunting was affected using the updated parameter estimates, which significantly altered the gas exchange (see below). These results agree with past work suggesting that an increasing ventilation/perfusion ratio preferentially alters N_2 exchange (West, 1962).

Estimated N_2 tension (PN_2)

Estimated tissue PN_2 was lower in all tissues using the species-specific input parameters tissue types, with the end-dive P_{N2} decreasing by 51.2±0.1% for brain (fast-loading tissue, Fig. 2), 39.6±0.4% for muscle (intermediate-loading tissue), and 22.6±7.7% for fat (slow-loading tissue).

Estimated PCO_2 and PO_2

The model output using the species-specific parameters had similar PCO_2 and PO_2 estimates in all tissue types as compared with the model output using parameters from terrestrial mammals. The end-dive PO_2 and PCO_2 decreased by <0.1% and 1.5% for brain, 5.6% and 0,5% for muscle, and <0.1% and 0.3% for fat, respectively (Fig. 3).

Lung Collapse Comparison

We varied the diving lung volume to fit the estimated alveolar collapse depth The model predicted that the diving lung volume had to be larger than 60% TLC to obtain alveolar collapse depths within the range presented by McDonald and Ponganis (2012). Also, changes in diving lung volume had a greater effect on alveolar collapse depth than changes in cardiac output.

Aim 2:

Dive data, and concurrent venous and arterial PO2 measurements, for California sea lions have been acquired from Drs. Paul Ponganis and Gitte McDonald and are being used to compare estimated and observed blood PO_2 levels during diving. An initial analysis of the venous P_{O2} estimates from the updated model show that they differ slightly from the empirically collected venous P_{O2} values.

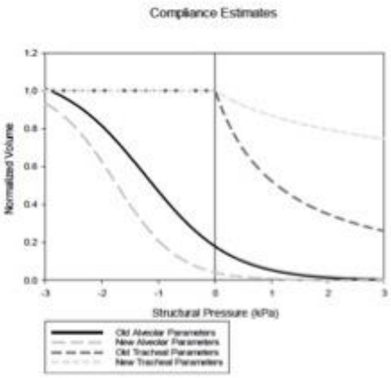

Fig. 1. Normalized lung volume estimates using the former parameter estimates (darker lines) and the updated parameter estimates (lighter lines).

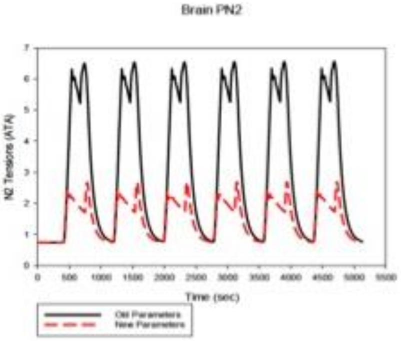

Fig. 2. Brain (a fast-loading tissue) PN_2 estimates using the former parameter estimates (black) and the updated parameter estimates (red) using a simulated dive profile from a California sea lion. (darker lines) and the updated parameter estimates (lighter lines).

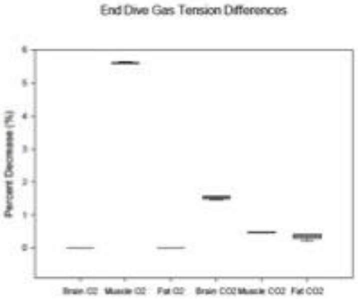

Fig. 3. End-dive PO2 and PCO2 differences ([old-new]/old*100%) between model runs with old and new parameters for fast-loading (brain), intermediate-loading (muscle), and slow-loading (fat) tissues

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Tag-based Heart Rate Measurements of Harbor Porpoises During Normal and Noise-exposed Dives to Study Stress Responses

Peter T. Madsen, PI; Birgitte I. McDonald, Post-doc
Dept. of Bioscience, Zoophysiology Section, Aarhus University
peter.madsen@bios.au.dk*, (+45) 5177 8771*

Background

To predict and quantify how marine mammals respond to natural and anthropogenic stressors, it is essential to understand their physiological limits, the potential plasticity of their diving physiology, and their physiological responses to stress. The typical mammalian startle or stress response to an acoustic stimulus is increased heart rate, cardiac output and ventilation rate (Graham 1979); all which are contrary to the typical marine mammal dive response (Scholander 1940). Information on the acute stress response during diving is essential to predict how potential stressors effect oxygen and nitrogen management and can provide information on the level of stress the animals routinely experience. This study directly addressed two of the ONR Marine Biology Program thrusts: Diving physiology and stress physiology. We investigated the relationship between dive behavior and the heart rate response in trained and wild harbor porpoises and documented the level of diving response in a wild cetacean in an urbanized environment. Understanding dive heart rate response during natural and noise exposure diving is critical for addressing how cetaceans manage O_2 and N_2, and how this process may be impacted by anthropogenic disturbance. Such data from relevant species are essential for use by the Navy in estimating the physiological consequences of naval exercises (i.e. mid-frequency sonar) on marine mammals.

Objectives

The specific objectives of this project were 1) Quantify the physiological and behavioral response to acoustic stimuli in captive porpoises by examining differences between the control and controlled noise exposure trials; and 2) study the dive heart rate, activity and ventilation rate of wild porpoises and examine the physiological and behavioral responses to absolute noise levels that they may be exposed to during routine behaviors. Using harbor porpoises as model species, this study provided novel information on the capability to use non-invasive, multi-sensor tags to quantify the impact of potential stressors such as sound on physiological systems in cetaceans in the wild.

