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RESEARCH ARTICLE

Whistling is metabolically cheap for communicating bottlenose dolphins (*Tursiops truncatus*)

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ABSTRACT

Toothed whales depend on sound for communication and foraging, making them potentially vulnerable to acoustic masking from increasing anthropogenic noise. Masking effects may be ameliorated by higher amplitudes or rates of calling, but such acoustic compensation mechanisms may incur energetic costs if sound production is expensive. The costs of whistling in bottlenose dolphins (*Tursiops truncatus*) have been reported to be much higher (20% of resting metabolic rate, RMR) than theoretical predictions (0.5–1% of RMR). Here, we address this dichotomy by measuring the change in the resting O_2 consumption rate ($\dot{V}_{O_2}$), a proxy for RMR, in three post-absorptive bottlenose dolphins during whistling and silent trials, concurrent with simultaneous measurement of acoustic output using a calibrated hydrophone array. The experimental protocol consisted of a 2-min baseline period to establish RMR, followed by a 2-min voluntary resting surface apnea, with or without whistling as cued by the trainers, and then a 5-min resting period to measure recovery costs. Daily fluctuations in $\dot{V}_{O_2}$ were accounted for by subtracting the baseline RMR from the recovery costs to estimate the cost of apnea with and without whistles relative to RMR. Analysis of 52 sessions containing 1162 whistles showed that whistling did not increase metabolic cost (P>0.1, +4.2±6.9%) as compared with control trials (−0.5±5.9%; means±s.e.m.). Thus, we reject the hypothesis that whistling is costly for bottlenose dolphins, and conclude that vocal adjustments such as the Lombard response to noise do not represent large direct energetic costs for communicating toothed whales.

KEY WORDS: Respiratory physiology, Sound production, Acoustic communication, Underwater noise, Vocal modifications, Toothed whales

INTRODUCTION

Marine mammals have evolved to use sound in multiple aspects of their life, from active biosonar-based localization of prey (Johnson et al., 2004; Kellogg, 1958), to passive localization of prey (Deecke et al., 2013) and predators (Cummings and Thompson, 1971; Curé et al., 2015; Deecke et al., 2002), and social communication (Janik, 2014) over short (Martin et al., 2018; Sørensen et al., 2018) and potentially long ranges (Payne and Webb, 1971). Because of such extensive reliance upon sound for a wide range of ecological functions, concerns have been raised about the consequences of anthropogenic noise and its effects on marine mammals (Southall et al., 2007). Prolonged exposure to high-intensity noise may result in auditory damage such as temporary (Mooney et al., 2009) or even permanent hearing loss (André et al., 2007), thus directly impacting critical auditory functions and individual fitness. Noise exposure may also elicit a variety of behavioral responses that have less severe impacts, such as spatial avoidance (Finley and Greene, 1993) and disruption of foraging (Blair et al., 2016; Wisniewska et al., 2018).

A variety of more subtle consequences of anthropogenic noise have proven difficult to link directly with long-term fitness costs of exposed animals. One of these is the concept of auditory masking (Clark et al., 2009; Erbe et al., 2016), where noise interferes with the detection or discrimination of acoustic communication signals. The impact of acoustic masking on communication can be estimated based on signal characteristics of the communication signal, hearing threshold, masking noise and reference background noise conditions (Clark et al., 2009; Jensen et al., 2009). Marine mammals are capable of partly ameliorating the masking effects of noise through compensatory mechanisms. Some marine mammals are seemingly able to compensate by increasing source amplitude at almost 1 dB per dB increase in noise via the Lombard response (Dunlop et al., 2014; Holt et al., 2009; Scheifele et al., 2005, though see also Kragh et al., 2019), which is at odds with the normal 0.4 dB dB^{-1} compensation for other vertebrates (Cynx et al., 1998; Roian Egnor and Hauser, 2006). Other studies have found that cetaceans are also capable of changing the frequency (Au et al., 1985; Parks et al., 2007) or increasing the redundancy (Buckstaff, 2004; Miller et al., 2000; Rendell and Gordon, 1999) of acoustic signals to partially overcome masking effects. These observations imply that, within some physiological limits, noise exposure may be compensated for through vocal adjustments, but it is currently unknown how effective such compensatory mechanisms are at ameliorating masking.

These vocal modifications may lead to indirect costs, as sound production is often associated with increased energy expenditure (Ophir et al., 2010), and has a somewhat low efficiency, ranging from 0.5 to 2.4% in anurans (McLister, 2001; Prestwich et al., 1989; Ryan, 1985) and from 0.4 to 3% for elk/red deer (Titze and Riede, 2010). The direct energetic costs of vocal modifications are well studied in terrestrial animals, and modifications such as increasing calling rate (Grafe, 1996; McLister, 2001; Prestwich et al., 1989; Taigen and Wells, 1985; Thomas, 2002; Wells and Taigen, 1989), song duration (Oberweger and Goller, 2001; Taigen and Wells, 1985) and signal amplitude (Oberweger and Goller, 2001; Russell et al., 1998) have been shown to come at additional energetic costs.

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However, the sound production mechanism in toothed whales is different from the laryngeal or syringeal sound production of terrestrial animals. Toothed whales produce sound by pneumatically inducing tissue vibrations (Madsen et al., 2012, 2013; Ridgway and Carder, 1988) in their nasal complex (Cranford et al., 1996). This *de novo* evolved sound production system then begs the question of whether sound production costs and efficiency of toothed whales are similar to those of their terrestrial counterparts, and subsequently what the direct energetic costs of vocal modifications to noise are. In recent years, efforts have been made to address these questions by estimating the energetic cost of sound production in bottlenose dolphins [*Tursiops truncatus* (Montagu 1821)]. Theoretical calculations of metabolic cost of whistling suggest a maximum increase in resting metabolic rate (RMR) of 0.5–1% (Jensen et al., 2012), but rely critically on an assumed sound production efficiency of 1%, similar to many terrestrial animals (Brackenbury, 1977; Fletcher, 2009; McLister, 2001; Prestwich et al., 1989; Ryan, 1985). By contrast, recent empirical studies have reported that whistling is surprisingly costly, with RMRs increasing by 20% (Holt et al., 2015; Noren et al., 2013). These findings in concert with the typical acoustic output of bottlenose dolphins (Janik, 2000; Jensen et al., 2012) imply that sound production efficiency of whistling bottlenose dolphins is more than two orders of magnitude poorer than values reported and modeled for terrestrial animals. If the previous findings of Holt et al. (2015) reflect the actual costs of increasing source level for toothed whales, the direct energetic costs of vocal compensation by increasing signal amplitude or calling rate in response to changes in background noise levels may be substantial (Holt et al., 2015).

Here, we investigate these contrasting claims and test the hypothesis that whistling is metabolically expensive in bottlenose dolphins by measuring the O_2 consumption rate ($\dot{V}_{O_2}$), a proxy of metabolic rate, in three post-absorptive bottlenose dolphins at rest that were either quiet or given a visual cue to whistle. We show that whistling adds no measurable increase in metabolism relative to a silent control period, demonstrating that the metabolic cost of sound production in delphinids is much lower than previously reported, and thus that direct energetic costs of vocal modifications in noise are small.

MATERIALS AND METHODS

Animal subjects and training

The metabolic cost of whistling was measured in three adult male bottlenose dolphins (*T. truncatus*) of varying age and body size (Table 1). The dolphins were kept on a diet of capelin and herring supplemented with multivitamins while housed at the Oceanogràfic in Valencia, Spain, where a total of 16 dolphins reside. For the study, all three dolphins were trained using operant conditioning to station near the side of a floating platform formed by two large buoyant mats while breathing into a custom-built pneumotachometer (Mellow Design, Valencia, Spain) for measurements of respiratory flow and expired O_2 and CO_2 content (Fahlman et al., 2015). The animals were trained to perform a voluntary apnea at rest by turning ventral side up, and either whistled when provided with a visual cue or remained silent. To capture the first breath after apnea, the dolphins were trained to hold their breath until the pneumotachometer was placed over the blowhole. Dolphins freely participated in the study and were not restrained or kept from leaving for the duration of the trial. Experiments were approved by the Animal Care Committee at Oceanogràfic (OCE-3-18).

