

RESEARCH ARTICLE

Vocal performance affects metabolic rate in dolphins: implications for animals communicating in noisy environments

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ABSTRACT

Many animals produce louder, longer or more repetitious vocalizations to compensate for increases in environmental noise. Biological costs of increased vocal effort in response to noise, including energetic costs, remain empirically undefined in many taxa, particularly in marine mammals that rely on sound for fundamental biological functions in increasingly noisy habitats. For this investigation, we tested the hypothesis that an increase in vocal effort would result in an energetic cost to the signaler by experimentally measuring oxygen consumption during rest and a 2 min vocal period in dolphins that were trained to vary vocal loudness across trials. Vocal effort was quantified as the total acoustic energy of sounds produced. Metabolic rates during the vocal period were, on average, 1.2 and 1.5 times resting metabolic rate (RMR) in dolphin A and B, respectively. As vocal effort increased, we found that there was a significant increase in metabolic rate over RMR during the 2 min following sound production in both dolphins, and in total oxygen consumption (metabolic cost of sound production plus recovery costs) in the dolphin that showed a wider range of vocal effort across trials. Increases in vocal effort, as a consequence of increases in vocal amplitude, repetition rate and/or duration, are consistent with behavioral responses to noise in free-ranging animals. Here, we empirically demonstrate for the first time in a marine mammal, that these vocal modifications can have an energetic impact at the individual level and, importantly, these data provide a mechanistic foundation for evaluating biological consequences of vocal modification in noise-polluted habitats.

KEY WORDS: Bottlenose dolphin, *Tursiops truncatus*, Metabolic cost, Oxygen consumption, Vocal effort, Vocal modification

INTRODUCTION

Animals routinely use specific sounds during foraging, predator avoidance and reproductive behavior (Bradbury and Vehrencamp, 1998). Noise that interferes with sounds involved in these vital biological functions has the potential to impact an individual's survival and reproductive success. Ambient noise decreases the signal-to-noise ratio of, or masks, sounds produced by animals during acoustic communication. Often, animals will modify their vocal behavior in response to fluctuations in environmental noise through changes in the amplitude, duration, repetition rate and/or frequency of sounds produced (Brumm and Slabbekoorn, 2005).

Vocal adjustments in some of these cases ameliorate masking noise effects. However, noise-induced vocal responses may come with a variety of biological costs that, to date, have largely remained unquantified. Biological costs might include increased detection by predators or competitors, degraded signal efficacy or function in social contexts as well as energetic costs related to changes in metabolic demands or activity budgets. A full understanding of such biological costs is necessary to inform conservation actions for animals living in noise-polluted environments.

As sound production results in an energetic cost to the signaler (Ophir et al., 2010), vocal responses to noise may affect the overall metabolic rate of an animal as a consequence of increased vocal effort [i.e. signaling louder (the Lombard effect; Lombard, 1911)], longer or more often (Hotchkiss and Parks, 2013; Scheifele et al., 2005). The energetic costs of acoustic signals have been well investigated in some vertebrate groups such as amphibians and birds (reviewed in Ophir et al., 2010; Stoddard and Salazar, 2011) while empirical measurements in mammals are more limited (Speakman et al., 1989; Russell et al., 1998). Most recently, Noren et al. (2013) found that dolphins vocalizing for a 2 min period have metabolic rates that are 1.2× resting metabolic rate, a similar increase to those found in echolocating bats hanging at rest and many birds producing sound with minimal body movement (Speakman et al., 1989; Franz and Goller, 2003; Ward et al., 2003). Total metabolic cost (over resting values) of sound production by dolphins varies between 163 and 2996 ml O₂ for a 2 min vocal bout and is positively related to the duration of the sounds produced, but these results are based on small sample sizes and the subjects were not specifically trained to vary their vocal effort across trials (Noren et al., 2013). Despite these few studies, the metabolic costs specifically associated with changes in vocal effort have remained empirically undefined in most taxa, with the few data limited to those measured in humans and birds (Russell et al., 1998; Oberweger and Goller, 2001; Zollinger et al., 2011). Studies on bird sound production, for example, have shown that oxygen consumption increases with increases in vocal repetition rate, loudness and duration (Horn et al., 1995; Oberweger and Goller, 2001; Franz and Goller, 2003). Zollinger et al. (2011) compared oxygen consumption in zebra finches (*Taeniopygia guttata*) as song amplitude varied in the presence of experimental noise. Oxygen consumption significantly increased as song amplitude increased in only one of the three subjects but the period of song production appeared to be short (approximately 10 s), the acoustic energy of the song bouts were not compared and the total metabolic cost of song production was not reported (Zollinger et al., 2011). In humans, oxygen consumption also increases with increases in the sound pressure level of spoken words (Russell et al., 1998).

Many marine species, most notably the marine mammals, rely on acoustic information in the ocean where noise pollution is a major concern because of anthropogenic inputs. Chronic sources of anthropogenic noise, such as those associated with vessel traffic

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Received 16 March 2015; Accepted 30 March 2015

near major urban ports (Bassett et al., 2012; Erbe et al., 2012), are particularly concerning given repeated exposure to local populations. Vocal responses to noise are well documented in many whale and dolphin species, including increases in whistle repetition rate in bottlenose dolphins during boat approaches (*Tursiops truncatus*; Buckstaff, 2004) and increases in call amplitude as noise levels increase in endangered killer whales (*Orcinus orca*; Holt et al., 2009) and North Atlantic right whales (*Eubalaena glacialis*; Parks et al., 2011). While mammalian sound production is most often accomplished by the larynx, odontocetes (toothed whales and dolphins) produce sound in the nasal complex (Cranford et al., 2011). They also face physiological challenges related to the aquatic lifestyle including a limited oxygen supply while diving. Generalizing the sparse data available on the metabolic consequences of vocal changes in terrestrial species is likely inappropriate given differences in physiology (e.g. for breath-hold diving) and sound production mechanisms. Furthermore, recent empirical estimates of the metabolic costs of whistle production in the bottlenose dolphin (Noren et al., 2013) do not agree with previous theoretical estimates based on the acoustic energy of the whistles and assumptions about the efficiency factor of sound production (Jensen et al., 2012).

