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The Metabolic Costs of Sound Production in Odontocete Cetaceans

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LONG-TERM GOALS

Animals often increase the amplitude (the Lombard effect), duration, and/or repetition rate of their acoustic signals as a strategy to help reduce the probability of masking from environmental sounds (NRC 2003). Although accumulating evidence from recent research (Scheifele et al. 2005, Holt et al. 2009, Parks et al. 2010) illustrates that several marine mammal species readily modify the parameters of their acoustic signals to compensate for masking noise, potential energetic costs of such compensation behavior are unknown. To our knowledge, there is no empirical data on the metabolic cost of sound production for any marine mammal species. Given that changes in vocal behavior in response to masking noise has been documented in several species, assessing the biological significance of these effects is paramount but also very difficult given the life histories of marine mammals. The Population Consequences of Acoustic Disturbance (PCAD) model has been proposed as a framework to address this challenging task (NRC 2005). Data on the energetic cost of dolphin vocalization from this study can be used to assess the biological significance of vocal compensation in response to sound exposure and populate transfer function 2 (transfer function between behavior change to life functions immediately affected) in the PCAD model.

OBJECTIVES

For the first year of this study (Phase 1), oxygen consumption was measured in two captive bottlenose dolphins during sound production of social signals and compared to resting metabolic rates (RMRs) and metabolic costs of other activities, such as performing surface active behaviors (SABs) and/or swimming. The phase of this work was completed in 2010 and results were reported in our ONR FY11 report. For the second year of this study (Phase 2, Jan – Dec 2011), we aim to measure oxygen consumption in these individuals while they produce the same type of sounds but at different levels and/or durations. This work was completed at the end of the calendar year 2011. These measurements

will quantify the potential metabolic cost of vocal compensation as an anti-masking strategy in response to anthropogenic sound exposure.

APPROACH

The metabolic cost of sound production is being measured in two captive male Atlantic bottlenose dolphins (*Tursiops truncatus*) maintained at Dr. Terrie Williams' Mammalian Physiology Laboratory at the University of California, Santa Cruz, Long Marine Laboratory. These individuals were trained by Traci Kendall (Program Manager/Research Training Supervisor) and Beau Richter (Head Trainer) to produce sounds on command while stationed under a metabolic hood to measure oxygen consumption. For Phase 2 of the study, the dolphins have also been trained to produce relatively higher and lower amplitude sounds of the same type on command using two different discriminative stimuli or cues (one for "loud" and one for "quiet"). The sounds of free-ranging Atlantic bottlenose dolphins have been described as clicks, whistles, buzzes, quacks, and pops (Jacobs et al. 1993). The trained sounds of the captive dolphins of the current study are representative of those found in wild, free-ranging populations.

Experimental trials are conducted in the morning. The dolphins are fasted overnight before experimental trials to eliminate the potential for the metabolic cost of digestion to confound oxygen consumption measurements. Thus, food rewards are given after the experimental trial is complete and only one experimental trial is conducted per dolphin per day. Briefly, each experimental trial consists of one dolphin remaining at the water surface under the metabolic hood (details described in next paragraph) for one 10-minute period of rest (to determine baseline metabolic rate), followed by two consecutive one-min bouts of sound production (the two bouts are separated by 15-20 sec of silence), and concluding with a recovery period (at least 10 minutes, or until oxygen consumption values return to resting values). For Phase 2 of the study, either "loud" or "quiet" trials are predetermined before the start of the trial in which the dolphin is asked to produce "loud" or "quiet" sounds during the vocalization period. Both trial types are run within one week's worth of data collection for each dolphin subject so that any seasonal effects of metabolic rates are not confounded with different trial conditions. During all trials, the dolphins are acoustically monitored in real-time and their sounds are recorded for further analysis as described below. The total duration of the rest period, sound production period, and recovery period are recorded for each experimental session. Respirations are also recorded during each of the three periods so that respiration rates can be calculated for the dolphins during rest, sound production, and recovery. The dolphin's behavior during each trial is also video recorded to ensure that body movement is keep to a minimum during all trial periods (baseline rest, vocal period, recovery). See figure 1 for a photograph taken during one experimental session.