Table 1. Overview of bottlenose dolphin subjects trained for participation in the study

Animal ID	Body mass (kg)	Straight length (m)	Date of birth	Place of birth
Tt7601	186.0±4.0	2.51	06/2004	Born in captivity
Tt9772	179.4±2.3	2.50	1992	Wild
Tt4560	165.5±2.0	2.45	05/2006	Born in captivity

Body mass presented as means±s.d.

Experimental design

An *a priori* Kruskal–Wallis power analysis, using a significance threshold (α) of 0.05 and a standard deviation (s.d.) in the increased $\dot{V}_{O_2}$ of 20%, suggested that 25 paired trials (control/whistle pairs) would provide a power >80% to detect the 20% increase in metabolic rate reported in earlier studies (Holt et al., 2015; Noren et al., 2013).

Trials were conducted after an overnight fast (from 18:00 h), and before the first feeding the following morning (between 10:00 and 11:00 h). All trials were performed with the animal stationed calmly (minimal to no movement) at the edge of a floating platform formed by two large buoyant mats stacked on top of one another (3×2.6 m beaching mat, Stark Mfg, San Diego, CA, USA). A custom-built hydrophone array made of hollow aluminium surrounded by seven floats was attached with Velcro strips to the side of the mat (Fig. 1). The hydrophones were submerged directly in front of the animal, at a depth of 0.5 m and at ranges of 2 m (near hydrophone) and 4 m (far hydrophone) from the melon of the dolphin.

Each trial consisted of a pre-apnea resting period, with the dolphin resting calmly at the surface breathing into the pneumotachometer (resting; Fig. 2A) to assess the RMR. The pre-apnea period lasted up to 5 min, with the last 2 min being used to determine the RMR. Next, the trainer instructed the dolphin to turn ventral side up for a 2-min apnea with the blowhole and melon submerged (depth of 38–42 cm) at a known distance of 2 m from the first hydrophone of the hydrophone array (apnea; Fig. 2B). In this position, the dolphin was given either a visual cue to whistle (whistling trial) or no cue (control trial). Following the 2-min breath-hold, the dolphin was again asked to turn dorsal side up, and the pneumotachometer was immediately placed over the blowhole to capture the first and subsequent breaths during the subsequent 5-min recovery period (recovery; Fig. 2C). During the resting and recovery phase, the dolphin was breathing into a pneumotachometer to measure the respiratory flow (Fig. 2D) and the exhaled O_2 and CO_2 content for each breath (Fig. 2E). During the trial phase [pre-apnea, apnea (whistling/control) and post-apnea], all sounds created by the dolphin were recorded, and quantified by the calibrated hydrophone array (Fig. 2F). The animal handling (turning, adjustment of stationing) was done by two trainers, who also assisted the dolphin. This helped minimize movement and reduced additional metabolic cost as it helped the animal stay afloat at the surface. Only one trial was performed per animal per day, and whistling/control trials were performed in a pseudorandom order.

Respiratory flow analysis

Respiratory flow was measured using a custom-built Fleisch-type pneumotachometer, housing a low-resistance laminar flow matrix (item no. Z9A887-2, Merriam Process Technologies, Cleveland, OH, USA). A differential pressure transducer (Spirometer Pod, ML 311, ADInstruments, Colorado Springs, CO, USA) was connected to the pneumotachometer with two firm-walled, flexible tubes (310 cm length of 2 mm I.D.). The differential pressure transducer was connected to a data acquisition system (Powerlab 8/35,

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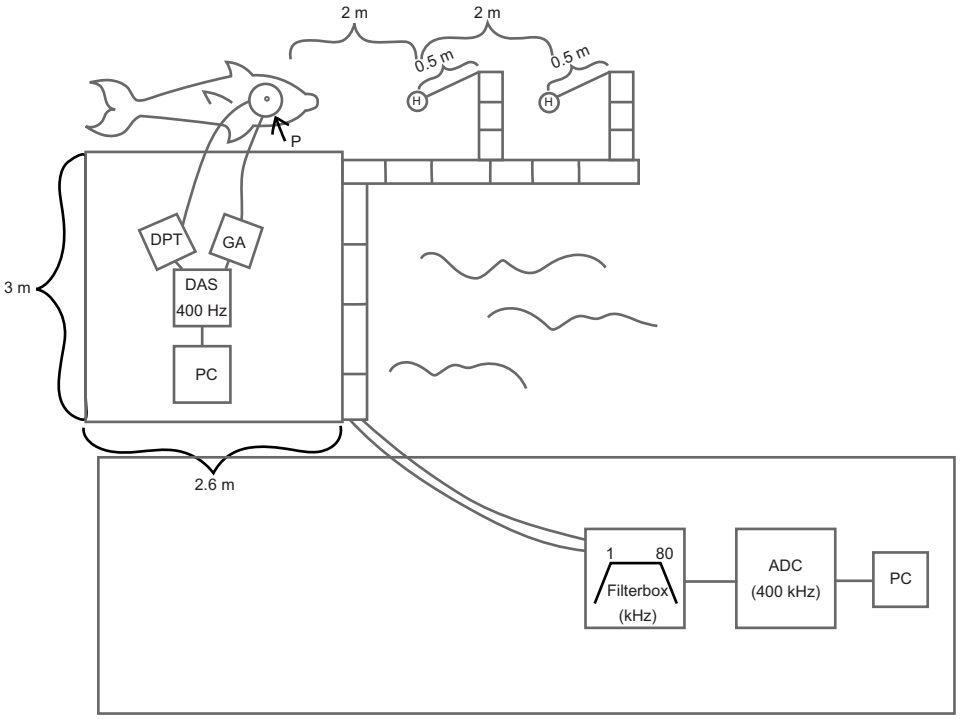

Fig. 1. Overview of the experimental setup. The bottlenose dolphin was located in an oval holding tank (23.5 m long, 12 m wide, 6 m deep) with gates to two adjacent holding pools closed during the experiment. The animal was stationed at the far side of two buoyant mats facing the hydrophone array and oriented along the length of the holding pool. Respirometry data were collected using a pneumotachometer (P) connected to a differential pressure transducer (DPT) and a fast-response gas analyzer (GA) and digitized to a PC using a data acquisition system (DAS; Powerlab). One side of an L-shaped hydrophone array was attached to the right side of the mats for stability, and the other side featured two arms going 45 cm out and 50 cm down. At the end of each arm at a depth of 50 cm, an HTI-96-Min hydrophone (H) was fixed at a distance of 2 and 4 m from the melon of the stationed dolphin. The hydrophones had a built-in one-pole high-pass filter at 1 kHz and were connected to a filtering box with an 80 kHz analog low-pass filter (bandpass filtered at 1–80 kHz, one pole). The filter box connected to an analog-to-digital converter (ADC; USB-6251) digitizing the data to a PC at 400 kHz, 16 bit, using custom-written LabView software.

ADInstruments), and the data were recorded at 400 Hz and displayed on a laptop computer running LabChart (v. 8.1, ADInstruments). The differential pressure was used to determine flow and was calibrated using a 7.0 liter calibration syringe (Series 4900, Hans-Rudolph Inc., Shawnee, KS, USA) before and after each trial. The signal was integrated, and the flow was determined assuming a linear response between differential pressure and flow (Fahlman et al., 2015). Breath-by-breath respiratory flow analysis using a pneumotachometer provides very similar results to conventional flow-through respirometry (Fahlman et al., 2015), and is capable of detecting both transient and cumulative increases in oxygen consumption rates (Fahlman et al., 2019; van der Hoop et al., 2018).