In the present study, we hypothesized that an increase in vocal effort by dolphins, manifested as an increase in the acoustic energy of sounds produced, would result in an increase in metabolic rate relative to resting. We tested this hypothesis by measuring metabolic rates during resting and variable levels of sound production in bottlenose dolphins, *T. truncatus* (Montagu 1821). The current study differed from the approach of Noren et al. (2013) in that the subjects were specifically trained to modify their vocalizations to produce either ‘soft’ sounds (when the ‘soft’ training cue was given) or ‘loud’ sounds (when the ‘loud’ training cue was given) within a trial, with a goal of ‘loud’ sounds being +10 dB relative to ‘soft’ sounds. Changes in vocal effort were then related to changes in

metabolic performance across trials. Here, we report new evidence of metabolic costs of increased vocal effort by bottlenose dolphins. This is the first study to experimentally measure the energetic consequences of vocal modifications in a marine mammal with implications for wild populations communicating in noisy environments.

RESULTS

Each dolphin was consistent in the type of sound he produced during trials but the vocalization type differed between dolphins. Dolphin A produced a whistle, which was a frequency-modulated tonal sound (Fig. 1A, C) while dolphin B produced a squawk, which was a broadband pulsed sound with individual pulses much longer in duration and lower in frequency than echolocation clicks (Fig. 1B, D). These vocalizations are described as social sounds produced by wild bottlenose dolphins (Jacobs et al., 1993; Herzing, 1996). In addition to increasing the loudness of their vocalizations (Fig. 1), both dolphins had a tendency to increase the duration of their vocalizations during ‘loud trials’ (when only the ‘loud’ training cue was given) compared with ‘soft trials’ (when only the ‘soft’ training cue was given). Dolphin A also produced more vocalizations during loud trials while dolphin B produced fewer, on average. Furthermore, vocal performance of both dolphins within a trial type showed considerable variation despite efforts to train consistent vocal behavior. Thus, the total sound energy of all vocalizations produced in a trial, reported as cumulative sound exposure level (cSEL), was the most consistent metric to relate to metabolic cost, irrespective of the trial type because the metric depends on the repetition rate, duration and amplitude of the sounds produced. In general, received cSEL was higher and the range was greater across trials in dolphin B (mean±s.d.=150.2±5.6 dB re. 1 $\mu\text{Pa}^2\text{s}$, minimum 141.2 dB re. 1 $\mu\text{Pa}^2\text{s}$, maximum 160.3 dB re. 1 $\mu\text{Pa}^2\text{s}$, $N=29$) relative to dolphin A (mean±s.d.=148.5±3.9 dB re. 1 $\mu\text{Pa}^2\text{s}$, minimum 139.0 dB re. 1 $\mu\text{Pa}^2\text{s}$,

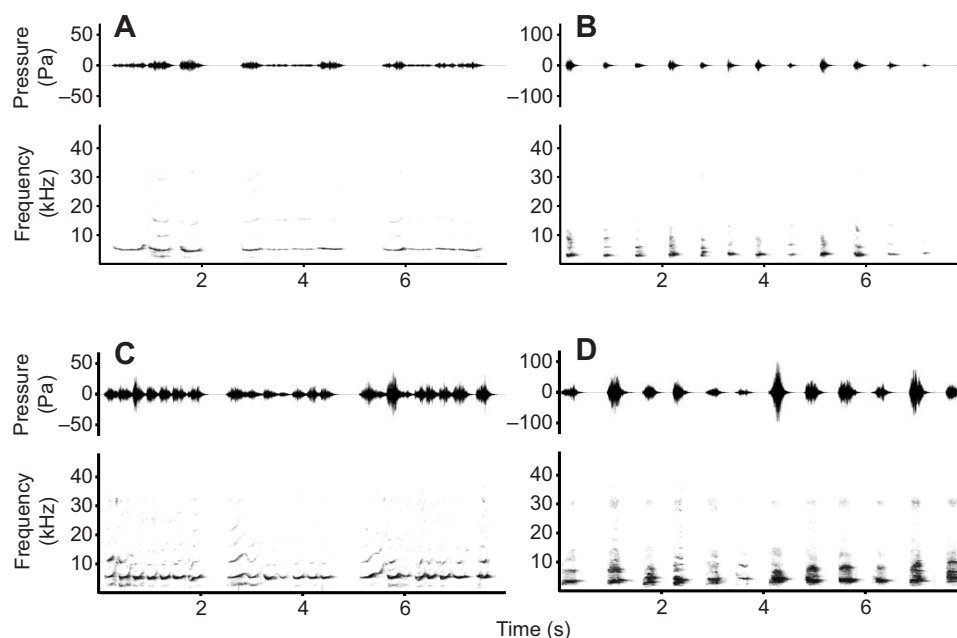


Fig. 1. Example spectrograms and corresponding time series of sounds made during the vocal period of ‘soft’ and ‘loud’ trials. Dolphin A (left panels) and dolphin B (right panels) took part in ‘soft’ trials (A,B) and ‘loud’ trials (C,D) and the sounds they produced were recorded from a contact hydrophone. Acoustic pressure and acoustic frequency data are shown.