Figure 1. Photograph taken during one experimental session showing the equipment set-up which includes the metabolic hood, the dolphin stationed under the metabolic hood, the acoustic recording equipment and operator, the dolphin trainer, and the assistant taking notes and recording respirations. During the trial the dolphin is acoustically monitored and all respirations are recorded during each of the three periods.

The method being used for determining metabolic rates from oxygen consumption values are similar to those used previously on bottlenose dolphins (Williams *et al.* 1993, Noren et al. 2011). For this study, the rate of oxygen consumption ($\dot{V}o_2$) is being determined for quiescent dolphins stationed at the water surface and for the same dolphins producing sounds at the water surface. Air is drawn into the hood at a flow rate of 300 L min^{-1}. The flow rate is maintained such that the content of oxygen in the hood will remain above 20%. Water and CO_2 from subsamples of excurrent air from the hood are absorbed using Drierite and Baralyme, respectively, prior to entering the oxygen analyzer. The percentage of oxygen in the sample line is monitored continuously using the FMS field metabolic rate system (Sable Systems International) and recorded by a laptop computer every second during the experimental sessions. $\dot{V}o_2$ for resting and vocalizing dolphins are calculated from the percentage oxygen data by respirometry software (Expedata data acquisition and analysis software, Sable Systems International). For each experimental trial, "baseline rest" MRs were calculated by averaging $\dot{V}o_2$ during the most level 5 min (determined by the "level" function in Expedata) of the last 8 min of the baseline resting period. Metabolic rates (MRs) during the 2 min of vocal bouts were calculated by averaging $\dot{V}o_2$ from the beginning of the first vocal bout to the end of the second vocal bout. Average MRs during the first 2 min of the recovery period (hereafter referred to as the "2-min post vocal bouts") were also calculated for comparison. "Recovered" MRs were calculated by averaging $\dot{V}o_2$ during the most level 5 min (determined by the "level" function in Expedata) of the recovery period. The total metabolic

cost of sound production (sound production costs plus recovery costs) above resting values and total recovery time were calculated by an automated macro analysis, specifically developed for this study. As stated previously, the primary focus of Phase 2 was to investigate the metabolic cost of modifying vocal performance. Consequently, trial components are only compared across the two trial types rather than statistically comparing trial components within each of the two trial types, as was done in Phase 1 of the study. Respiration and oxygen consumption data are compared across trial types using one way repeated measures analysis of variance or one way repeated measures analysis of variance on ranks when normality and/or equal variance tests fail. When results are significant, pairwise comparisons are made using the Holm-Sidak method for repeated measures ANOVA and the Tukey Test for repeated measures ANOVA on ranks. The low sample size of trials combined with the high variability of oxygen consumption values obtained from dolphins (due to apneustic breathing patterns and other factors) often resulted in statistical results with low power. Because of this, a p-value of 0.10 was considered to be the critical statistical level of significance to avoid erroneously concluding that results were insignificant when trends were present. Dr. Dawn Noren is responsible for collecting and analyzing the respiration rate and oxygen consumption data.

Sound production during all trials is acoustically monitored in real-time and also recorded using calibrated equipment to quantify the received sound pressure level (SPL in dB rms re: 1 μPa), duration (in sec), repetition rate (phonations/min), and received acoustic energy of the phonations of the dolphins. A contact hydrophone is placed on the dolphin's melon during trials to carefully quantify the received SPL of sounds. This method is being used because the dolphin is stationed at the air-water interface under the hood and small changes in dolphin position can affect how much sound energy is transmitted under water. This allows comparisons between trials and experimental conditions. The recording equipment includes two calibrated Reson hydrophones. One is positioned in the pool as a monitoring hydrophone (Reson TC 4033) and the other is molded into a small suction cup for contact (TC 4013). The position of both hydrophones is always the same among trials and trial periods (rest, sound production, and recovery). Both hydrophones are connected through a series of filters and amplified (Reson VP 2000) and digitized using the MOTU traveler at a sampling rate of 96 kHz and then recorded (2 channels) and monitored in real-time in the time and frequency domain. Calibration is checked through the entire recording chain on a regular basis with a pistonphone connected to a custom adaptor (42AA with RA78, GRAS Sound & Vibration). Hydrophone placement is the same during all periods (rest, sound production, and recovery) of each experimental session. All sounds produced during trials are analyzed using Avisoft SASlab Pro (v5.1.17). A high pass filter at 1.5 kHz and 2 kHz for trials run with Puka and Primo, respectively, is first applied to the recordings to reduce low frequency extraneous sounds (breaths and water sounds) that occur below the frequency range of dolphin vocalizations. Then, the automated measurement option is used to window each vocalization during a trial period. These windows are manually checked and modified as needed. A number of acoustic parameters are measured in both the time and frequency domains for each vocalization, and these values are averaged across the entire vocalization period for each trial. Means of these means are then calculated and summarized. Dr. Marla Holt is responsible for collecting and analyzing the acoustic data.