Methods

Heart rate response to acoustic distrubance

Dive heart rate and behavioral response to acoustic stimuli was examined in two harbor porpoises housed in net pens (size: 35x10x5m) at the Fjord&Bælt center using two protocols: 1) experimental and 2) opportunistic. For the experimental protocol porpoises were trained to perform prey capture dives in a range of dive durations while instrumented with an ECG Dtag3 data logger. Experimental sessions consisted of a block of 4-6 experimental dives (2-3 controls and 2-3 noise exposure in random order). There were 4 experimental treatments that varied in 1) dive duration (predictable short dives or random medium duration dives) and 2) acoustic stimuli (sonar or startle). Session order was mixed to prevent habituation. Additionally, we obtained opportunistically noise exposure data when porpoises were instrumented with Dtags during free swims while pile driving was taking place.

Wild porpoise heart rate

Two porpoises accidentally caught in pound nets were in 2016 instrumented with an EKG Dtag3 data logger upon release. The tags were deployed with four suction cups programmed to release after ~12-48 hours. Recovery from small boats was aided by built in VHF antennas following establish procedures (Soto et al. 2008). Additionally, due to the acoustic data collection capabilities of the data loggers we will be able to examine the heart rate response to acoustic stimuli while at sea.

Results

Heart rate response to acoustic disturbance

In all dives (control and noise), f_H decreased compared to surface rates; however, the relationship between dive f_H and dive behavior was complex. Dive f_H was variable even in short duration dives, suggesting the conscious ability to regulate f_H in relation to dive type, duration, and activity (Elmegaard et al., 2016). Noise exposure treatment (control, sonar exposure, startle exposure) did not influence overall dive heart rate for either porpoise ($P>0.05$). Neither treatment or duration influenced post dive respiration rate ($p > 0.05$).

We also examined the fine scale heart rate response in a 10 second window centered around the noise exposure. Immediately after the first sonar exposure (received level 155 dB re 1µPa (rms)) both porpoises exhibited a pronounced decrease in heart rate (24 beats min^{-1} (Freja) and 38 beats min^{-1} (Sif)), despite no obvious behavioral response in the video or acceleration signal (MSA) during or immediately following the stimulus (Fig. 1). After the initial exposure, the porpoises did not display any consistent heart response, even when they had a clear behavioral response suggesting that they quickly habituated to the stimulus (Fig. 2). Our data suggest that evolutionary pressures may have resulted in a physiological stress response opposite that for terrestrial mammals, which may conserve or maintain oxygen and nitrogen management when diving, allowing the animals to stay at depth longer.

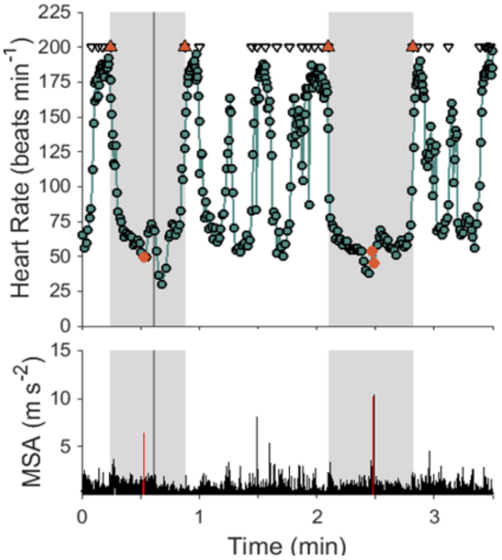


Figure 1. Dive heart rate and activity during control and noise exposure dives from a captive porpoise. In all dives heart rate decreased (top panel), but during noise exposure trials, heart rate did not change, or decreased more after the acoustic stressor. This figure shows the heart rate response to the first sonar exposure (received level 155 dB re 1μPa rms). Dives are highlighted with grey shading, acoustic stimuli is indicated by the dark grey line, respirations are indicated with triangles, and prey capture is indicated by the orange shading. The bottom panel shows activity of the porpoise using minimum specific acceleration as an activity index (MSA).

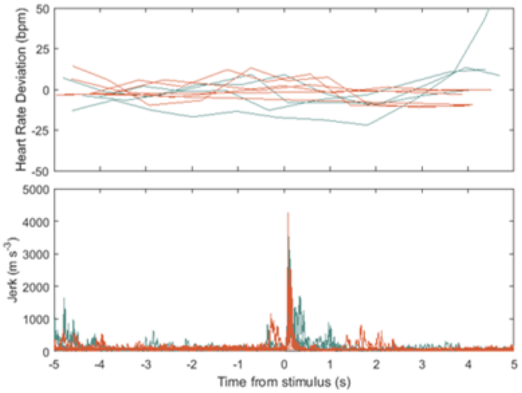


Fig 3. Heart rate deviation and jerk in the 10 second window centered around the start of the startle stimulus for two porpoises. There was no consistent heart rate response when porpoises were exposed to the startle stimulus (received levels ~ 150-160 dB re 1μPa rms), despite a clear behavioral response which is evident in the jerk (m s-3) signal and video recordings. Orange is Freja, and green is Sif.

Wild porpoise heart rate

In 2016 we have deployed two suction cup ECG Dtag3s on wild porpoises allowing us to investigate the heart rate response in relation to diving, exercise, and foraging behavior in a wild cetacean. The dataloggers remained on the porpoises until programed release, collecting 12 and 38 hours of data. The porpoise exhibited several hours of foraging behavior. Bottom heart rates were similar in both foraging and non-foraging dives and typically ranged between 55-75 beats/min, despite an increase in activity associated with foraging behavior. Conversely, surface heart rates were significantly different. After non-foraging dives, surface heart rates ranged between 160-180 beats/min and heart rate decreased between respirations. After foraging dives, heart rate ranged between 190-210 beats/min and remained elevated between respirations. In contrast to studies that report an exercise modulated dive response, we found that, while porpoises exhibited a similar dive response in most dives, they increased heart rate at the surface after active foraging dives, likely to offload CO_2. We are now in the process of testing for effects of anthropogenic noise on diving f_H across a range of behaviors.