Table 1. Summary of vocal parameters averaged across all trials

Subject	No. of trials	No. of sounds produced	Sound duration (s)	Interval between start of each sound (s)	Received SPL (dB re. 1 μ Pa)	Received cSEL (dB re. 1 μ Pa ² s)
Dolphin A	27	57.2 \pm 14.2	1.23 \pm 0.45	2.27 \pm 0.65	129.0 \pm 2.8	148.5 \pm 3.9
Dolphin B	29	199.2 \pm 21.3	0.248 \pm 0.062	0.607 \pm 0.071	130.8 \pm 6.2	150.2 \pm 5.6

cSEL, cumulative sound exposure level; SPL, sound pressure level, based on root mean square. Means are presented \pm 1 s.d.

maximum 156.6 dB re. 1 μ Pa² s, $N=27$). Acoustic parameters of vocal effort during the 2 min vocal period averaged across all trials for both dolphins are shown in Table 1.

With increasing cSEL, the percentage increase in metabolic rate over resting metabolic rate (RMR) during the vocal period ($F_{1,27}=8.883$, $P=0.006$; Fig. 2B) and 2 min following the vocal period ($F_{1,27}=13.466$, $P=0.001$; Fig. 2D), and the total metabolic

cost ($F_{1,18}=13.602$, $P=0.002$; Fig. 2F) increased in dolphin B. The percentage increase in metabolic rate over RMR during the 2 min following the vocal period was also greater in dolphin A as cSEL increased ($F_{1,25}=6.457$, $P=0.018$; Fig. 2C). The percentage increase in metabolic rate over RMR during the vocal period and total metabolic cost had a tendency to increase as cSEL increased in dolphin A but these results were not significant ($F_{1,25}=0.553$,

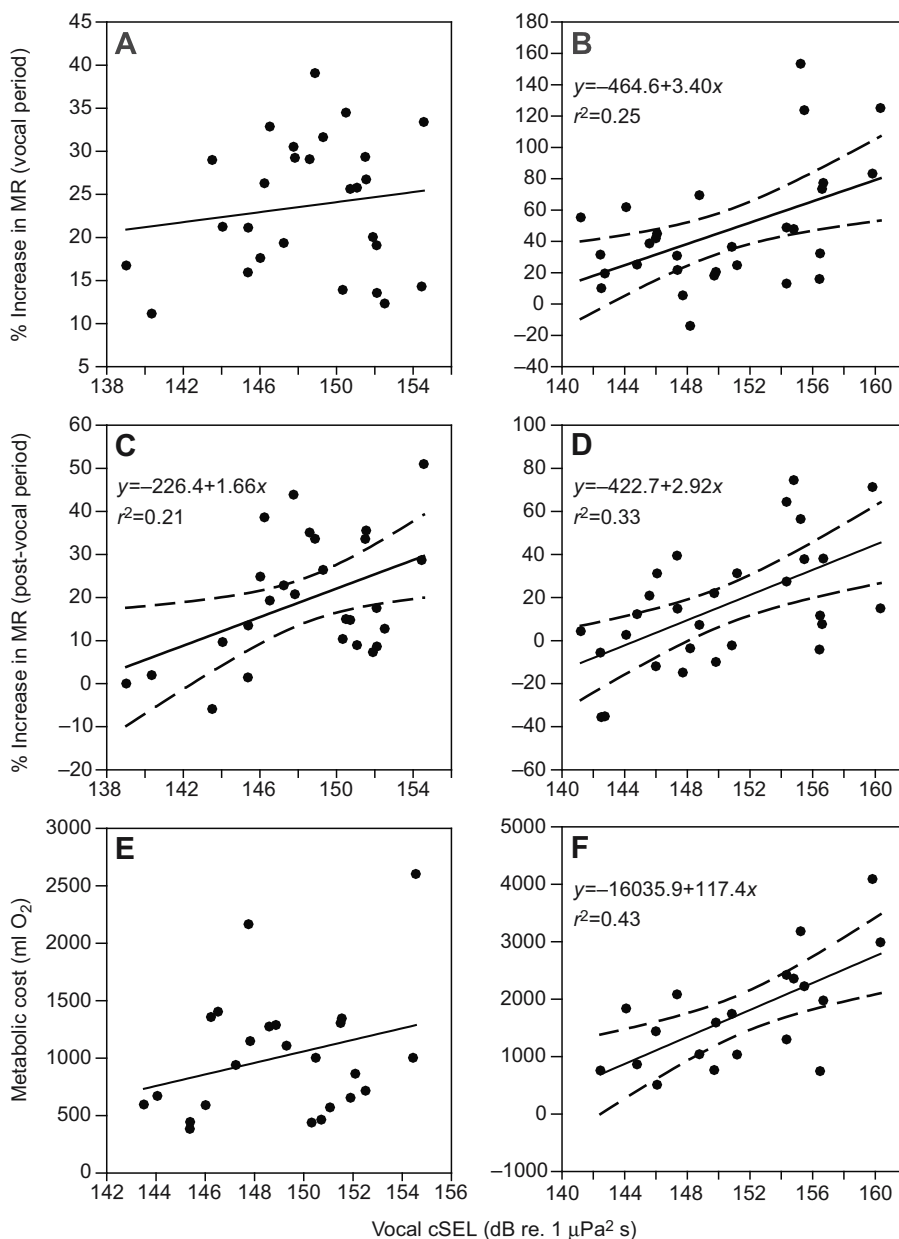


Fig. 2. Percentage increase in metabolic rate over resting metabolic rate and total metabolic cost during the trials.