WORK COMPLETED

Data collection for Phase 2 of the study was completed in 2011. Data were collected over six, one week periods in Dr. Williams' Lab in which 60 trials have been conducted (30 trials per dolphin).

Metabolic, respiration, acoustic, and video data have been preliminarily analyzed and are discussed in the next section.

RESULTS

During the study, each dolphin produced the same sound type during his vocal bouts, but the sounds produced were qualitatively different between the two dolphins. Specifically, Primo produced a whistle while Puka produced what we describe as a pulsed squawk or squeak-like sound as illustrated in the spectrograms of Fig. 2. Puka's pulsed sound is similar to the quack sounds described by Jacobs et al. (1993).

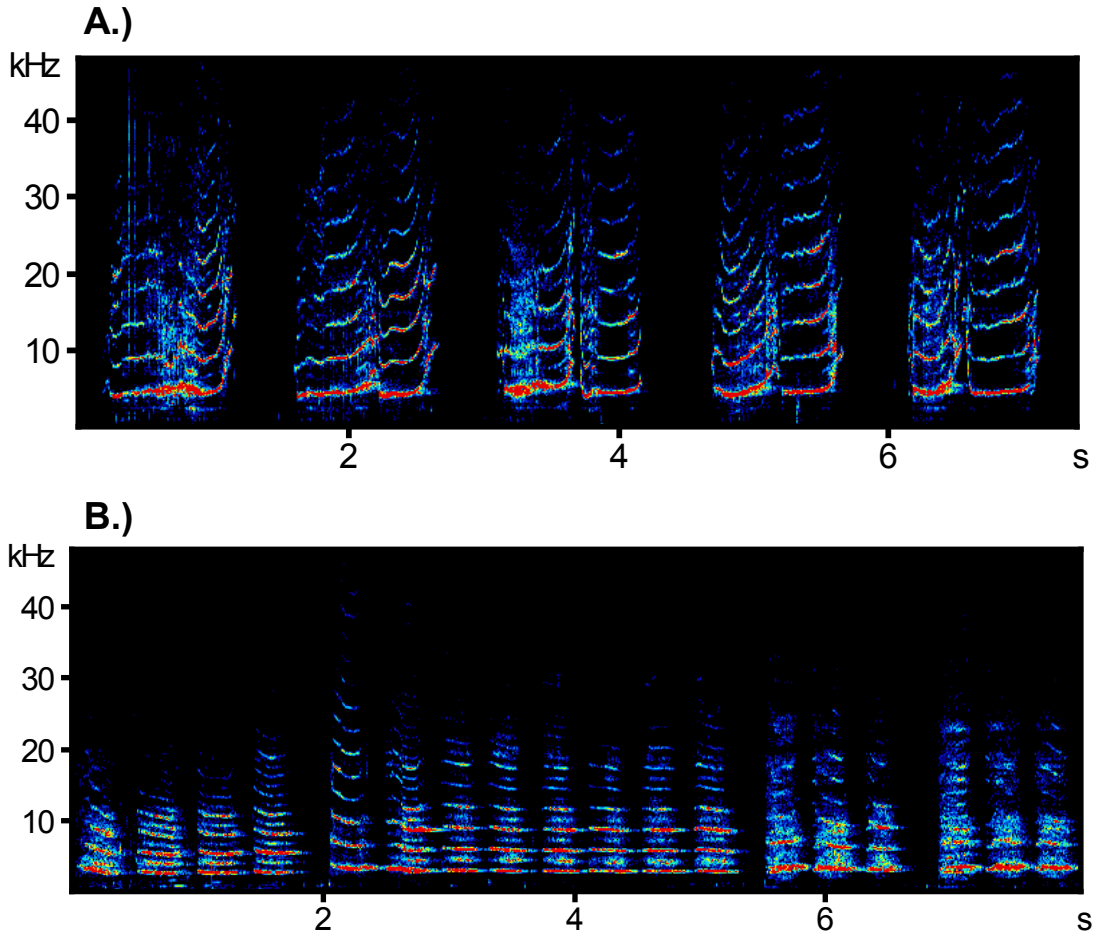

Figure 2. Spectrograms showing 8 second examples of vocalizations performed by A.) Primo which are five whistles and B.) Puka which are 18 pulsed squeak-like sounds. Both spectrograms show visual representations of vocalizations performed during oxygen consumption data collection with time from 0-8 seconds on the x-axis and frequency from 0-48 kHz on the y-axis. The colors denote relative level or amplitude differences with red indicating higher levels and blue indicating lower levels.

A total of 27 and 29 trials for Primo and Puka, respectively are being included in the analysis. Vocal performance between the two trial types for each dolphin are shown in Table I. The average received SPL difference between "quiet" and "loud" trials was 10.7 dB for Puka, but only 4.6 dB for Primo. Although efforts were made to train each dolphin to produce louder vocalizations during "loud" trials while keeping the number of vocalizations, duration, and repetition rate constant between trial types, both dolphins had a tendency to lengthen their vocalization during "loud" trials as well. Primo also produced more vocalizations during his "loud" trials while Puka produced fewer vocalizations, on average, during his "loud" trials. To account for these differences in vocal performance between trial types, an analysis of sound energy integrated over the entire vocal period within each trial (quantified as cumulative sound exposure level in dB re 1 μPa^2*s) is warranted in addition to exploring increased amplitude or other vocalization parameters as separate effects on metabolic rates and total metabolic cost. The average cumulative received SEL difference between "quiet" and "loud" trials was 5.8 dB for Primo and 10.0 dB for Puka (Table I).