Notes:

MEASURING COMPARTMENT SIZE AND GAS SOLUBILITY IN MARINE MAMMALS

Michael Moore, Andreas Fahlman, Yara Bernaldo de Quiros
Woods Hole Oceanographic Institution
266 Woods Hole Road, MS 50, Woods Hole, MA, 02543 Phone: 508-289-3228

Background

Gas bubble lesions consistent with DCS similar to human divers have been described in beaked whales stranded in temporal and spatial association with military exercises. There is a growing consensus that exposure to military sonar may trigger a behavioral response in beaked whales that may lead to bubble growth through decompression as the whales alter their diving behavior and/or physiology. Theoretical studies can be used to model different scenarios and to estimate tissue gas burden, thus pointing out those behavioral changes that may affect risk. The principal challenge of these models is to incorporate realistic parameters for the different species and to be calibrated against empirical data. Parameters that require further study for marine mammals include compartment size and gas solubility in the different tissues.

Objectives

1. To improve available morphometrics data of body composition and body compartments of marine mammal species.
2. To develop methods for the determination of gas solubility for various tissues of marine mammals
3. To develop methods to measure gas content in tissues of marine mammals and to relate this content with amount and gas composition of bubbles.

Methods

Access to different marine mammal species was achieved through collaborations with IFAW, University of North Carolina Wilmington, and the University of Las Palmas de Gran Canaria (ULPGC).

1. **Morphometric data.** Dissections were carried out according to McLellan et al. (2002). Tissues were weighed separately. Muscle samples were taken from different muscle groups to quantify myoglobin content. The mass of each body compartment was reported as a percentage of the total body mass (TBM) in accordance to Grand (1977) and grouped first attending to morphology (body composition: integument%, muscle%, bone%, and organs%), and then to a five-compartment model: fat (bones and blubber), central circulatory (heart, kidneys and liver), muscle (muscle and other abdominal and thoracic organs not included in the central circulatory compartment), brain, and blood (not measured in this project).
2. **Methods for the determination of gas solubility in tissues of marine mammals.** Solubility studies were carried out following Zhou and Liu (2001) but inside a glove box filled with argon, and using evacuated tubes to prevent atmospheric nitrogen contamination and to favor the diffusion of nitrogen into the head-space.
3. **Methods to measure gas content in tissues of marine mammals.** Tissues were sampled and ground using an “anaerobic” tissue grinder. The ground tissue was transferred to an anaerobic glass tube. Once tissues were transferred anaerobically, we analyzed the gas content using the headspace method.

Results

1. **Morphometric data.**
   Mass data for 19 marine mammals of 7 species were collected: 1 *M. bidens*, 1 *M. densirostris*, 1 *K. sima*, 1 *K. breviceps*, 5 *D. delphis*, 6 *S. coeruleoalba*, and 6 *S. frontalis*, The targets of the study were fresh (decomposition code 2) adult animals in good body condition. However mass dissection data were taken from animals with different body condition given available specimens. The mass dissection data from the animals with poor body condition (two *D.delphis* and one *M. densirostris*) contribute to our understanding of the health status of these animals. The loss in body mass

(18-50%) implied an increase of mass-specific basal metabolic rate (8-61%) and a concurrent reduction in relative muscle mass and oxygen storage capacity (35-49%). Body condition has direct implications for body compartments. Further studies should be performed to study the implications in the dive capabilities and susceptibility to decompression sickness. These animals were removed from the body composition and body compartments study.

Differences in body composition were found between deep divers and shallower divers: deep divers presented very low relative weight of organs and low relative weight of bones. Additionally, Mesoplodon sp. and *K. sima* presented the largest muscle mass % (Table-1). These results showed the importance of using specific-species data. The five-compartment model used did not properly reflect the different body composition of the species. Although it showed a clear difference in relative brain size between shallow and deep diving species (Table-1).

Myoglobin concentration presented a heterogeneous distribution between and within the muscles. Myoglobin concentration was significantly higher in marine mammals´ locomotor muscles compared to non-locomotor muscles. There was not a good unique location for myoglobin calculations and nitrogen solubility studies. The mean myoglobin concentration values between the epaxial axilla and *rectus abdominis* yielded the closest overall myoglobin mean values in 4 out of 6 animals.

2. **Methods for the determination of gas solubility for various tissues of marine mammals.** We have tested our method using olive oil in order to validate the method. In four assays we were able to obtain results within the numbers published in the literature, but we haven't been able to reproduce the assays. This part of the project will be continued at the ULPGC.
3. **Methods to measure gas content in tissues of marine mammals.** We have developed an anaerobic excision and grinding device based on Scholander (1941). The device still needs some adjustments. No results have been reached yet, since we need first to know the solubility coefficient. This part of the project will be continued at the ULPGC.

Notes: This project has been continued thanks to the funding of National Project CGL2015/71498P and the Canary Islands Government, which has funded and provided support to the stranding network, as well as the ULPGC that funded a postdoc fellowship for Y.B. The results here presented are a result of the collaboration of the two projects.

Table 1: Body composition and body compartments expressed as % of total body mass (TBM). Asterisks represent data drawn form the literature (McLellan et al., 2002; Mallette et al., 2016; Pabst et al., 2016)

	Body composition (% of TBM)				Body compartments (% of TBM)			
	Integument	Muscle	Organs	Bones	Fat	Muscle	CC	Brain
D. delphis (n=3)	18.9	45.6	13.6	10.2	27.1	56.2	4.1	1.0
S. frontalis (n=5)	16.2	49.5	16.2	10.5	24.8	61.6	4.9	1.2
S. coeruloalba (n=6)	16.1	47.1	12.9	12.2	26.6	57.0	3.4	1.2
*P. phocoena**	27.2	33.5	17.8	9.6	36.8	45.9	4.4	1.0
*T. truncatus**	22.5	36.9	17.5	11.0	32.1	43.9	1.3	1.6
K. breviceps (n=1)	21.4	41.0	9.1	8.9	29.0	48.2	6.4	0.3
K. sima (n=1)	24.0	53.7	12.0	12.0	34.1	61.3	9.6	0.4
M. bidens (n=1)	25.0	53.1	6.6	9.3	33.0	59.0	4.4	
Mesoplodon sp.*	19.4	48.5	5.0	9.0	28.4	49.8	3.6	0.2

Notes:

Sponsor: ONR
Status: ONGOING

Blood oxygen conservation in diving sea lions: How low does oxygen really go?