The increase in metabolic rate during the vocal period (A,B) and 2 min following the vocal period (C,D), and the total metabolic cost (above resting values, E,F) are shown as a function of cumulative sound exposure level (cSEL) of sounds produced. Data for dolphin A and B are shown in the left and right panels, respectively; 95% confidence intervals are shown as dashed lines. For A and E, although the results are not statistically significant, trend lines are included to illustrate consistency of the positive relationship between dolphins.

$P=0.464$, $F_{1,22}=2.009$, $P=0.170$; Fig. 2A,E). Respiration rate (breaths min^{-1}) during the vocal period and other phases of the trial, as well as total recovery duration did not change with increasing cSEL in either subject ($P>0.05$ in all cases). When measured outside of the context of a metabolic trial, water-propagated source levels of dolphin A's individual whistles ranged from 121 to 146 dB_{rms} re. 1 μPa at 1 m (SEL range: 121–149 dB re. 1 μPa^2 s). The source levels of the louder whistles are within the range of those measured in wild bottlenose dolphins (Jensen et al., 2012). Water-propagated source levels of dolphin B's individual squawks ranged from 117 to 135 dB_{rms} re. 1 μPa at 1 m (SEL range: 105–127 dB re. 1 μPa^2 s). There are no published values of squawk source levels measured in wild bottlenose dolphins for comparison. Video analysis revealed no difference in body posture (dolphin A: $t=1.545$, $P=0.138$; dolphin B: $U=59.000$, $P=0.689$) or number of fluke motions (dolphin A: $U=46.000$, $P=0.331$; dolphin B: $U=51.500$, $P=0.255$) between soft and loud trials.

DISCUSSION

This study provides the first experimental evidence of metabolic consequences of vocal modifications in a marine mammal that are consistent with behavioral changes in noisy environments in wild populations (Buckstaff, 2004; Holt et al., 2009; Parks et al., 2011). When the dolphins continuously vocalized over a 2 min period, there was a significant percentage increase (Fig. 2) in metabolic rate over RMR during the 2 min following sound production as cSEL increased in both subjects. We also found that there was a significant percentage increase in metabolic rate over RMR during sound production and in total oxygen consumption (representing the total metabolic cost of sound production plus recovery costs excluding baseline resting cost) as cSEL increased in dolphin B. The maximum cSEL of sound production was higher and the range was greater in dolphin B relative to dolphin A. The restricted range of vocal changes in dolphin A likely contributed to a lack of significance in some statistical results but positive trends were apparent in these cases (Fig. 2A,E). To investigate this further, dolphin B's data were restricted to within the range of dolphin A's vocal performance (i.e. only data for cSEL<156 dB as shown in Fig. 2E) and re-analyzed. We found a positive trend but no significant increase in total oxygen consumption as cSEL increased; this supports the assumption that the restricted range of cSEL measured for dolphin A precluded finding significant relationships. Differences in statistical outcomes could also be partly due to the different sound types produced between the dolphins. Unfortunately, both animals could not be trained to squawk and whistle because extensive time and effort were required to train variations in vocal effort within a sound type.

The metabolic results varied widely by individuals and across trials. Some of this variability is statistically related to differences in vocal performance across trials. Specifically, variation in vocal cSEL accounted for 43% of the variation in total metabolic cost across trials in dolphin B (i.e. $r^2=0.43$; Fig. 2F) and accordingly the remaining proportion of variation in metabolic cost is due to other factors. Variability might also be inherent to measuring metabolism in apneustic subjects that are adapted for breath-hold diving. However, respiration rates during the vocal period and other trial components did not change with increasing cSEL in either subject. Thus, differences in metabolic cost as related to vocal performance in this investigation are likely due to differences in metabolic demand rather than to differences in breathing patterns. Variability might also be due to slight differences in body movement between trials but we found no difference in body posture or level of

movement from the video analysis. A portion of the increase in metabolic rate relative to RMR might also be due to general arousal or excitement from being signaled by the trainers to vocalize. However, the percentage increase in metabolic rate over RMR during the vocal period or 2 min following the vocal period was low or close to zero for some trials, particularly for trials with lower vocal cSEL (Fig. 2A–D), even though the dolphins were signaled to vocalize on all trials. Thus, the increase in metabolic rate during vocalizations cannot be fully attributed to an aroused state from being signaled to vocalize, per se. The few negative values for the percentage increase above RMR during the vocal period or 2 min post-vocal period (Fig. 2A–D) could be attributed to the subject not reaching a true state of rest for various physiological or psychological factors. Elevated estimates of RMR, as a result, would not likely influence our estimate of total metabolic cost (Fig. 2E,F) as total metabolic cost could not be calculated when the dolphin's baseline RMR was higher than the post-vocal RMR (see Materials and methods). Nonetheless, varying degrees of excitement or anticipation during the trial likely contributed to some of the variability in metabolic rates that is not explained by vocal effort.