Table I. Vocal performance between the two trial types for each dolphin

Subject	Trial type	Mean no. voc	Mean duration (sec)	Mean interval (sec)	Mean received SPL (dB re 1 μPa)	Mean Cumulative received SEL (dB re 1 μPa^2*s)	N
Primo	Quiet	54.6	1.16	2.37	110.9	130.2	14
	Loud	60.0	1.30	2.17	115.5	136.0	13
	Difference:	5.4	0.14	-0.19	4.6	5.8	
Puka	Quiet	209.4	0.20	0.57	109.9	129.9	14
	Loud	189.7	0.29	0.64	120.5	139.9	15
	Difference:	-19.6	0.08	0.07	10.7	10.0	

As was demonstrated in Phase 1 of the study, the oxygen consumption data (ml O2 min^{-1} kg^{-1}) from Phase 2 also show that both types of vocalizations impact a measurable metabolic cost to dolphins and that recovery to baseline levels occurs gradually after the vocalization period ceases (Figs. 3, 4). For Primo, even though MRs (ml O_2 min^{-1} kg^{-1}) measured during the 2 min vocal bouts and 2 min post vocal bouts appear to be higher during the "loud" trials, there were no statistically significant differences in any of the four trial components ("baseline rest", 2 min vocal bouts, 2 min post vocal bouts, and "recovered state") across the two trial types ("quiet" and "loud", Fig. 3). It is interesting to note that the difference in MRs during the 2 min post vocal "quiet" and "loud" bouts was only marginally insignificant ($P = 0.130$), and the power of the test was low (0.208). For Puka, even though MRs measured during the 2 min vocal bouts and 2 min post vocal bouts both appear to be higher during the "loud" trials, the only significant difference across the two trial types was during the 2 min post vocal bouts ($P = 0.065$, Fig. 4). Respiration rates during loud vocal bouts were significantly greater than respiration rates during quiet vocal bouts for Puka ($P = 0.068$), but not for Primo. The total oxygen consumed above resting values during the vocal bouts plus required recovery duration were greater during "loud" trials, but this was only significant for Puka ($P = 0.079$). Indeed, Puka's total metabolic cost (ml O_2) of "loud" vocal bouts was nearly double that of quiet vocal bouts. The required recovery duration following loud vocal bouts was also nearly double that of the required recovery duration following quite vocal bouts for Puka ($P = 0.006$). Results from the video analysis showed that there were no significant differences in dolphin posture, the number of fluke beats, or the