Respiratory gas composition

The concentration of expired O_2 and CO_2 was subsampled via a port in the pneumotachometer and passed through a firm-walled, flexible tube (310 cm length of 2 mm I.D.) and Nafion tubing (30 cm length of 1.5 mm I.D.) fed into a fast-response gas analyzer (Gemini O_2/CO_2 analyzer, part no. 14-1000, CWE Inc., Allentown, PA, USA) at a flow rate of 250 ml min^{-1}. The gas analyzer was calibrated before the experiments using a commercial mixture of 5% O_2, 5% CO_2 and 90% N_2 (UN1956 Air Liquide, USA) and ambient air. Air temperature, pressure and humidity were measured during each trial and were used for STPD (standard temperature, pressure and dry) conversion of inhalations, and exhalations were STPD corrected assuming the gas was at body temperature and saturated with water vapor (Quanjer et al., 1993).

Data analysis

The respiratory gas signals were phase corrected for each trial (approximately by 3 s depending on the flow rate) so that the change in gas concentrations matched the change in flow from the respirations. The O_2 and CO_2 content were multiplied by the expiratory flow to calculate the instantaneous $\dot{V}_{O_2}$ and CO_2 production ($\dot{V}_{CO_2}$) rates, which were then integrated over each breath to calculate the total volume (l) of O_2 and CO_2 exchanged with each breath (Fig. 3A–D, Fahlman et al., 2015). From these data, the RMR was calculated as the $\dot{V}_{O_2}$ during the pre-apnea resting period by summing the volume of O_2 and CO_2 of all breaths that occurred during the last 2 min of the pre-apnea resting period and dividing by the duration. The metabolic cost owing to apnea and either whistling or no whistling was calculated as the difference in metabolic cost during the apnea and post-trial period minus the pre-trial period. For this, the expected metabolic cost owing to rest [RMR (resting $\dot{V}_{O_2}$ or $\dot{V}_{CO_2}$)×(apnea+post-trial duration)] was subtracted from the accumulated volume of O_2 and CO_2 during the recovery period. The resulting volume was divided by the apnea duration and a value different from 0 either indicated a metabolic rate higher or lower than the pre-trial RMR (Fahlman et al., 2008).

Whistles produced during trials were recorded using two calibrated HTI-96-Min hydrophones (High Tech, Inc., MS, USA; one-pole high-pass filter at 1 kHz). The hydrophones were calibrated in a tank relative to a Reson TC4034 (RESON A/S, Slangerup, Denmark) with a known sensitivity of −215 dB re. 1 V μPa^{-1} using the ratio of the average peak-to-peak signal over four cycles, with a delay between cycles of 200 ms. The hydrophones were mounted on a custom-built array made of hollow aluminium, which was attached to the floating mats with Velcro. The hydrophones were connected to a custom-made filtering box (80 kHz first order analog low-pass filter, Aarhus University Electronics lab), a two-channel analog-to-digital converter (USB-6251, National Instruments, Austin, TX, USA) sampling at 400 kHz with a 16-bit resolution. Data were recorded and stored on a laptop using a custom-written sound acquisition software (LabView, National Instruments, written by K. Beedholm) with a built-in digital high-pass filter (500 Hz, second-order Butterworth filter). All acoustic data analysis was performed using custom-written code in MATLAB R2013b (MathWorks, Natick, MA, USA). First, the data were digitally resampled to 60 kHz for further analysis. All recordings were digitally bandpass filtered using a fourth-order

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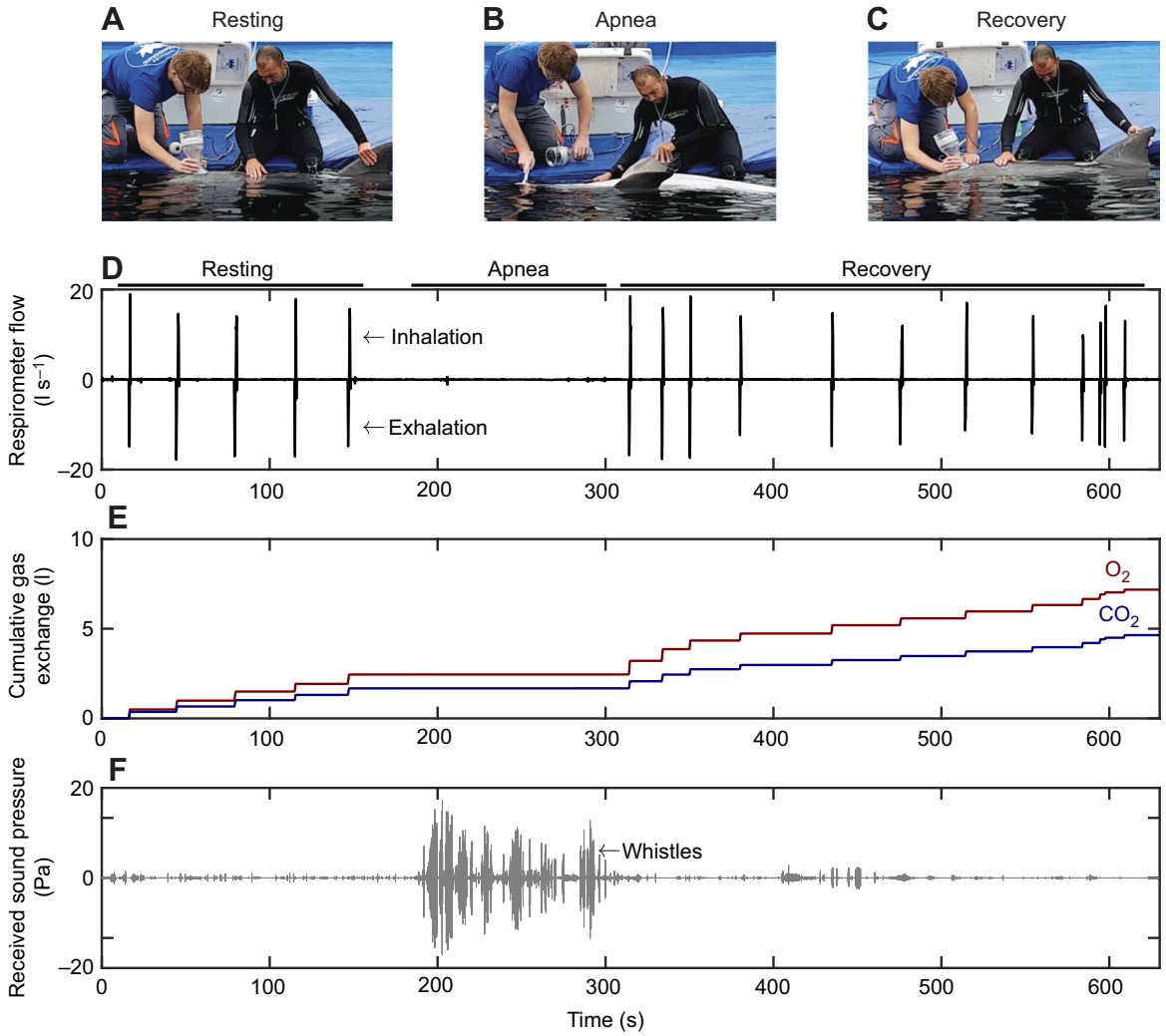

Fig. 2. Experimental overview of a complete session. Each session was divided into (A) a 2-min pre-apnea resting period, (B) a 2-min apnea period, where the animal was turned ventral side up and either continuously whistling or silent, and (C) a 5-min post-apnea recovery period. (D) When resting and recovering, the dolphin was breathing into a pneumotachometer, which recorded flow (l s^{-1}). Negative values are expirations, and positive are inspirations. (E) The flow and change in O_2 and CO_2 tension were used to calculate the cumulative gas exchange (l) integrated over each breath. (F) During the trial period, whistles were recorded on the hydrophones, which were used to calculate their energetic content.