Most studies do not report metabolic costs of vocal activity as the total metabolic cost of sound production plus recovery costs (excluding baseline resting costs). Rather, investigators most often report metabolic rates during vocal activity and compare vocal metabolic rates with RMRs. To put our data in a comparative perspective, metabolic rate during the vocal period was, on average, $1.2\times$ RMR in dolphin A and $1.5\times$ RMR in dolphin B. The relative increase in metabolism is similar to that found in bats and birds when vocal activity was measured during minimal body movement (Speakman et al., 1989; Franz and Goller, 2003; Ward et al., 2003). Variability in metabolic costs across trials and individuals reported in other studies is similar to that reported in the current investigation and, in some cases, is also attributed to differences in vocal performance such as the duration of vocal activity (Oberweger and Goller, 2001; Franz and Goller, 2003; Ward et al., 2003). Although both bats and birds have a much smaller overall body mass and produce sound in a different medium compared with dolphins, it appears that all endotherms have similar costs of sound production relative to their RMR. This might be unexpected based on comparisons of the acoustic energy output corrected for differences in reference level, acoustic impedance and total body mass between dolphins and smaller terrestrial animals (e.g. Madsen and Surlykke, 2013). However, theoretical predictions of the metabolic cost of acoustic signaling based on the acoustic energy released to the environment are inconsistent with empirical results (Noren et al., 2013). Comparisons of costs between smaller terrestrial endotherms and larger marine dolphins are also complicated by other differences including sound production anatomy. For example, the nasal complex structures involved in sound production in dolphins appear to be orders of magnitude greater in mass than the vibrating membranes of the larynx of similar-sized mammals (Cranford et al., 2011). Teasing out the individual processes involved in sound production to relate to energetic cost, such as powering the lungs to produce sound, activating the sound-producing muscles and adjusting the vocal tract, is complex and better understood in some terrestrial taxa than in dolphins (Titze and Riede, 2010). Furthermore, it appears that the size of the muscles that are active during sound generation is an important variable to consider for comparative purposes. As the proportion of the mass of sound-producing muscles relative to total body size increases, so does metabolic

scope (Ophir et al., 2010). Sound-producing muscle mass in birds is approximately 0.2% of total body mass, with birds showing the lowest metabolic scope of calling (similar to phonating dolphins) among compared taxa (Ophir et al., 2010). Active muscles during sound generation in dolphins include the posterior internus, anterior internus, nasal plug, diagonal membrane and palatopharyngeal muscles (Ridgway et al., 1980). Although Green et al. (1980) present sectional data of the bottlenose dolphin nasal region, many of the photographs are not sufficient in detail to accurately determine the volume of these sound-producing muscles to estimate mass [specifically, the palatopharyngeal muscle is cut off ventrally in multiple sections and two sections in the series (17 and 18), which include the nasal plug muscle, are not shown in Green et al. (1980)]. Other muscles are likely involved as well but their activity during sound generation has not been measured (Green et al., 1980; Ridgway et al., 1980; Cranford et al., 2011). Therefore, with the currently available data, we cannot determine whether the same relationship reported by Ophir et al. (2010) holds true in dolphins. Investigations that include precise estimates of the mass of all muscles involved in sound generation, as well as analyses to shed light on muscle performance (e.g. oxidative capacity and fiber type), are needed to better understand the physiology of dolphin sound production.

Vocal activity and concomitant oxygen consumption measurements took place under a reverberant metabolic hood, which precluded source level measurements of the sounds produced during the experimental trials. Thus, the received levels reported in Table 1 should not be interpreted as approximates of source levels of water-propagated sounds. Water-propagated source levels were measured outside of the context of the trials in order to evaluate whether the signals that the subjects produced were comparable to those reported in the literature. The results demonstrated that the louder whistle levels of dolphin A were similar to those reported in wild populations, indicating that the vocal ability of this dolphin is representative of free-ranging animals. The results might also be used to estimate the efficiency of social sound production in bottlenose dolphins. For example, the trial with the most energetic bout contained approximately 0.08 J of energy (assuming sounds were radiated omni-directionally with a cSEL equal to 160 dB re. 1 μPa^2 s in source level) and the total cost of sound production ranged between 383 and 4087 ml O_2 (Fig. 2E,F) or 7691 and 82,067 J (using 20.08 J ml^{-1} O_2). The ratio of sound energy output to metabolic cost, both in joules, indicates an extremely low calculated efficiency factor range (less than 0.1%), especially compared with values for terrestrial animals (Ophir et al., 2010; Prestwich, 2007). This indicates that dolphins either have very poor sound production efficiency or the metabolic cost is due to processes beyond sound production alone. Other factors besides just the actuation of the muscles in the nasal region indeed contributed to increases in metabolic rate during vocal activity as metabolic rate was measured at the level of the whole animal and unavoidable physiological or psychological factors undoubtedly influenced metabolic rate estimates, leading to a large degree of scatter in the data (Fig. 2). The validity of the calculations on efficiency should also be questioned given that the study was not designed to test hypotheses about the efficiency of dolphin sound production to compare this with terrestrial animal values. First, the vocal cSELS reported in Fig. 2 are received levels and not source levels and conversions need to be made to account for this fact. Dolphins can also change the energy levels of their emitted sounds over several orders of magnitude (Jensen et al., 2012; Madsen et al., 2013) and vocal performance varied considerably within and

between bouts during both metabolic trials and source level measurements. In fact, vocal behavior was likely different between the two contexts because the sound production and reception pathways occurred in different media (occurring partly in air under the hood during trials and wholly underwater during source level measurements). In addition, most studies that report sound production efficiency are based on estimates of metabolic power during active calling and acoustic power averaged across many individual acoustic signals (Prestwich, 2007) as opposed to the estimates given here on the total metabolic cost of a vocal bout (including recovery costs) with vocal performance quantified as the cumulative energy of all the sounds produced in that bout. The current experiment was specifically designed to determine whether increases in vocal effort would result in increases in metabolic cost. So, while the above caveats might affect an estimate of sound production efficiency, the magnitude of the change in metabolic cost with change in vocal effort was estimated as accurately as possible, given that an animal's metabolic rate varies naturally, considering the methodological constraints of the experiment, and assuming that variability in the regression model not explained by vocal effort was randomly distributed among trials.