intensity of fluke beats across trial types for either dolphin. Thus, the increase in oxygen consumption is likely due to increased metabolic costs associated with the modification of the acoustic signals. Additional analyses will be conducted to assess the factors contributing to differences in metabolic costs that are related to differences in vocal performance. The ultimate goals are to assess whether the metabolic cost of sound production and vocal compensation are biologically significant.

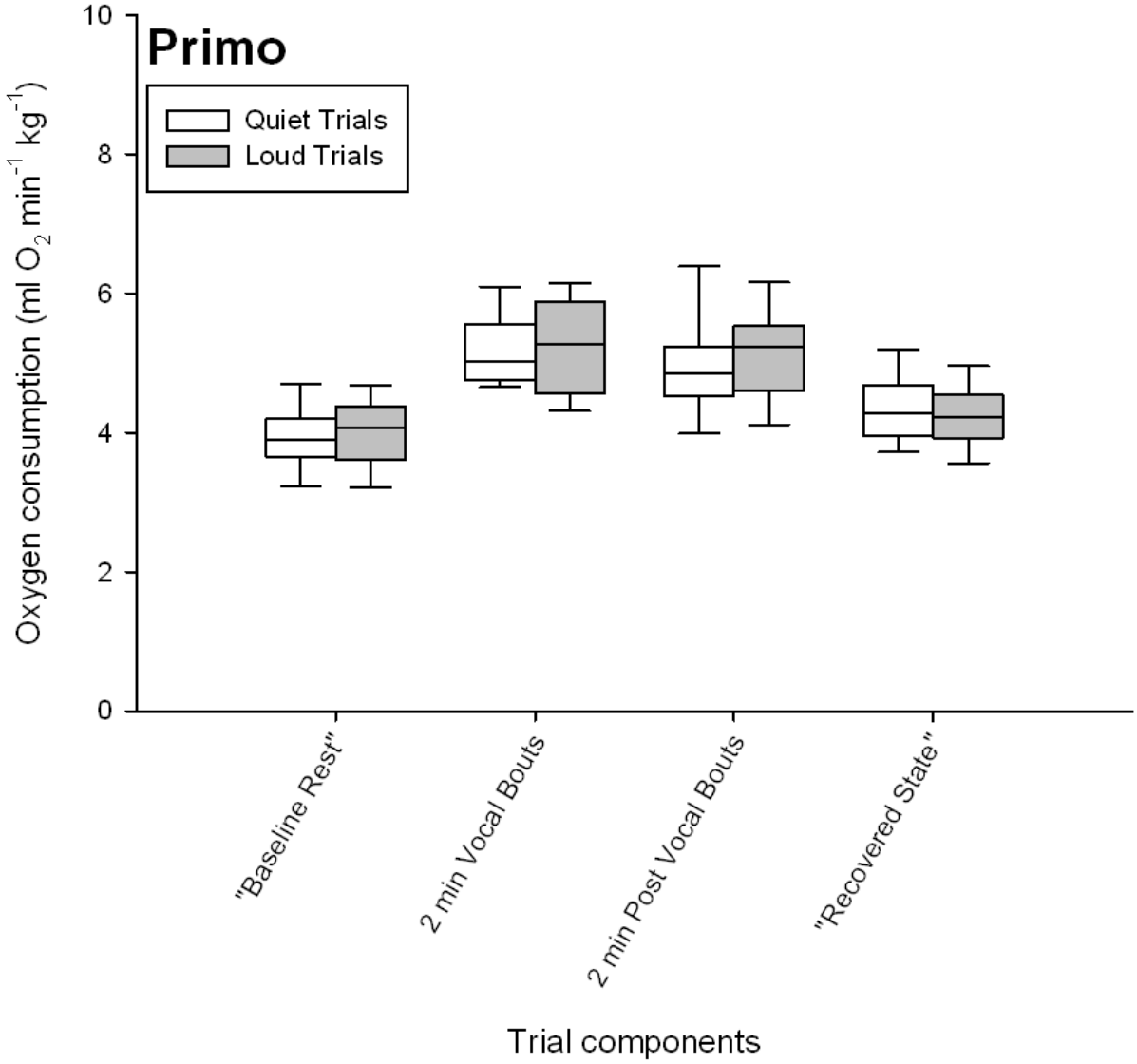

Figure 3. Oxygen consumption (ml O_2 min-1 kg-1) measured during four components of fourteen and thirteen quiet and loud experimental trials, respectively, for Primo. Quiet and loud trials are designated by white and gray bars, respectively. For each box plot, the boundary of the box closest to zero indicates the 25th percentile, the solid line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers (error bars) above and below the box indicate the 90th and 10th percentiles, respectively. During both quiet and loud trials, oxygen consumption tended to increase during vocal bouts and remained elevated for at least 2 min post vocal bouts. There were no significant differences in oxygen consumption values across quiet and loud trials for any of the four trial components (baseline rest, 2 min vocal bouts, 2 min post vocal bouts, recovered state).