Butterworth filter from 3 to 18 kHz, and the signal envelope was calculated as the absolute value of the analytical signal (Hilbert transform), over which a 100 ms running average was computed. All points where the signal envelope exceeded a threshold of 110 dB re. 1 µPa for at least 100 ms were deemed to be potential whistles and were inspected manually in a spectrogram. The detector triggered on whistles from the test animal, whistles by other animals and pump noise. Only whistles that followed the expected transmission loss of approximately 6 dB from the near hydrophone to the far hydrophone were used for further analysis, with all other detections being discarded. Whistles produced by other animals were rare and of very low amplitude as the other animal in the pool was stationed on the far side of the array with the melon above water, and with gates to other pools closed. Even if vocalizations from these animals triggered the energy detector, their received level would be higher on the far hydrophone rather than the near hydrophone. Whistles produced by each of the test animals were also extremely stereotyped (see Fig. S1), and thus the fundamental frequency contour served as an additional validation that signals were from the test animal. In total, <20 of potential detections (1.8%) were discarded as being from other animals, so this was a very rare issue.

For each whistle, the root mean square (RMS) source level (Madsen, 2005) over the 95% energy window and the centroid frequency (F_c, defined as the frequency which evenly splits the power spectrum in two halves with equal energy; Au, 1993) was calculated. For each whistle, the directivity index (DI) was estimated from F_c using a modified relationship between directivity and frequency for dolphin whistles ($DI=0.20F_c+4.3$; Branstetter et al., 2012). The DI was subtracted from the calculated RMS source level to convert it into the equivalent omni-directional sound source radiating the same acoustic power (Madsen and Wahlberg, 2007; Urick, 1983). The energy flux density (EFD) was calculated by adding $10\log(T)$ to the equivalent omni-directional RMS source level, where T is the duration of the 95% energy window in seconds (Madsen, 2005). The energetic content in mJ was calculated by dividing the squared pressure on a linear scale with the specific acoustic impedance Z and multiplying with the surface area of a sphere with a 1 m radius. When multiplied by the duration of the signal in seconds, this provides the acoustic energy radiated

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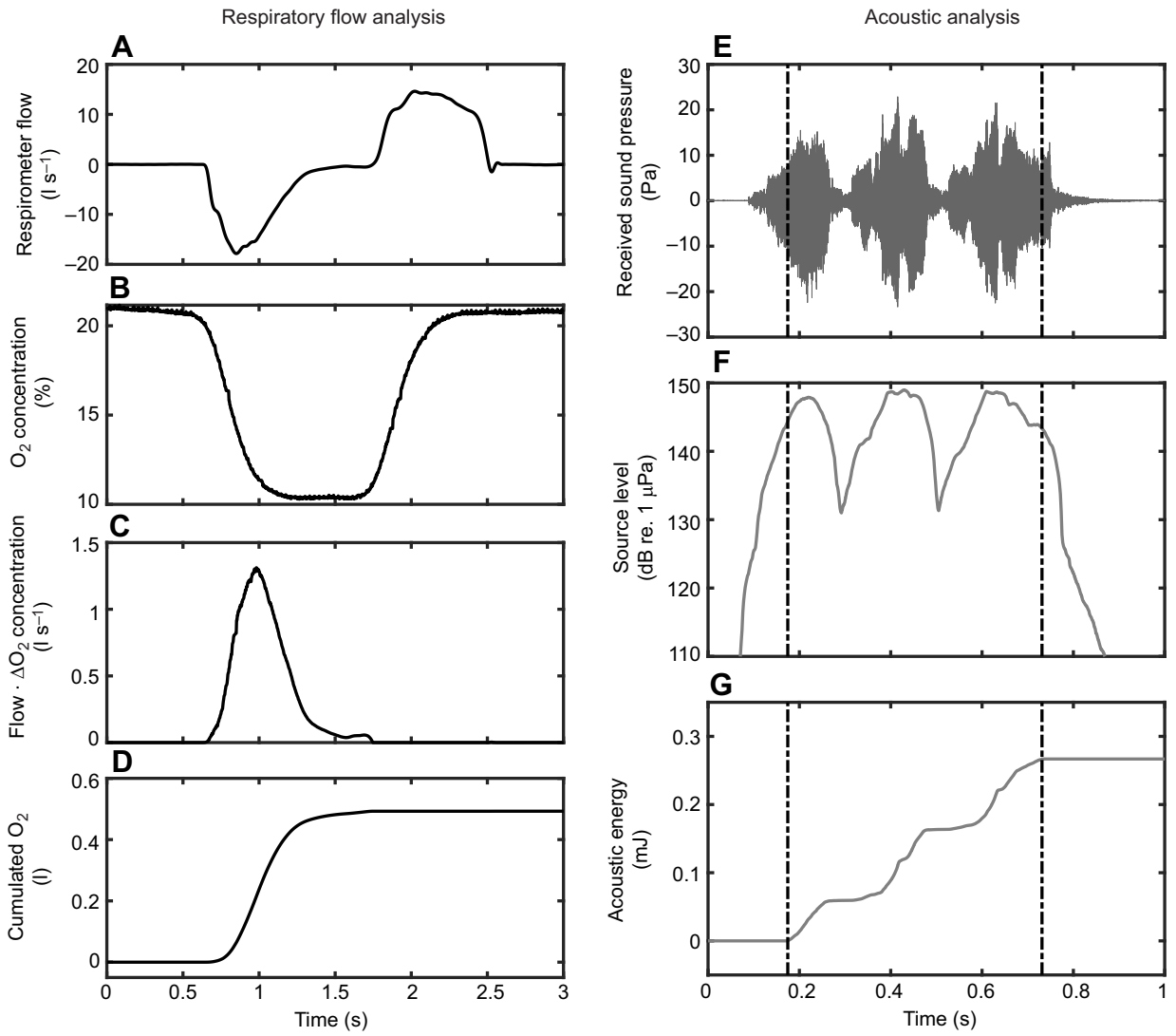

Fig. 3. Data analysis overview. (A) Flow data of a single breath. (B) The change in O_2 concentration following an exhalation and subsequent inhalation. (C) The change in oxygen concentration multiplied by the respirometer flow, giving the instantaneous O_2 consumption rate ($\dot{V}_{O_2}$). (D) The cumulated O_2 consumption, integrated over the instantaneous $\dot{V}_{O_2}$. (E) The received sound pressure wave form of a single whistle. (F) The signal envelope of the whistle source level. (G) The cumulative energy of the waveform in mJ over the 95% energy window (vertical dashed lines) calculated from the directivity corrected whistle.

(Fig. 3E–G) (Madsen and Wahlberg, 2007). Finally, the total radiated acoustic energy per session was calculated by summing the acoustic energy from all whistles, and the cumulative EFD (EFDc; dB re. 1 µPa^2 s) per session was calculated by summing the EFD on a linear scale before log transformation back to dB.

Statistical analysis

We used mixed-effects models to determine the relationship between the metabolic cost of apnea and emitted acoustic energy while accounting for correlation between multiple measurements performed on the same individual (Littell et al., 1998). Mixed-effects models were implemented using the lme4 package (Bates et al., 2015) in R (ver. 3.4.0, https://www.r-project.org/). We calculated *P*-values for each test using the Satterthwaite approximation for degrees of freedom implemented in the package lmerTest (Kuznetsova et al., 2017). The Satterthwaite approximation was used, as it offers low type I error rates, even for small sample sizes, when compared with alternative methods of calculating *P*-values of mixed-effects models (Luke, 2017). For each test, 95% confidence intervals were calculated using the confint function in R, using a bootstrap method with 1000 simulations for each parameter.