As vocal effort increased, there was a significant increase in metabolic rate over resting during the 2 min following sound production in both dolphins, and in the total oxygen consumption in the subject (dolphin B) that showed a wider range of vocal effort across trials. Specifically, there was an increase of 117.4 ml of oxygen consumed for every dB cSEL increase in vocal performance over a 2 min period in dolphin B, at least for the range of vocal performance of approximately 20 dB cSEL that was observed in the subject (Fig. 2F). Higher energy vocalizations from increased vocal effort likely necessitate higher energy requirements in muscles that actuate the sound-producing organs (Cranford et al., 2011), thereby resulting in higher metabolic costs. Although dolphin B produced squawks, we assume that the results are applicable to whistling dolphins as well, given that the two sound types are likely produced by the same sound-production mechanisms and the lack of a significant result in the whistle data is attributed to a restricted range of vocal performance of dolphin A.

Bottlenose dolphin whistle rate at the onset of vessel approach is double the rate when no boats are present (Buckstaff, 2004). In such a scenario, the cSEL would increase by 3 dB and would result in an increase of 352.2 ml of O_2 consumed. The cost of such vocal modification would be the equivalent of 7 kJ in caloric content. For comparison, the average energy content of food consumed by adult bottlenose dolphins in captivity ranges between 36,438 and 48,219 kJ day^{-1} (Kastelein et al., 2002, 2003). It is also important to emphasize that in the current study, dolphins A and B vocalized at a relatively high average repetition rate of 28.6 whistles min^{-1} and 99.6 squawks min^{-1} , respectively, during oxygen consumption measurements (Table 1). Field reported whistle repetition rates in free-ranging bottlenose dolphins are usually much lower, including those measured during vessel approaches, and depend on the behavioral context (Buckstaff, 2004; Janik and Sayigh, 2013). Metabolic costs at such repetition rates are predicted to be lower as well, based on extrapolating the results of the current investigation, although the accuracy of extrapolated metabolic costs for much lower whistle repetition rates is questionable. The driving force behind sound generation in bottlenose dolphins is pressurized air in the bony nasal passage (Ridgway et al., 1980; Amundin and Andersen, 1983). Whistles are much longer and require close to twice the nasal air pressure that echolocation click generation does (Ridgway and Carder, 1988; Ridgway et al., 2001; Cranford

et al., 2011). Thus, it is predicted that whistle production for communicative purposes would be energetically more costly than click production for biosonar and may explain why whistle repetition rates in wild bottlenose dolphins are usually much lower than those observed in this investigation. Indeed, the signal type most often associated with extremely high repetition rates in dolphins is broadband clicks, which is not surprising given that clicks are used for foraging and navigation (Au, 1993; Ridgway, 1983; Wahlberg et al., 2011; Branstetter et al., 2012). Field studies have shown that other delphinid species adjust their vocal amplitude in the presence of vessel noise (Holt et al., 2009), but the Lombard effect in bottlenose dolphins has not been demonstrated. Dolphins living in high noise level environments do not increase the source level of their whistles compared with other individuals living in lower noise conditions (Jensen et al., 2012). Thus, one should not assume that bottlenose dolphins would continuously vocalize and raise the source level of their communicative signals by 1 dB for every 1 dB increase in noise level when interpreting the results of the current study. Responses by free-ranging animals to human disturbance are complicated and individuals may wait for silent periods or avoid noisy areas when acoustic communication is critical instead of incurring the costs of increased vocal effort.

The costs of modified vocal behavior in response to noise are estimated to be quite modest under the hypothetical scenario we present here, although caution should be exercised in extrapolating these results to free-ranging animals for the reasons given above. Nonetheless, in coastal habitats near major ports, vessel transits typically occur many times per day, resulting in a high percentage of time that vessels increase ambient noise levels (Bassett et al., 2012; Erbe et al., 2012). Even modest metabolic costs of modified vocal behavior in chronically noisy habitats could have negative effects on certain individuals, particularly those who fail to meet their daily energy requirements during energetically vulnerable periods such as reproduction and lactation. Other responses, such as performing energetically expensive surface active behaviors (Noren et al., 2009), can also occur in the presence of vessels and associated noise. In such cases, the cumulative impact could be significant when the costs of vocal modification occur along with other metabolically costly responses as a consequence of the anthropogenic disturbance (Lusseau and Bejder, 2007).

Our findings provide new experimental evidence of a metabolic cost associated with increases in the acoustic energy of sounds produced by dolphins but the cost depends on the extent of the vocal modification. Increases in acoustic energy are a consequence of increasing the amplitude, duration and/or repetition rate of acoustic signals, which are consistent with behavioral modifications in noisy environments in free-ranging animals. Furthermore, our results in dolphins are consistent with those of bird and human studies that have found that oxygen consumption increases with increases in vocal repetition rate, loudness and duration (Hom et al., 1995; Russell et al., 1998; Oberweger and Goller, 2001; Franz and Goller, 2003). The current investigation provides the critical energetic link between vocal modification in noisy marine environments and potential population-level consequences for individuals that rely on acoustic communication for fundamental life functions. These data also represent an important step towards quantifying the collective biological consequences of animals living in noise-polluted habitats.