Paul J. Ponganis
Scripps Institution of Oceanography / pponganis@ucsd.edu / 858 822 0792

Background

The physiology of deep (> 300 m) dives of California sea lions (*Zalophus californianus*) is relevant to the development of the sea lion as a model to investigate deep diving physiology and the avoidance of decompression sickness in a marine mammal. Such a model is essential to better understand the etiology of the stranding of beaked whales after exposure to naval sonar as well as to evaluate the value and accuracy of the many numerical models of diving physiology and gas uptake and distribution in these animals. This project focuses on the relationship of blood oxygen depletion patterns during dives to heart rate and muscle workload.

Objectives

1. Compare anterior vena cava (AVC) and posterior vena cava PVC) oxygen depletion profiles during dives to examine the relationship of oxygen depletion to heart rate and flipper stroke rate, and also evaluate for arteriovenous (a-v) shunting and the potential of a central venous oxygen store to maintain arterial oxygenation during “lung collapse” at depth.
2. Determine magnitude of blood oxygen depletion in deep dives.
3. Examine heart rate regulation and the relationship of heart rate to stroke effort and depth.

Methods

Backpack data recorders (partial pressure of oxygen (P_{O2}), electrocardiogram (ECG), tri-axial acceleration) were applied to sea lions on San Nicolas Island (CA) to investigate heart rate, flipper stroke effort and blood oxygen depletion in the anterior and posterior venae cavae during dives at sea. P_{O2} data were converted to hemoglobin saturation data with O_2-hemoglobin saturation curves.

Results

Fig.1 provides an overview of heart rate, stroke rate, and blood oxygen depletion in the aorta, AVC and PVC during deep dives. These profiles are consistent with a) a-v shunts, lack of muscle blood flow and arterialization (high oxygen saturation) of both venae cavae prior to dives and in early descent, b) severe hypoperfusion of the posterior body but maintenance of cerebral blood flow and a-v shunts into the AVC during the low heart rates of the later part of descent, c) reperfusion of tissues during the increased heart rate and stroke rate of ascent, and d) incomplete blood oxygen store depletion (45% decline) during the deepest, longest dives of sea lions. Furthermore, some gas exchange must still occur during the estimated period of lung collapse as oxygen profiles in both venae cavae are not high enough to maintain aortic saturation during lung collapse.

Fig.1. **Representative heart rate, stroke rate, and blood oxygen saturation (S_{O2}) profiles in the aorta, anterior vena cava and posterior vena cava during deep dives by different sea lions to 400-m depth.**

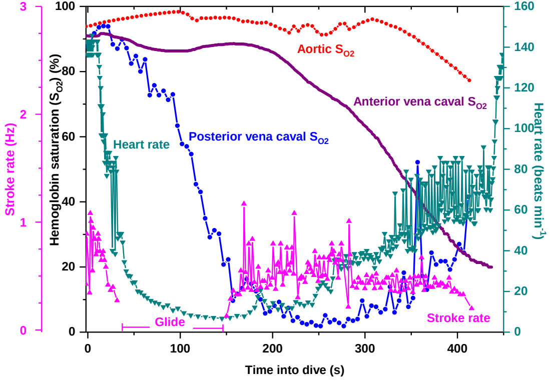
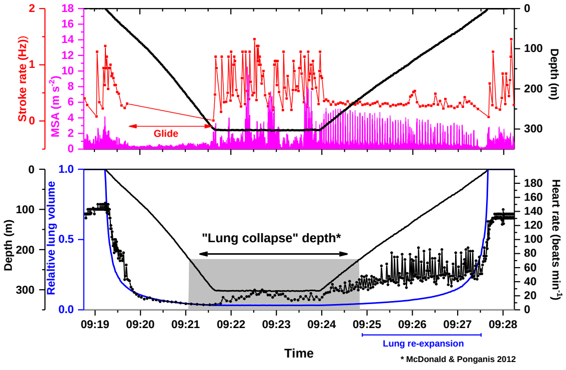


Fig. 2 illustrates the relationship of heart rate to two indices of stroke effort (stroke rate, and minimum specific acceleration (MSA)) and to relative lung volume during a deep dive of a sea lion. The heart rate profile most closely resembled the change in relative lung volume. Heart rate had only a weak correlation with stroke effort; in addition, the instantaneous heart rate and stroke effort profiles did not reflect each other. From a review of neuroregulation of heart rate, it was concluded that these changes in heart rate were primarily controlled by the parasympathetic nervous system via the dive response with additional input from lung stretch receptor reflexes, exercise, and volitional control.

Fig. 2. **Stroke rate, minimum specific acceleration (MSA), relative lung volume, heart rate and depth profiles during a 305-m deep, 8.5-min dive of a sea lion. Depth of lung collaped estimated from McDonald & Ponganis 2012.**



In addition, the project provided data for a publication on blood pH regulation and anesthesia in sea lions. Use of project recorders also allowed evaluation and planned publication of the ECG and heart rate of three mid-sized whales in captivity (killer whales, beluga whales, pilot whales).