Metabolic costs of whistling

To test whether there was a significant increase in metabolic rate during whistling trials, we modeled the estimated metabolic rate of the trial session as a function of trial type (control or whistling) with individual modeled as a random effect on the intercept. No interaction term between individual and emitted acoustic energy was included, as different individuals were not expected to have different sound production efficiencies. To account for fluctuations in daily RMRs, we calculated the change in metabolic rate during trials relative to the measured RMR during the pre-apnea period. We modeled this relative change in metabolic rate as a function of trial type (control or whistling) with individual as a random factor. To directly compare our results with previously reported values of the cost of whistling in dolphins (1.2-fold increase in RMR), we subtracted 20% from whistling trials on a relative scale. Assuming

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Table 2. Overview of acoustic parameters from whistling trials

Dolphin ID	Whistling trials (*n*)	Centroid frequency (F_c; kHz)	Omnidirectional $SL_{95\%RMS}$ at 1 m (dB re. 1 µPa)	Omnidirectionally radiated SL_{EFDc} (dB re. 1 µPa^2 s) per trial	Directionally radiated SL_{EFDc} (dB re. 1 µPa^2 s) per trial	Acoustic energy per trial (mJ)
Tt7601	10	5.3±0.3	126±3 (range: 117–137)	138.8±5.7	144.3±5.7	6.2±4.3
Tt9772	14	8.9±0.8	123±1 (range: 115–133)	135.7±3.4	141.9±3.4	2.4±1.5
Tt4560	4	6.2±0.7	129±5 (range: 117–137)	139.8±4.5	145.4±4.5	7.1±7.0

Source levels are presented as equivalent omnidirectional source level (SL) over the 95% energy window, calculated from the centroid frequency (F_c), by subtracting the estimated directivity index (Branstetter et al., 2012). The omnidirectionally and directionally radiated cumulative energy flux density (EFDc) represents the average total acoustic energy per whistling trial when correcting or not correcting for whistle directivity, respectively. Acoustic energy (mJ) is calculated from the omnidirectionally radiated energy flux density. Values are presented as means±s.d. per session.

that a 20% increase in metabolic rate is correct, there should be no significant difference between control trials and whistling trials after subtracting 20% from whistling trials.

Metabolic costs of whistling as a function of radiated acoustic energy

To test whether the costs of sound production varied with total acoustic output, we modeled the estimated metabolic rate of each trial as a function of total radiated acoustic energy for each session, with individual as a random effect on the intercept. Next, we again accounted for daily fluctuations in metabolic rate by modeling the relative change in metabolic rate as a function of total radiated acoustic energy, with individual modeled as a random effect on the intercept.

Detecting increasing metabolic rates for varying acoustic efficiency

To test how low sound production efficiency would have to be to reliably detect the added cost of whistling given the sample size and variation in the cost of apnea and total emitted acoustic energy measured in this study, we performed a power analysis with an α of 0.05 in MATLAB R2013b. The cost of apnea was converted into mJ using measured respiratory quotient values of 0.83±0.08 (mean± s.d.) so that sound production efficiency could be modeled as the inverse of the slope. Sound production efficiencies were simulated in the range 0.0001–100%, with 10,000 repetitions at each interval. At each interval, we generated 52 points from the mean and standard deviation of the cost of apnea, and added the measured acoustic energy multiplied by the inverse of the efficiency to obtain the added cost of sound production. From this, the likelihood of measuring the cost of sound production for the given efficiency was calculated.

RESULTS

In total, 59 trials were carried out on three animals over the study period. Of these, seven trials were terminated as the animal aborted the trial, leaving 52 trials that were used for further analysis. Average acoustic variables for the dolphin whistles of the three animals are summarized in Table 2, and average metabolic and respiratory variables are summarized in Table 3. Means are presented ±s.d.

Metabolic cost of whistling

The metabolic cost of the 2-min breath-hold was not different from the RMR for that day. There was no difference in metabolic cost of a 2 min apnea during whistling (1228±86 ml O_2, 95% CI=837–1611) as compared with control trials (1067±110 ml O_2, 95% CI=862–1276; $t_{2.9,48.18}$=1.87, P=0.06; Fig. 4A). When accounting for daily fluctuations in RMR, we found no significant increase above pre-apnea metabolic rates for whistling trials (+4.2±6.9%, 95% CI=−21.9–29.1%) compared with silent control trials (−0.5± 5.9%, 95% CI=−12.8–11.1%; $t_{5.87,48.83}$=0.668, P=0.51; Fig. 4B). This value was significantly smaller than previously reported 20% increases in metabolic rate (Holt et al., 2015; Noren et al., 2013) ($t_{5.87,48.83}$=−2.2, P=0.03).

Metabolic costs of whistling as a function of radiated acoustic energy

Each trial differed in the amount of acoustic energy emitted owing to variation in the source level and directivity of each whistle as well as the number of emitted whistles per trial. To capture this variation, we performed a linear mixed-effects model using total emitted acoustic energy in mJ as a fixed effect and individual as a random effect to explain the variation in the relative costs of apnea. The relative metabolic cost of apnea did not change significantly as a function of increasing radiated acoustic energy (−1.1±0.9 mJ, 95% CI=−2.8–0.8 mJ) in the overall model ($t_{2.92,49.85}$=−1.26, P=0.23), nor for any individual animals. Two animals (Fig. 5A,C) had negative regression slopes, whereas a positive slope was found for the third animal (Fig. 5B), but the 95% CI illustrates that no slope deviated significantly from a null line for any of the three animals in the study.

Detecting increasing metabolic rates for varying acoustic efficiency

Given the limited sample size and variation in radiated acoustic energy, the likelihood of finding a significant relationship between radiated acoustic energy and increase in metabolic energy was no better than chance (i.e. 5%) until a sound production efficiency of 0.001% or

Table 3. Pre-apnea resting metabolic rates estimated metabolic rates during apnea and respiration rates prior to and after the 2-min breath-hold for control and whistling trial types

Dolphin ID:	Tt7601 Control	Tt7601 Whistling	Tt9772 Control	Tt9772 Whistling	Tt4560 Control	Tt4560 Whistling
Number of trials (*n*)	10	10	10	14	4	4
Pre-apnea (resting) metabolic rate (ml O_2 min^{-1})	618±137	733±142	486±97	467±83	496±121	528±126
Apnea metabolic rate (ml O_2 min^{-1})	570±124	669±154	487±109	530±138	527±100	504±105
Pre-apnea (resting) respiration rate (breaths min^{-1})	4.0±2.4	5.3±2.9	6.2±1.7	6.3±2.0	3.5±0.2	3.1±1.1
Post-trial (recovery) respiration rate (breaths min^{-1})	3.5±1.7	5.9±3.1	6.9±1.4	7.7±2.9	4.7±1.6	4.1±0.6

Values are presented as means±s.d. across sessions.

