

Respiratory patterns and oxygen consumption in singing zebra finches

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Summary

Song production in birds is driven by temporally complex respiratory patterns. In zebra finches (*Taeniopygia guttata*), song consists of repetitions of a stereotyped sequence of distinct syllables (motif). Syllables correspond to distinct expiratory pulses, which alternate with short deep inspirations. We investigated the effect of the song motor pattern on respiration using a newly developed mask system to measure oxygen consumption while simultaneously monitoring subsyringeal air sac pressure. The metabolic cost of song is closely related to song duration (mean=85.7 $\mu\text{l O}_2\text{g}^{-1}\text{min}^{-1}$ over pre-song levels) and confirms earlier estimates for this species. When motif duration is controlled for, there is only small interindividual variability in oxygen consumption per motif. The oxygen measurements were also used to evaluate various methods of estimating and reporting the metabolic cost. Up to 20 s before song, respiratory activity and oxygen consumption increased. Shortly before and

during the introductory notes of the song bout, respiration and oxygen consumption decreased markedly. In some individuals, significant hyperventilation occurred during song, causing almost complete apnea after the song. In three different birds, we measured tracheal airflow and air sac pressure during song. Birds with higher airflow during song relative to flow during quiet respiration had a more pronounced decrease in respiratory activity after the song bout. These results suggest that gas exchange continues in the lung during the song bout and that long expiratory pressure pulses of the song motif can lead to hyperventilation but that no oxygen debt resulted from song. This research allows a first assessment of respiratory constraints that may influence the evolution of song complexity.

Key words: song, bird, respiration, metabolic cost, zebra finch, oxygen consumption, *Taeniopygia guttata*.

Introduction

Most sound production in birds involves a conversion of fluid dynamic energy (airflow) into acoustic energy (sound). Airflow past the vocal organ, the syrinx, is generated by contraction of respiratory muscles while syringeal muscles regulate the airway resistance (for a review, see Suthers et al., 1999; Goller and Larsen, 2002). In addition, the sound characteristics are typically also controlled by muscle activity. For example, increasing fundamental frequency of sound is closely correlated with an exponential increase in the electromyogram (EMG) activity of the ventral syringeal muscles (Goller and Suthers, 1996a). Similarly, some amplitude modulation is generated by active opening and closing of the syringeal valve (Goller and Suthers, 1996b). Once sound is generated in the syrinx, it can be modified by upper vocal tract filtering. Of the various theoretically possible mechanisms for altering the filter properties of the upper vocal tract, beak movement is the best described. Opening of the beak shows a positive correlation with sound frequency and, possibly, with sound amplitude (e.g. Westneat et al., 1993; Hoese et al., 2000; Williams, 2001). The summed activity of the various motor systems involved in singing represents the direct cost of sound production.

This direct cost can be estimated by assessing changes in metabolic rate associated with vocal behaviour. To date, the metabolic cost of song production in passerine birds has been investigated in four species. The estimated increase in oxygen consumption during song, relative to resting metabolic rate (RMR), was 1.77–3.44-fold in zebra finches, canaries (*Serinus canaria*) and European starlings (*Sturnus vulgaris*) (Oberweger and Goller, 2001) but reached 9-fold in Carolina wrens (*Thryothorus ludovicianus*; Eberhardt, 1994). However, the wide range found in different species is more likely to reflect methodological difficulties in studying song in a respirometry chamber than actual differences in the metabolic cost of song production (Eberhardt, 1996; Gaunt et al., 1996; Oberweger and Goller, 2001).

Aside from the metabolic cost of singing, gas exchange is also of interest because respiratory patterns during song are very different from the rhythmic pattern of quiet breathing (e.g. Suthers and Goller, 1998). Typically, song is generated during expiration, and the airflow is driven by increased expiratory pressure. The duration of individual expiratory pulses may vary greatly within a song. Inspirations taken in between expiratory pulses are typically of short duration, and

inspiratory pressure exceeds that during quiet respiration to enable short but deep inhalations. Thus, both the rhythm and intensity of respiratory movements are drastically altered for song production. Many aspects of gas exchange during the respiratory pattern of song are unexplored. Gas exchange may, however, play an important role in song organization. For example, respiratory needs may dictate the duration of syllables, syllable sequence and the temporal pattern of sound and silent intervals (e.g. Hartley and Suthers, 1989; Suthers and Goller, 1998). Such a role would, therefore, put physiological constraints on how sexual selection can influence the evolution of the temporal pattern of song (e.g. Podos, 1996).