Fig. 3. **The relationship of blood pH to the partial pressure of carbon dioxide (P_{CO2}) in anesthetized sea lions.**

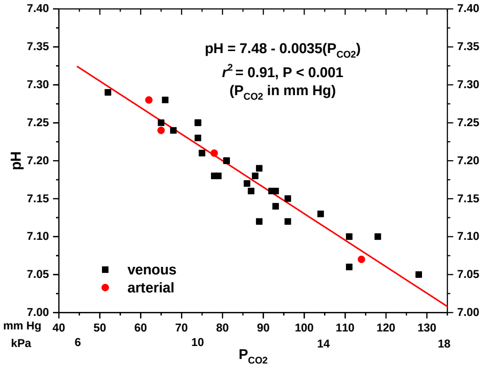
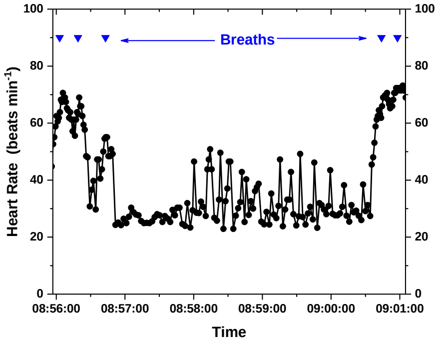


Fig.4. **Heart rate during a 4-min dive of a beluga whale. Similarity of the oscillations in heart rate to those of sea lions in Figs. 1 and 2 emphasize the value of the sea lion as a model for diving physiology research.**



Publications:

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PONGANIS, P. J., MCDONALD, B. I., TIFT, M. S., GONZALEZ, S. C., DAVALLE, B., GLINIECKI, R. A., STEHMAN, C. C., HAUFF, N., RUDDICK, B. & HOWARD, R. 2017a. Effects of inhalational anesthesia on blood gases and pH in California sea lions. *Marine Mammal Science*.

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Notes:

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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

Laura A. Thompson[1], Tracy A. Romano[1]
[1] *Mystic Aquarium, a division of Sea Research Foundation, Mystic CT 06355*
LThompson@mysticaquarium.org, (860) 572-5955 ext. 155

Background

The development of decompression sickness in humans is linked to activation of immune responses (Brenner *et al.,* 1999), particularly complement and inflammatory processes (Ward *et al.,* 1987; Nyquist *et al.,* 2004; Barack and Katz, 2005; Montcalm-Smith *et al.,* 2007), in response to the presence of gas bubbles during decompression.

The extent to which bubbles may occur, or under what circumstances bubbles may be symptomatic vs. silent, is unknown in naturally diving marine mammals. Recent work however, suggests marine mammal dive behavior may allow bubble formation ((e.g. supersaturation of tissue nitrogen in sperm whale (Jepson *et al.,* 2003), bottlenose dolphins, beaked whales and blue whales (Houser *et al,* 2001; modelling efforts predicting bubble growth with repeated dives, short surface intervals (Zimmer and Tyack, 2007)).

Should bubbles be more common than previously thought, adaptive non-responsiveness of the immune system has been suggested to be one mechanism by which marine mammals avoid the development of injury (Fahlman *et al.,* 2006). This project aims to address this hypothesis by investigating the effects of nitrogen (N2) bubble exposures on immune function and activation of inflammatory responses in marine mammals.

This project builds on previous ONR funded research (award No # N00014-13-1-0768) which demonstrated that a physiological stress response, or altered dive behavior, (potential consequences of anthropogenic sound exposure) can modulate the response of marine mammal immune cells to increased pressure (simulated dives; Thompson and Romano, 2015; 2016; *in prep*). While adding to our understanding of marine mammal dive physiology, this study is directly relevant to understanding non-lethal, chronic effects of sound on marine mammal health.

Objectives

The objective of this project is to evaluate whether adaptation of immune responses is a viable mechanism through which marine mammals avoid pathologies related to bubble formation following decompression. Specifically, this will be accomplished by;

1) *Measuring phagocytosis and respiratory burst, as well as granulocyte activation, in marine mammal blood samples following in vitro exposure to* N_2 *bubbles.*
2) *Monitoring complement activation in marine mammal blood samples following in vitro exposures to* N_2 *bubbles.*
3) *Evaluating the role of exercise modulation on the response of immune cells and complement activation to in vitro exposures to* N_2 *bubbles.*
4) *Validating the presence of complement proteins in blow for potential use in monitoring immune status in belugas.*

Methods

Beluga samples were obtained from animals resident at the Mystic Aquarium between 2009 and 2016, as well as from the Bristol Bay, AK population during live capture/release health assessments in 2014 and 2016. Harbor seal samples were obtained from animals admitted to the Animal Rescue Program at Mystic Aquarium between 2015 and 2016.

All N2 bubble exposures were done *in vitro* using a micro-flowmeter to monitor flow rates.

Phagocytosis and respiratory burst were measured using the protocol detailed in Spoon and Romano (2012) which was developed for use with marine mammal samples at the Mystic Aquarium. A commercial mouse-anti-canine antibody for CD11b, previously used within our lab, was used to monitor expression of this protein as a marker of granulocyte activation. C5a was measured using a commercial ELISA based kit, produced for this project by MyBioSource.com using harbor seal protein, in order to evaluate complement activation and validate the presence of C5a in beluga blow samples.

One beluga at Mystic Aquarium was successfully trained to participate in active and stationary dive sessions, of up to 3 minutes duration, prior to sample collections.

Results

Twelve N2 bubble exposures, including three durations (30 minute, 3 minute, 30 seconds) and four gas flow rates (0.02 ml/min, 0.06 ml/min, 0.5 ml/min and 1 ml/min) were targeted. This abstract will focus on the 30 minute exposures.