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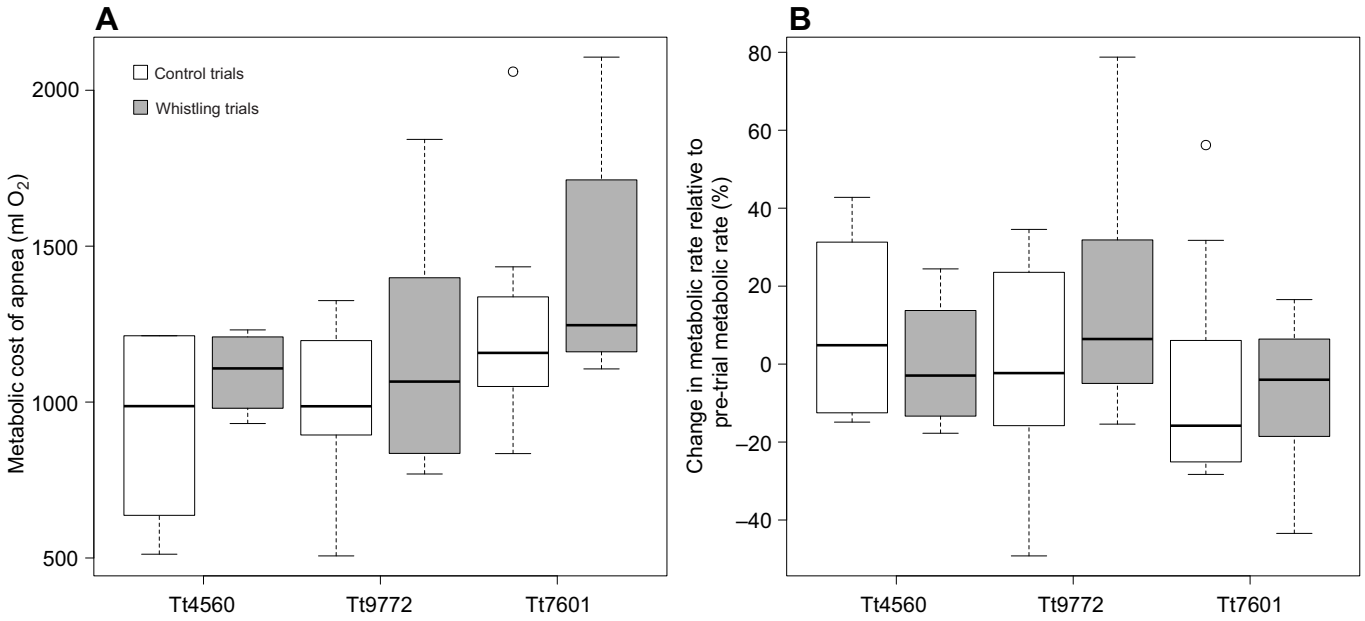

Fig. 4. Calculated oxygen consumption of the 2 min static surface apnea of the 52 experimental trials. Represented as (A) the absolute cost of apnea and (B) relative to the resting metabolic rate measured on the same day. No significant differences between control and whistling periods were found. Solid line represents the median of the dataset and the box boundaries are the 1st and 3rd quartiles and 75th percentiles. Whiskers extend to 1.5 times the interquartile range from the 1st and 3rd quartiles. Outliers depicted as circles.

lower was reached (Fig. 6). To find a significant result with statistical power of 50% or greater, sound production efficiency would have to be lower than 0.0002%, and for statistical power of at least 80%, sound production efficiency would have to be lower than 0.00015%.

DISCUSSION

Previous experimental studies on sound production costs in bottlenose dolphins have concluded that whistling is energetically demanding, eliciting a 20% increase from RMR, and with costs of producing a separate burst-pulse 'squawk' vocalization as high as 50% (Holt et al., 2015; Noren et al., 2013). This implies that dolphin sound production efficiency is more than two orders of magnitude poorer than for terrestrial animals and that the fairly small muscles involved in toothed whale sound production (Ridgway and Carder, 1988) must have very high power outputs and extremely high oxygen consumption. Here, we tested the hypothesis that whistle production in dolphins is expensive using a high-resolution respirometry setup, and an experimental protocol with both experimental and control trials. We show that sound production is associated with no detectable metabolic cost compared with pre-apnea RMRs (Fig. 4). This discrepancy is not simply a matter of sample size – our data are significantly different from a mean 20% increase and thus inconsistent with findings reported in previous studies (Holt et al., 2015; Noren et al., 2013). We therefore reject the hypothesis of expensive whistle production and conclude that communication is metabolically cheap for bottlenose dolphins. This is in line with theoretical predictions of the cost of whistling in this species, which estimates that continuous sound production at realistic source levels should only marginally increase metabolic rate by 0.5–1% (Jensen et al., 2012).

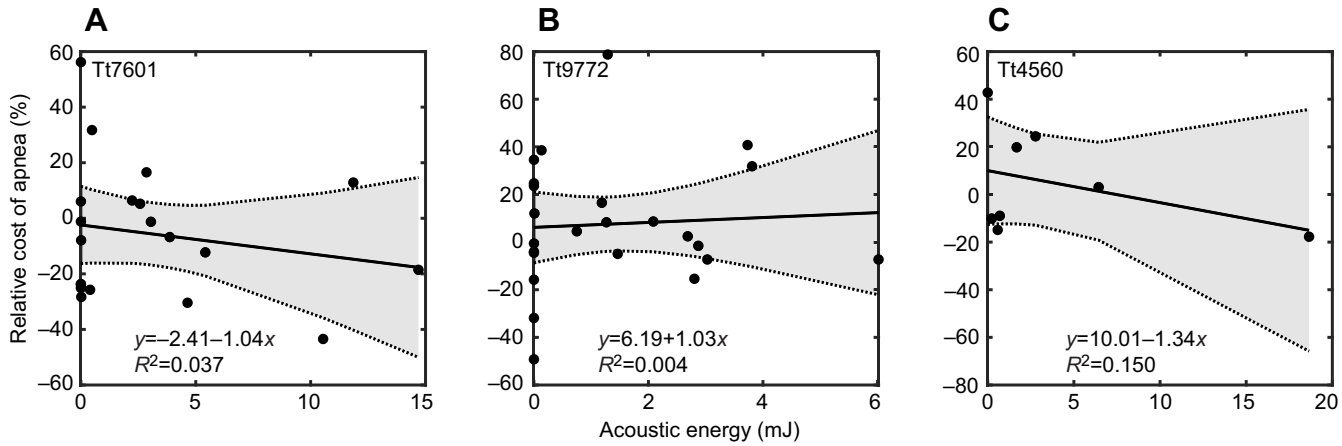

Fig. 5. Relative change in metabolic cost of apnea as a function of total radiated acoustic energy in mJ. The least-squares regression line (solid black line), its equation and R^2 value are presented in each plot, and the shaded gray area represents the 95% confidence intervals of the slope. Slight positive tendencies were found for one animal (Tt9772; B), whereas negative trends were found for Tt7601 (A) and Tt4560 (C), but none of these relationships were significant.