MATERIALS AND METHODS

Subjects and experimental procedure

The subjects were two male Atlantic bottlenose dolphins, dolphin A and B, that were 32 and 26 years old, respectively, and were maintained at a healthy

mass in outdoor pools (water temperature: 19–21°C) at Long Marine Laboratory in Santa Cruz, CA, USA. They were previously trained, using operant conditioning techniques and positive reinforcement, to rest and produce sounds on command while stationing, with minimal body movement, at the water surface under a metabolic hood (Noren et al., 2013). Experimental participation was voluntary (the dolphins were free to leave the hood or stop vocalizing at any point during the trial). Experimental trials were conducted with each dolphin separately following an overnight fast as in Noren et al. (2013). Each trial consisted of three consecutive phases: (1) initial baseline period when the dolphin remained still and quiet at the water surface for 10 min to measure RMR; (2) vocal period when the dolphin produced sound for approximately 2 min; and (3) recovery period when the dolphin again remained quiet for at least 10 min or until oxygen consumption returned to resting values. The dolphin was reinforced with food after completing the entire trial under the metabolic hood.

Both dolphins were trained over 6 months prior to data collection to produce either higher or lower amplitude sound (of the same sound type) using two different discriminative training cues, with a criterion of higher amplitude sounds being +10 dB relative to lower amplitude sounds. Only one training cue ('soft' or 'loud') was given during the vocal period of a given experimental trial. Both trial types (referred to as 'soft trials' and 'loud trials') were run within the same week in pseudorandom order. Vocalizations produced during trials were acoustically monitored in real-time and recorded using calibrated equipment as described below. All procedures were approved by the University of California, Santa Cruz Institutional Animal Care and Use Committee and conducted under US National Marine Fisheries Service permit No.13602.

Metabolic data collection and analysis

During each trial, oxygen consumption (\dot{V}_{O_2}) was measured with a Field Metabolic System (Sable Systems International, Las Vegas, NV, USA) using flow-through respirometry. Ambient air was drawn into the metabolic hood at 300 l min⁻¹, a rate sufficient to keep oxygen content in the hood above 20%. Excurrent respiratory gases were pulled through a sample line, dried and scrubbed of CO₂ using alternating tubes of Drierite (W. A. Hammond Drierite Co., Xenia, OH, USA) and Sodasorb (Sodasorb, Chemetron, St Louis, MO, USA) before entering the oxygen analyzer (FMS model, Sable Systems International). The oxygen analyzer was calibrated daily with dry ambient air (20.95% O₂) and the entire system was checked for leaks and the lag time measured weekly using the N₂ dilution method (Fedak et al., 1981). The percentage of oxygen (%O₂) in the sample line was monitored continuously during a trial and recorded every second by a laptop using Expedata acquisition and analysis software (Sable Systems International). The start and end time of all trial components (baseline, vocal and recovery) were marked on the computer and later adjusted using the measured lag time of the system prior to analysis. \dot{V}_{O_2} was calculated from the %O₂ data using eqn 4b in Withers (1977) and a respiratory quotient of 0.77. Respirations (number of breaths) were also recorded during each phase of the trials.

Metabolic rates were calculated for the following phases of each experimental trial: (1) RMR during the baseline period, (2) the 2 min vocal period and (3) 2 min following the vocal period. RMR was calculated by averaging \dot{V}_{O_2} during the most level 5 min of the baseline period (determined by the 'level' function in Expedata). Data from the first 2 min of the trial were excluded to remove any potential metabolic effects of the dolphin entering and stationing under the hood. Metabolic rates during vocalizations (vocal metabolic rates) were calculated by averaging \dot{V}_{O_2} during the 2 min vocal period. Metabolic rates during the post-vocal period (post-vocal metabolic rates) were calculated by averaging \dot{V}_{O_2} during the 2 min immediately following the vocal period to demonstrate that \dot{V}_{O_2} often remained elevated after sound production concluded. The metabolic cost of vocal activity was estimated according to the methods described in Noren et al. (2013). Briefly, the percentage increase in metabolic rate over RMR during the vocal period and 2 min post-vocal period were calculated by determining the percentage increase of vocal metabolic rate and post-vocal metabolic rate relative to RMR of the same trial. Although convenient for comparison with most previous investigations, the relative increases in metabolic rate do not take into

account the total cost above resting of sound production given that \dot{V}_{O_2} increases during sound production and often remains elevated for several minutes after sound production ceases (Noren et al., 2013). Thus, the total metabolic cost of sound production (in ml O_2 and representing the cost of sound production plus recovery costs above baseline resting cost) and total recovery duration (in min) were calculated according to methods described in Noren et al. (2013). Oxygen consumption was first integrated against time using the Expedata integration tool. Two parallel linear regressions were then fitted to the integrated data. The first linear regression was fitted to the 5 min of level baseline data (the baseline RMR) and the second was fitted to the final 10 min of the recovery period when the dolphin was presumed to have fully returned to the resting metabolic state. The difference in the y -intercepts between the regressions was then equal to the total cost above the resting rate established in the baseline period. The time to return to the resting metabolic state was calculated by finding the point at which the metabolic costs had reached 95% of the total costs for the vocal plus recovery period. The method described above assumes that the RMR before and after the vocal period are equivalent. In several trials, the dolphin's baseline RMR was higher than the post-vocal RMR and thus violated this key assumption. This occurred in 3/27 and 9/29 trials run in dolphin A and dolphin B, respectively, and these trials were excluded from this part of the analysis. Vocal performance was also statistically related to metabolic cost as the percentage increase of metabolic rate over RMR during both the vocal period and post-vocal period so that data from all trials could be included in the statistical analysis.