OBJECTIVE 1: Phagocytosis and respiratory burst, as well as CD11b expression have been measured in belugas (n=2) and healthy harbor seals (n=5) following N2 exposures. No statistical analyses can be run on beluga data due to the small number of animals. Additional samples have been requested from other facilities. While phagocytosis results for belugas display large variability both within and between individual whales, overall results suggest slight increases in both phagocytosis and respiratory burst following N2 exposures. No apparent pattern in CD11b expression was noted for belugas. No statistically significant changes were detected in harbor seal phagocytosis, respiratory burst or CD11b expression following any of the 30 minute N2 exposures (GLM; $\alpha=0.05$).

OBJECTIVE 2: Changes in C5a, as an indication of complement activation following N2 exposures were measured in aquarium belugas (n=7), free ranging Bristol Bay belugas (n=9) and stranded harbor seals at the time of admit to rehabilitation and again at release (n=9). Aquarium belugas showed no significant changes in C5a following N2 bubble exposures, while Bristol Bay animals showed a small but significant increase in C5a only following exposures to a N2 flow rate of 1 ml/min (GLM; p=0.019). Bristol Bay animals also displayed larger changes in C5a following N2 exposures than aquarium belugas. Harbor seals displayed no significant changes in C5a for admit samples, but a significant increase in release samples following N2 exposures to 0.02 ml/min (GLM: p=0.045). Large changes, and variation, were also noted in release samples for flow rates of 0.06 and 0.5 ml/min.

OBJECTIVE 3: A single beluga at Mystic Aquarium has been successfully trained to perform active and stationary dives for up to 3 minutes. Blow samples were obtained pre-dive, immediately post-dive and 1 hr post-dive for 5 replicates of active diving, and 4 replicates of stationary diving. Due to animal health concerns, blood samples were only drawn immediately post-dive, and this has been carried out for 2 active dives and 1 stationary dive. An additional stationary dive is scheduled for September; with another round of paired active/stationary dives planned for Fall 2017.

Preliminary results suggest higher phagocytosis and respiratory burst activity following active dives (n=2) as compared with a baseline average, as well as greater N2 bubble induced changes in phagocytosis, respiratory burst and CD11b expression following active dives as compared with stationary dives.

Preliminary C5a data has been run for a single pair of active/stationary dives. Serum C5a immediately following dive behaviors is similar to baseline measures for this animal, however increased C5a was detected following N2 exposures and this change appears larger than baseline aquarium belugas, Bristol Bay belugas, and the individuals own baseline response. Additionally, C5a in blow remained constant before and following the active dive, while increased C5a was detected immediately and 1 hr following the stationary dive. Approximately 30 blow samples have been collected for this aim and will be run following further validation of C5a kits as per objective 4.

OBJECTIVE 4: The C5a kits being utilized for serum are being tested for use with blow samples. Initial tests suggested C5a is present in blow. Due to the variability in sample volumes obtained, increments of 5-10µl per well were tested up to the recommended 50µl, as per assay protocol. The % variability of C5a measures in these series was 14.27%, indicating that even low volume samples (<100µl or 50µl per well in duplicate) could provide useful information. C5a measures in blow samples show a significant positive correlation with blood C5a ($r_s=0.824$, p=0.001) however, blow C5a is not always less than that measured in serum. We propose this may be due to 1) dynamics of C5a in blood vs. blow, 2) in situ production of C5a in the lungs, or 3) unknown interference in the assay. Further validation steps are underway before final analysis of blow samples from objective 3.

Notes:

Full C5a data (Objective 2) are currently being analyzed and
a manuscript on the complement response to N2 bubbles is currently *in prep.*

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Sponsor: ONR
Status: ONGOING

HIGH RISK BEHAVIORS IN MARINE MAMMALS: LINKING BEHAVIORAL REPOSNSES TO ANTHROPOGENIC DISTRUBANCE TO BIOLOGICAL CONSEQUENCES

Terrie M. Williams
University of California- Santa Cruz
williams@biology.ucsc.edu*, 831-459-5123*

Background

Over the past decade the number of mass stranding events involving marine mammals in U.S. waters has more than doubled and involved thousands of animals. Despite little understanding of how marine mammals respond to anthropogenic disturbance, oceanic noise and Navy sonar have generally been listed as causal factors in due to their potential to elicit unusual swimming behaviors and physical exertion by the animals. This explanation is problematic because we do not know how such “escape” behaviors push an animal so far past normal exertion levels and physiological capabilities that they strand due to exhaustion, tissue injury or both. Only after we have determined the physiological costs, risks and safety factors associated with extreme physical performance while submerged will we be positioned to make the connection between “risky” abnormal behaviors, “loud oceanic noise,” sub-lethal impacts, and ultimately stranding. Specifically, this project focuses on the physiological costs and potential risks of three common responses by cetaceans to oceanic noise, 1) high-speed swimming, 2) elevated stroke frequencies, and 3) rapid ascent from depth. We are testing the central hypothesis, that **extreme behaviors requiring marine mammals to perform outside of preferred physiological states represent a risk to metabolic and cardiovascular homeostasis during diving, and account for species-specific vulnerabilities to anthropogenic perturbation.** By combining data from previous studies as well as from ongoing experiments, we are providing a comprehensive evaluation of biological capacities and “safety zones” for diving marine mammals. We are developing key physiological metrics to identify those marine mammal species or specific attributes of different species that are associated with susceptibility to acoustically mediated disturbance and tissue damage. By identifying high risk and low risk behaviors, and the specific triggers for tissue injury and cardiac instability, we accomplish the overall objective of improving the protection of marine mammals during naval operations. Thus, the study is designed to help mitigate the current misperception regarding lethal and sub-lethal impacts of anthropogenic disturbance and related controversy regarding Navy sonar and other marine operations by providing a comprehensive understanding of how marine mammals specialized for swimming or diving maintain physiological homeostasis in the face of perturbation. This will allow us to achieve our overall goal of enabling Navy personnel to develop schedules for acoustic activities that take into account the likely lethal and sub-lethal effects on marine mammal populations.