Zebra finch song is characterized acoustically by a variable number of short-duration introductory notes and a stereotyped sequence of distinct syllables (motif; Zann, 1996). Males often repeat motifs to form a song bout. The acoustic sequence corresponds to a distinct respiratory pattern (Fig. 1). Most syllables are generated during an expiratory pressure pulse, but a few high-frequency syllables may also be generated during inspiration (Goller and Daley, 2001; Franz and Goller, 2002). The pattern of respiratory pressure is characteristic for each syllable of the motif. The song motif consists of an alternating sequence of stereotyped expiratory and equally stereotyped inspiratory pressure pulses (minibreaths; Wild et al., 1998).

To further investigate the metabolic cost of singing and the effect of song motor patterns on gas exchange, a method is required that allows a higher temporal resolution of oxygen consumption and avoids other problems of assessing metabolism in respirometer chambers (Oberweger and Goller, 2001). To circumvent the problems with a respirometer chamber, we used a breathing mask system. The results confirm earlier estimates of the direct metabolic cost of singing but also explore how the respiratory patterns of song affect gas exchange.

Materials and methods

Birds

Zebra finches (*Taeniopygia guttata* Gould) were bred in a flight aviary at the University of Utah. Adult males (age >120 days) were used for experimental testing. During the experiment they were kept individually in 32 cm×23 cm×30 cm wire cages on a 13 h:11 h L:D cycle. Directed song was elicited by placing a female in a separate cage in front of the male.

Air sac pressure recording

After a male was removed from the aviary and placed in an individual cage, an elastic belt with a Velcro tab on the back was placed around the thorax. A leash was attached to the Velcro and led through the top of the cage. It was connected to a tether arm, which was counterbalanced to support the additional mass from the backpack (2.5–3 g after transducers were attached; see below), allowing free lateral and vertical movement in the cage. Birds were also able to rotate freely but were limited to 2–3 full rotations in the same direction while

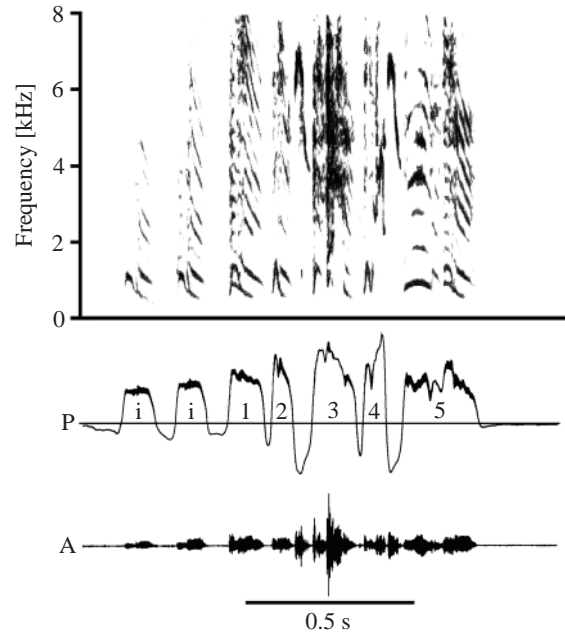


Fig. 1. Zebra finch song consists of a stereotyped sequence of distinct syllables (motif). Song is illustrated as oscillogram (A) and spectrogram (top panel). The air sac pressure (P) pattern for song is equally stereotyped; the horizontal line indicates ambient pressure. Numbers mark the different expiratory pressure pulses of the motif, which is preceded by two introductory notes (i).

data were recorded. Typically, singing resumed 1–3 days after males were placed on the tether system.