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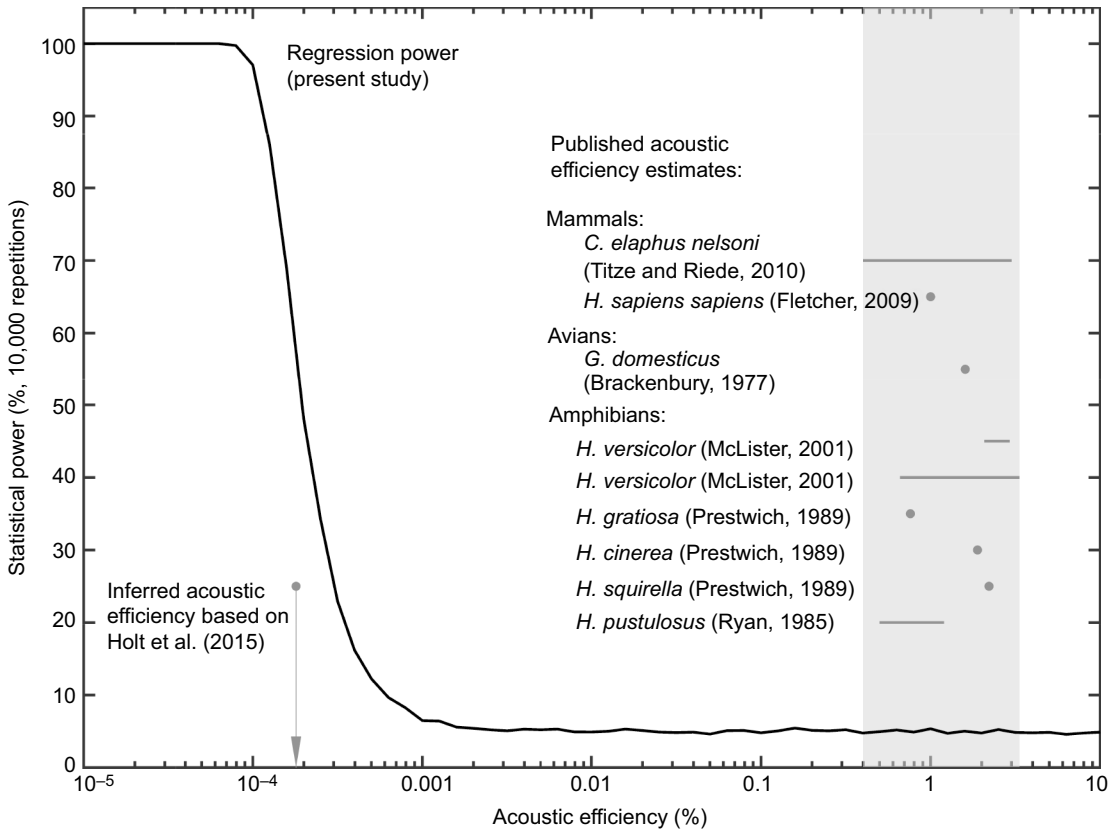

Fig. 6. Statistical power as a function of the modeled sound production efficiency given the variation in metabolic rate and emitted acoustic energy measured in this study, and efficiencies reported and calculated from values in prior studies. Shaded area denotes upper and lower bounds of sound production efficiency of all studies, excluding inferred efficiency from Holt et al. (2015), using the reported regression coefficient and a respiratory quotient of 0.77. If bottlenose dolphins are as efficient at producing sound as all other animals, the likelihood of detecting a significant increase in metabolic cost does not deviate from random chance (5%).

Although the biomechanics and organs of sound production are different, experimental evidence in birds has also indicated relatively low costs of sound production, with changes in metabolic rate of 2–36% of baseline (Oberweger and Goller, 2001; Ward, 2004; Ward et al., 2003), or as undetectable (Chappell et al., 1995). By contrast, in amphibians, a 2- to 15-fold increase in metabolic rate has been reported (Grafe, 1996; McLister, 2001; Prestwich et al., 1989; Wells and Taigen, 1989). Such differences in the metabolic cost of sound production across taxa are likely caused by the differences in metabolic rate for ectotherms versus endotherms and by differences in the relative mass of the vocal organs. Amphibians dedicate as much as 10% of their body mass to sound producing muscle (Ophir et al., 2010), and their standard metabolic rate is an order of magnitude lower than that of a similarly sized endotherm (Ruben, 1995). By contrast, birds dedicate much less body mass to sound-producing muscle (Ophir et al., 2010), and have high RMRs. Less sound-producing muscle, coupled with a much higher RMR relative to amphibians likely results in the much lower metabolic scopes of sound production for birds. Similarly, toothed whales have high RMRs owing to their large body size, endothermy and possibly a life in water with a high heat conductivity, and they do not dedicate a significant proportion of body mass to muscles associated with sound production (Ridgway and Carder, 1988). In concert, this leads to predicted low sound production costs in keeping with our measured values.

In contrast, Holt et al. (2015) reported that the metabolic cost increases substantially with increasing acoustic output levels for bottlenose dolphins, in line with some studies of birds and amphibians (McLister, 2001; Oberweger and Goller, 2001). While this is to be expected if sound production costs are a significant part of the total metabolic rate during the vocal phase, the rate of increase implies a sound production efficiency several orders of magnitude less than what has been reported for humans, amphibians and fowl, and modeled for elk/red deer (Brackenbury, 1977; Fletcher, 2009; McLister, 2001; Prestwich et al., 1989; Ryan, 1985; Titze and Riede, 2010) (Fig. 6). Although the sound production organs are different in cetaceans as compared with terrestrial mammals, whistles are produced by pneumatically induced tissue vibrations (Madsen et al., 2012), a mechanism that is similar to vibrating vocal cords or syringeal membranes. It therefore appears parsimonious to surmise that it would give rise to a similar sound production efficiency in keeping with the low costs of sound production demonstrated here.

Wild dolphins are capable of emitting calls of much greater amplitude (169 dB re. 1 µPa; Janik, 2000) than we recorded in the present study. However, calls of this amplitude still only contain 0.67 J of acoustic energy, assuming they are radiated omnidirectionally and are 1 s in duration. At an efficiency of 1%, one whistle would only require 67 J of metabolic energy, which is insignificant relative to the RMRs of these animals. This then begs the question of why we find such substantial differences in the metabolic cost of low-energy acoustic signals compared with Holt and coauthors (2015). Dolphins in our study used acoustic output levels before accounting for effects of directivity that were

comparable with previous work (Holt et al., 2015; Noren et al., 2013), so the differences in measured costs likely stem from differences in methodology. One potential cause could arise from conflating the cost of sound production with extraneous factors such as locomotive costs and costs of posture. Indeed, one animal in their study was trained to produce burst-pulse squawks at high rate, and it is also possible that the dolphins trained to whistle continuously are not respiring normally, as both cannot be done simultaneously, thus inducing a period of apnea. Thus, a reduction in alveolar ventilation during the 2 min whistling period could result in an accumulated O_2 debt that would be paid back during the period of recovery. The experimental protocol employed by Holt et al. (2015) and Noren et al. (2013) lacked an appropriate control period, where the dolphins performed the entire trial without producing sound, only correcting for daily variation in metabolic rates. Additionally, Holt et al. (2015) employed a recovery period of a non-standard duration, which makes it difficult to decouple locomotive and stationing costs from vocalization costs, and may allow for small movement costs to accumulate over time. We sought to overcome these problems by employing an experimental design that included both control trials and a consistent recovery period of 5 min with minimal animal movement. We show that a 5 min period was sufficient to fully recover from a 2-min breath-hold, as the RMR during apnea was very similar to pre-apnea metabolic rates. This is further corroborated by recent experimental evidence, showing that 1.2 min is sufficient for a bottlenose dolphin to recover from a 159 s breath-hold (Fahlman et al., 2019). Failing to account for such extraneous factors, as may be the case in Holt et al. (2015), has occurred before: high costs of bird song (with 2.7- to 8.7-fold increases above basal metabolic rate) were initially reported for a small passerine bird, the Carolina wren (Eberhardt, 1994). Later studies on passerines, which employed control periods, found much smaller metabolic scopes of sound production (Oberweger and Goller, 2001; Ward, 2004; Ward and Slater, 2005; Ward et al., 2003). Appropriate control periods, where everything except sound production is kept the same, therefore seem essential for teasing apart the metabolic costs of signaling from extraneous factors. Indeed, a study on the cost of echolocation in bottlenose dolphins found that click production is cheap when compared with a control period (Noren et al., 2017), and this is also supported by field estimates of the minute air volumes used for sound production in echolocating pilot whales (Foskolos et al., 2019).