Objectives

Four specific aims are being addressed:

1. Determine physiological costs and risks of high speed behaviors.

2. Evaluate species-specific costs and cardiovascular risks of high stroke frequency responses.

3. Determine physiological capacity and costs of rapid ascents by marine mammals

4. Integrate behavioral, energetic, and cardiac risk factors associated with preferred and maximum performance by cetaceans.

Methods

This study achieved the above goals by conducting a series of novel exercise experiments using custom-designed instrumentation to simultaneously monitor behavior, energetic costs, cardiac responses, stroking mechanics and depth on trained and wild cetaceans and pinnipeds. Stroking costs were determined for phocid seals, and odontocetes ranging in body mass from 42 kg harbor porpoises to 2738 kg killer whales by simultaneously measuring oxygen consumption using open-flow respirometry and 3-D acceleration in animals performing trained and free-ranging swimming and diving behaviors.

The data were subsequently used to model the cost of escape by two deep diving wild cetaceans, beaked whales exposed to simulated sonar (Williams *et al*., 2017) and wild narwhals escaping from net entanglement and stranding (Williams *et al.*, submitted).

Results

Comparative evaluation of the metabolic cost of stroking in large and small cetaceans.

We completed our analyses of stroking costs for cetaceans varying in body mass, style of swimming, preferred depths, and level of exercise. Stroking costs determined for odontocetes ranging in body mass from 42 kg harbor porpoises to 2738 kg killer whales varied with swimming gait pattern. For the odontocetes examined, the energetic cost per stroke was not constant as reported for stepping costs in terrestrial mammals. Rather, it changes with stroke frequency, speed, and body mass. The locomotor cost of stroking (LC), which separates the energy expended for maintenance functions from the energetics of moving the flukes, was described by two allometric regressions based on preferred swimming and maximum aerobic performance levels (Williams *et al.,* 2017). Because not all strokes have similar costs, this finding has marked implications for the energetic cost of flight by cetaceans exposed to unanticipated noise.

Predicting the physiological cost of beaked whales exposed to anthropogenic noise.

An important objective of this research was to develop an integrative physiological model incorporating the elements of a fear/escape response in cetaceans to whales exposed to simulated Naval sonar. Our initial energetics model focused on beaked whales, a species of concern due to its sensitivity to oceanic noise. Using the allometric regressions for stroking costs described above, we examined how the energetic cost of a dive changes prior to and after exposure to anthropogenic noise in the Cuvier's beaked whale (*Ziphius cavirostris*) exposed to experimental mid-frequency sonar. (Fig. 1). Predicted stroking costs were applied to accelerometer–depth data from previous studies for dives prior to and after exposure to sonar, and revealed the high energetic cost of escape dives. The model is currently being used to evaluate how flight responses of other species of deep-diving cetaceans affect overall energetic cost of diving and aerobic dive limits inherent in PCoMS models.

Assessment of the relationship between cardiac variability and kinematics in deep-diving cetaceans. We developed and successfully deployed submersible, kinematic-linked ECG recorders on a wild, deep-diving cetacean (Fig. 2), the narwhal (*Monodon monoceros*). Following a comprehensive analysis of the ECG responses of 9 animals and deployment records of up to 50 hours in duration, we have found that the aerobic cost of escape dives often exceeds the aerobic dive limits of the animals (Williams *et al*., submitted, Fig. 3). This collaboration with Mads Peter Heide-Jørgensen (Greenland Institute of Natural Resources), and Susanna Blackwell (Greenridge Sciences Inc.), together with collaborations with Ari Friedlaender (Oregon State University) and Brandon Southall (SEA, Inc., and UCSC) have enabled us to develop a comparative kinematic and cardiac data-base for a wide variety of cetacean species. These analyses are being used as a physiological baseline for studies planned with free-ranging cetaceans exposed to unanticipated noise. Together, the studies demonstrate the capacity of these deep diving cetaceans to respond to anthropogenic impacts in their environements.

Notes: Resulting Publications

Williams, T.M., Blackwell, S., Richter, B., Sinding, M., Heide-Jørgensen, M.P. In review. Paradoxical escape responses by narwhals (*Monodon monoceros*) when diving in a seascape of fear. For **Science**.