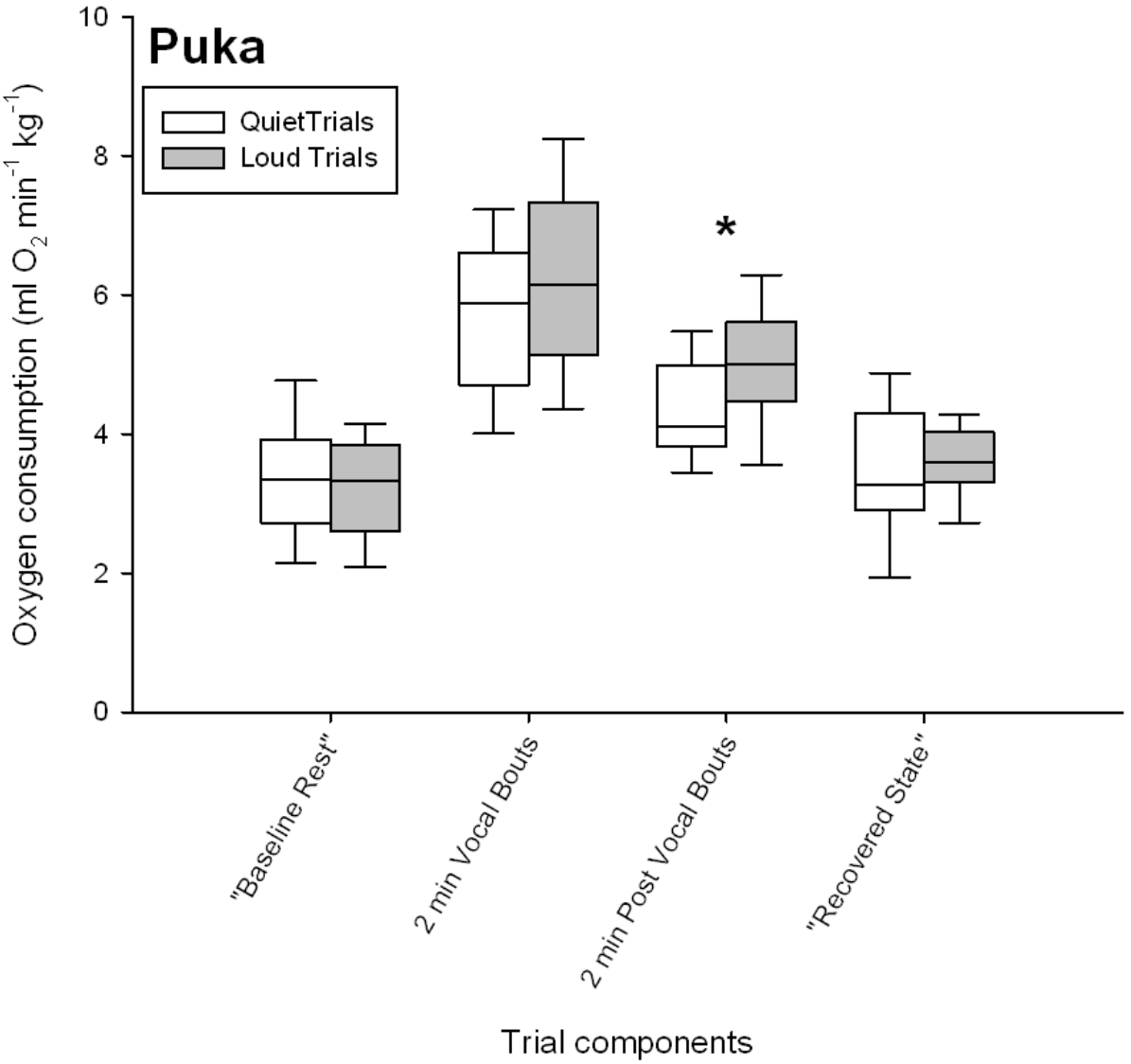

Figure 4. Oxygen consumption (ml O_2 min^{-1} kg^{-1}) measured during four components of fourteen and fifteen quiet and loud experimental trials, respectively, for Puka. Quiet and loud trials are designated by white and gray bars, respectively. For each box plot, the boundary of the box closest to zero indicates the 25th percentile, the solid line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers (error bars) above and below the box indicate the 90th and 10th percentiles, respectively. Asterisks designate significant differences ($P < 0.10$) across trial type during trial components. During both quiet and loud trials, oxygen consumption tended to increase during vocal bouts and remained elevated for at least 2 min post vocal bouts. Oxygen consumption measured for two min post vocal bouts during loud trials were significantly greater than those measured for two min post vocal bouts during quiet trials.

IMPACT/APPLICATIONS

Currently, there is no empirical data on the metabolic cost of sound production in any marine mammal species. Theoretical assessments of such costs need to factor in variables such as efficiency factors and the relationships between physiological processes and metabolic costs associated with behaviors given that they often do not simply scale according to linear relationships. However, such data needed for theoretical modeling on this topic are also lacking. Empirical data collected from this study provide valuable information about sound production costs in odontocetes including costs of modifying acoustic signals in response to anthropogenic sound exposure. Analyses of the data from both phases of this study demonstrate that there is a measurable metabolic