Given that whistling has no measurable cost, the direct metabolic costs of increasing vocal amplitude as a response to increasing anthropogenic noise (Au et al., 1985; Dunlop et al., 2014; Holt et al., 2009) or an increased call rate (Buckstaff, 2004) are likely to be very small. However, noise may still have adverse effects not related to direct energetic costs. Recent evidence has shown that one of the consequences of increased noise is a reduction in whistle contour complexity (Fouda et al., 2018). Reduced contour complexity may jeopardize the ability to recognize individuals as the frequency contours carry information used to identify individuals (Janik et al., 2006). Additionally, the ability to compensate to increased noise is not unlimited. The upper limit of source levels reported for bottlenose dolphins' whistles is 169 dB re. 1 µPa (Janik, 2000). For that population, which had a mean back-calculated source level of 158 dB re. 1 µPa, this only allows for 11 dB of potential compensation on average, and measurements have shown that wild bottlenose dolphins only compensate with 0.1–0.3 dB per dB of noise increase (Kragh et al., 2019). Additionally, vessel noise is both spatially and temporally heterogeneous, and loud noise at the location of a recipient would not necessarily induce a sufficient Lombard response in the sender. Of greater concern are alterations in the activity budget of wild marine mammals, and their potential direct or indirect energetic effects. Escape from noise may alter activity and increase metabolic cost, which may also increase the risk of gas bubble formation (Fahlman et al., 2014). Although dolphins are capable of ameliorating noise partially by increasing call rates (Buckstaff, 2004), the increased time spent calling cannot be spent foraging or on other behaviors important for fitness, and as such noise may indirectly reduce the time spent foraging, offsetting a positive energy balance (Pirotta et al., 2018). Additionally, energetic intake may be reduced more directly through disturbances that limit feeding opportunities (Christiansen et al., 2013; Williams et al., 2006; Wisniewska et al., 2018). The lost foraging opportunities and reduced foraging efficacy caused by noise are much more likely to negatively affect their energy budget, compared with the very small direct energetic consequences of vocal adjustments reported here.

Conclusions

This study demonstrates that when movement costs and daily fluctuations in metabolic rate are accounted for, whistle production does not significantly increase the metabolic rate of bottlenose dolphins. The energetic costs of sound production reported here are significantly smaller than those reported in previous studies in spite of similar total radiated acoustic energy, and we therefore conclude that sound production is metabolically inexpensive for bottlenose dolphins. As a consequence, the direct energetic consequences of a Lombard effect in wild bottlenose dolphins are unlikely to be a concern. More research is needed to define the physiological limits of vocal compensation and the efficacy of compensation mechanisms for spatially heterogeneous noise sources. Additionally, we need to elucidate to what extent anthropogenic noise indirectly affects the energy balance of wild odontocetes by altering activity budgets through increased calling rates as well as reducing feeding efficacy.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: M.B.P., A.F., P.T.M., F.H.J.; Methodology: M.B.P., A.F., P.T.M., F.H.J.; Software: M.B.P., F.H.J.; Validation: M.B.P., A.F., F.H.J.; Formal analysis: M.B.P., A.F., A.B., F.H.J.; Investigation: M.B.P., A.F., A.B., F.H.J.; Resources: A.F., P.T.M.; Data curation: M.B.P.; Writing - original draft: M.B.P., P.T.M., F.H.J.; Writing - review & editing: M.B.P., A.F., A.B., P.T.M., F.H.J.; Visualization: M.B.P., A.F., F.H.J.; Supervision: A.F., F.H.J.; Project administration: M.B.P., A.F., P.T.M., F.H.J.; Funding acquisition: M.B.P., A.F., P.T.M.

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Data availability

Data used in this article can be found online on OSF.io using the link https://osf.io/avtqd/

Supplementary information

Supplementary information available online at
http://jeb.biologists.org/lookup/doi/10.1242/jeb.212498.supplemental

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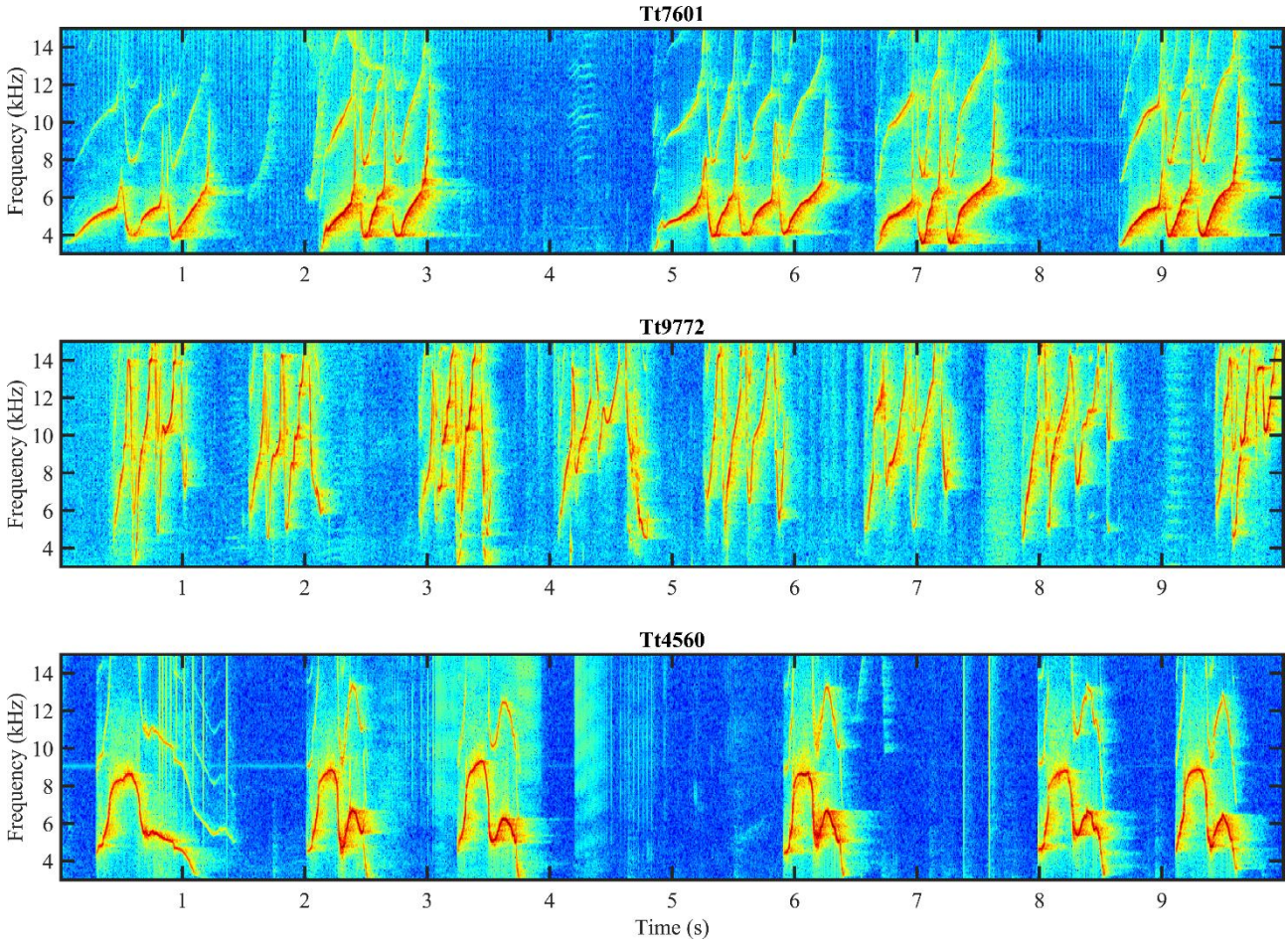

Figure S1 – Example spectrograms of whistles from the three study animals. Each dolphin produced whistles with unique frequency modulation pattern. The whistles are highly stereotyped, and the fundamental contour was used as part of the validation process for inclusion in the study. Spectrogram settings FFT size 1024, 95% overlap.