Surgery was performed once singing resumed. Birds were deprived of food and water for 1 h before surgery. Using isoflourane anaesthetic, a small hole was made in the abdominal wall into the left posterior thoracic air sac, and the tip of a flexible cannula (Silastic tubing; 1.65 mm o.d., 6 cm length) was inserted into this hole. The cannula was sutured to the rib cage. The cannula insertion site was sealed with tissue adhesive (Nexaband) to prevent leakage of air. The free end of the tube was connected to a piezoresistive pressure transducer (FPM-02PG; Fujikura, Tokyo, Japan), which was mounted on the Velcro tab.

Oxygen measurements

Oxygen consumption was measured using a custom-made mask system. Each bird was fitted with a head net (Fig. 2) made from elastic thread (approximately 1 mm diameter) several days before surgery. The head net was fitted to the specific dimensions of each individual bird, such that an elastic ring was situated at the base of the beak and the other segments provided a tight enough fit to prevent movement. A 1 cm-long piece of polyethylene tubing (1.57 mm o.d., 1.14 mm i.d.) was sewn to the top of the head net to serve as an air outlet, and a 2 cm-long piece at the bottom of the net served as an air inlet.

The elastic beak ring served as an anchor for a rigid plastic ring (1.2 cm diameter), around which the mask, made of balloon material, was stretched. Mask volume was

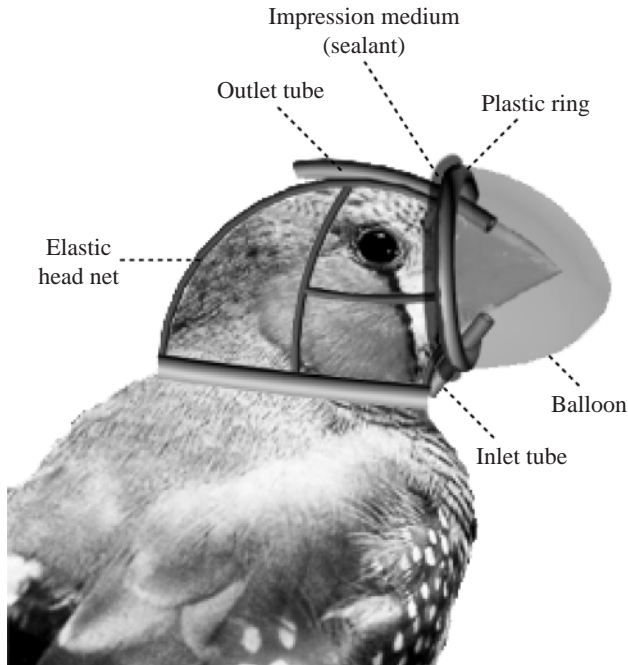


Fig. 2. Schematic illustration of the mask system used to measure oxygen consumption. The key components are labelled. Inlet and outlet tubes were routed from the mask to a backpack and then to the oxygen analyzer.

approximately 1 ml. The plastic ring was attached to the elastic beak ring of the head net using dental impression medium (Reposil, hydrophilic vinyl polysiloxane impression material, Type I, very high viscosity), forming a seal around the beak against the face. The ring was large enough that the bird could open and close its beak freely and beak movements during singing were not impeded. The mask outlet tube was connected to Silastic tubing (0.76 mm i.d., 1.65 mm o.d.), which was guided over the top of the head to the backpack and from there up the length of the leash. A second set of identical tubing was run from the backpack to the 'reference' channel of the oxygen analyzer (see below). For both channels, tubing from the bird was run through a small chamber filled with desiccant (Drierite) and CO₂ absorbent (Ascarite). From there, tygon tubing (10 cm) led to the oxygen analyzer through a partially inflated condom to buffer small fluctuations in pumping pressure (Ellington et al., 1990). All tubing was kept as short as possible to optimize the temporal resolution of the system.