Acoustic data collection and analysis

Vocalizations produced during trials were acoustically monitored in real time and recorded using a calibrated Reson TC-4013 hydrophone (nominal sensitivity: -211 dB re. $1 \text{ V } \mu\text{Pa}^{-1}$, ± 3 dB from 0.02 to 170 kHz; Reson, Inc., Goleta, CA, USA) connected to an external voltage preamplifier (Reson VP2000, model EC6081, gain: 30–40 dB). The hydrophone, which was molded into the contact suction cup, was positioned securely on the midline of the dolphin's melon at 10 cm from the anterior edge of the blowhole using a measuring tape before each trial. The signal was digitized at a sampling rate of 96 kHz (16-bit resolution, MOTU Traveler, Cambridge, MA, USA) and then recorded onto a PC laptop using Ishmael software (Mellinger, 2001). Recordings during the vocal period of each trial were analyzed in Avisoft SASLab Pro (v.5.1.17; Avisoft Bioacoustics, Berlin, Germany). The acoustic recordings of each vocal period were first digitally high-pass filtered (128 taps, Hamming window, filter setting: 1.5 and 2.0 kHz for dolphin B and dolphin A, respectively) to remove extraneous low-frequency sounds. Each vocalization was then windowed, numerically labeled and measured according to the following: start and end time, duration, interval between start of each sound, received root mean square (rms) pressure (averaged over the defined duration, in μPa) and received acoustic energy (in $\text{Pa}^2 \text{ s}$). Measurements in the frequency domain (e.g. peak frequency at start, end and maximum) were also determined but showed relatively little variation within and between trials. From the individual vocalization measurements, the total number of sounds produced, mean duration, mean interval between the start of each sound, mean received acoustic pressure (adjusted for gain) and received cSEL (in dB re. $1 \mu\text{Pa}^2 \text{ s}$, adjusted for gain) were calculated for each trial. Note that source levels of vocalizations could not be measured during trials because sounds were produced under a reverberant hood at the air–water interface. Thus, received levels of vocal signals are used to measure variations in vocal performance across trials within a subject and these should not be interpreted as approximations of source levels of entirely water propagated signals.

Estimation of source levels

To determine whether the vocal ability of the tested dolphins might be comparable with that of free-ranging individuals reported in the published literature, source levels of the sounds produced underwater by each dolphin were estimated outside of the context of an experimental trial but within the period of the study. For the source level estimates, the trainer was positioned on a float in the middle of the test pool away from the pool edge and the contact hydrophone (Reson TC-4013) was placed in the same position on the dolphin as during trials. Another calibrated Reson hydrophone

(TC-4033) connected to an external preamplifier (Reson VP2000) was positioned at a depth of 0.5 m. The trainer stationed the dolphin underwater at an estimated depth of 0.5 m with the dolphin facing the calibrated hydrophone at 0 deg and at a horizontal distance of 1 m. The trainer then gave the cue to produce either soft or loud vocalizations for a 15 s interval. The dolphin was then allowed to surface and given a short break before repeating the exercise 3–4 times for both soft and loud vocalizations. The sounds were recorded using the same analog-to-digital acoustic equipment and settings, and analyzed using the same software as for metabolic trials.

Video analysis

During each trial, the dolphin's stationing position under the metabolic hood was recorded using a video camera (DCR-HC21 model, Sony Corporation, Tokyo, Japan). Videos were later analyzed to quantitatively assess any differences in body posture and movement across trial types. Specifically, the number of fluke beats and the number of times the dorsal fin broke the water surface during vocal periods were scored for each trial by an observer who was unaware of the experimental trial type (soft or loud). A fluke beat was counted if the dolphin moved the fluke and the base of the fluke in a large undulating motion. A dorsal fin break was counted if any part of the dorsal fin broke the water surface after being completely submerged.

Statistical analysis

Linear regression analyses were performed to determine the relationship between metabolic performance (percentage increase over RMR during the vocal period, percentage increase over RMR 2 min post-vocal period, total metabolic cost of sound production above resting, and total recovery duration) and vocal performance (measured as received cSEL) for each dolphin separately given that they produced two different sound types. Linear regression analyses were also run to test the relationship between respiration rates during each of the trial periods (baseline, vocal and post-vocal periods) and vocal performance. Assumptions of the linear regression model were fulfilled by testing for linearity via residual plot inspections, normality via the Shapiro–Wilk test and homogeneity of variance via the Constant Variance tests before linear regression analysis was performed. For the video analysis, a Student's t -test or Mann–Whitney U -test, if t -test assumptions failed, was used to determine whether fin breaks (as a proxy of body posture) or fluke motions differed across soft and loud trial types within a dolphin subject. All statistical tests were run using SigmaPlot 12.3 (Systat Software Inc., San Jose, CA, USA).

Acknowledgements

We thank the volunteer staff from UCSC Long Marine Laboratory for animal care and assistance during data collection. We especially thank Traci Kendali and Beau Richter for providing their expertise in animal training and handling, which made this study possible. Maia Goguen (UCSC) and Annie Fadely (NOAA NMFS NWFSC) provided assistance with respiration and video data analysis. We are grateful to Drs Sam Ridgway and Ted Cranford for thoughtful discussions on dolphin anatomy and muscle activity involved in sound production. We also thank two anonymous reviewers for their helpful suggestions on previous versions of the manuscript.

Competing interests

The authors declare no competing or financial interests.

Author contributions

M.M.H. and D.P.N. conceived the study. M.M.H., D.P.N. and R.C.D. designed and executed the study. M.M.H., D.P.N., R.C.D. and T.M.W. interpreted the findings. M.M.H., D.P.N., R.C.D. and T.M.W. drafted and revised the article.

Funding

This work was funded by the US Office of Naval Research awards N0001410IP20067 and N0001411IP20017 to M.M.H. and D.P.N., N000141110341 and N000140811273 to T.M.W.

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