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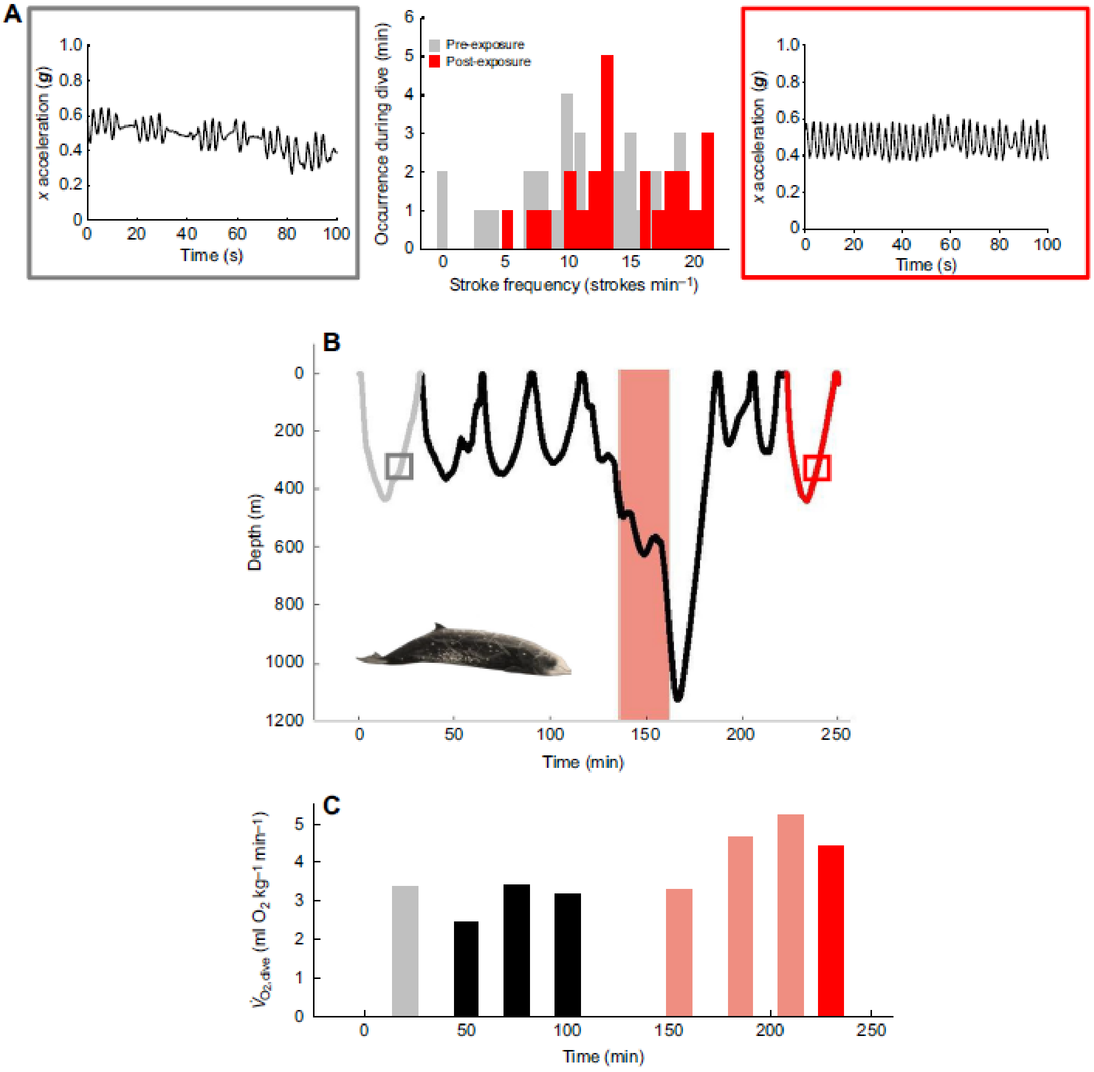

Figure 1. The effects of unanticipated noise on beaked whales. Kinematic (A), behavioral (B) and energetic (C) responses to exposure to mid-frequency sonar by a diving Cuvier's beaked whale are shown. Panel B shows the 4-h sequential dive pattern of an adult beaked whale before, during and after exposure to sonar (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 panel B correspond to the colored boxes in A which show the prevalent stroking gait pattern from an accelerometer tag during each period. The middle graph in A illustrates the shift in the range of stroke frequencies for these two matched pre- (grey) and post-exposure (red) dives. Bar height corresponds to the occurrence of each level of stroke frequency (calculated per minute) during the entire dive. In C, the calculated rate of oxygen consumption in relation to sequential dive time as shown in B is compared for pre-exposure dives (grey and black bars), and dives taking place during and immediately after exposure (pink bars), and nearly 2 h post-exposure (red bar). (From Williams et al., 2017).

Fig. 2. Deployment of a submersible ECG-accelerometer monitor on free-ranging narwhals.

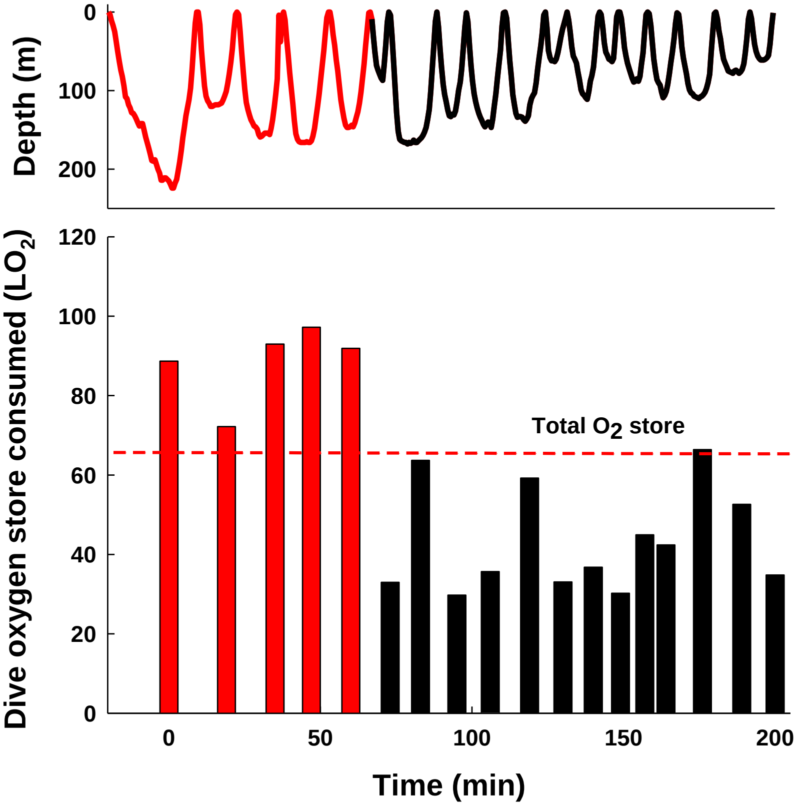

Figure 3. Energetic costs for escape reactions by narwhals. The estimated oxygen consumed during individual dives (lower panel bars) is shown for sequential escape (red) and post-escape (black) dives (upper panel) following release of an adult female narwhal (907F). The horizontal dashed red line denotes the total oxygen store calculated for the narwhal.