cost for bottlenose dolphins producing sound. Furthermore, preliminary analysis of data from Phase 2 suggest that modification of acoustic signals can impart an additional metabolic cost, but the significance of this cost is likely related to the magnitude of the change in vocal performance. For example, the difference in both mean received SPL and mean cumulative received SEL between the two trial types was nearly double in one dolphin compared to that of the other dolphin of the study. The associated increase in the total metabolic cost of loud vocal bouts, relative to quiet vocal bouts, was only significant for the dolphin with the greatest change in vocal performance. Thus depending on the magnitude of vocal modification, along with the duration of time that animals modify their vocalizations, there could be significant metabolic costs associated with vocal compensation in response to anthropogenic sound exposure. Depending on the extent of these costs, the energy balance of individuals may be impacted, which could, in turn affect survival and reproduction. This study will provide important input data to populate transfer function 2 in the PCAD model which can then be used to assess the biological significance of such responses to anthropogenic sound exposure.

RELATED PROJECTS

Dr. Terrie Williams' Marine Mammal Physiology Project involves other studies on the two dolphins used in this study. The goal of one related study is to assess the changing energetic demands in cetaceans, and in particular, determine the principle factors in regulating the variable metabolism of cetaceans over the seasons.
http://www.mmpp.ucsc.edu/The_Marine_Mammal_Physiology_Project/Home.html

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