A flow control unit (R-2; Applied Electrochemistry, Pittsburgh, PA, USA) was used to pull air through the analyzer. The percentage difference in oxygen content between the air of the two channels was measured with an Applied Electrochemistry S-3A/2 oxygen analyzer (N 37M sensor). All oxygen measurements were taken at room temperature (21–23°C). The sensor was calibrated with room air (20.95% oxygen) before each recording session. The flow rate was kept at 860 ml min⁻¹. Once the sensor reading had stabilized at zero, the analyzer was connected to the mask on a bird. Recording started after an adjustment period of 15–20 min and lasted for

up to 2 h. All birds were observed throughout the recording period, and information about their locomotor activity was noted for each song bout.

Testing the mask system

Our system for measuring oxygen consumption was tested for several possible sources of error: (1) leakage of air at the mask attachment site, (2) pressure conditions inside the mask during breathing and (3) the possibility of partial re-breathing of exhaled air. To test for the occurrence of the first two potential sources of error, we attached a piezoresistive pressure transducer *via* a T-connector to the inlet tube. The pressure conditions inside the mask were slightly subatmospheric during both respiratory phases. This indicates that there were no leaks in the mask system. At the same time, it confirms that breathing activity does not affect the pressure inside the mask to the extent that flow reversals occurred during either respiratory phase.

We used tracheal flow measurements (see below) to determine the volume of air that was exchanged during quiet breathing and song syllables. Extrapolating from the highest flow rate during song, we estimate the exchanged volume to be maximally 180 ml min⁻¹ (data from three birds). Because the flow rate through the mask system was 860 ml min⁻¹ and the volume of the mask was 1 ml, the air in the mask was sufficiently replenished to prevent re-breathing of exhaled air by the bird.

Data recording

Song was recorded with a microphone (AT8356; Audiotechnica, Stow, OH, USA) placed in front of the cage. The microphone output was amplified (100×; Brownlee 410, San Jose, CA, USA). The analogue output voltage from the oxygen analyzer (percentage difference between the two air channels) was recorded simultaneously with song and the voltage output of the pressure transducer using a multi-channel digital recorder (135T; TEAC, Tokyo, Japan). All signals were recorded with a 24 kHz digitization rate.

Oxygen analysis

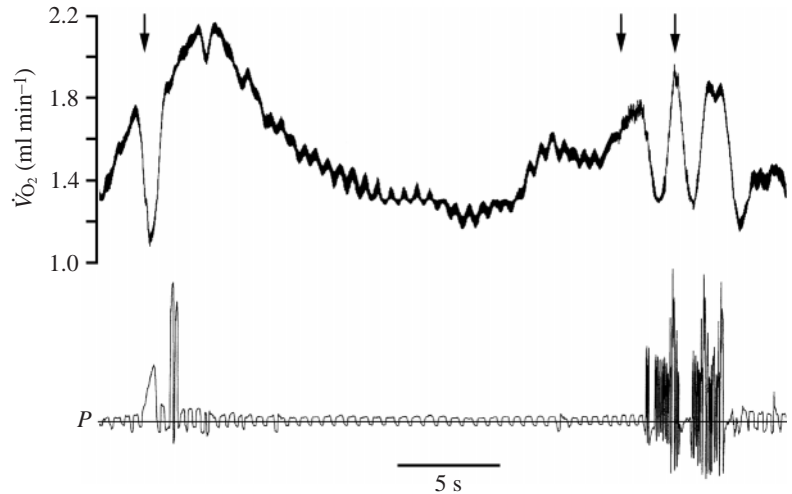
Air sac pressure and oxygen signals were digitized (sample rate = 5 kHz) for analysis using SIGNAL 3.1 (Engineering Design, Belmont, MA, USA) software and a DT-2821 AD board (Data Translation, Marlboro, MA, USA). Recorded oxygen values were corrected for standard conditions. The following equation for a mask system (Withers, 1977) was used to calculate the volumes of consumed oxygen (ml min⁻¹):

$$\dot{V}_{O_2} = \dot{V}_E (F_{I_{O_2}} - F_{E_{O_2}}) / (1 - F_{I_{O_2}}), \quad (1)$$

where \dot{V}_{O_2} is the rate of oxygen consumption, \dot{V}_E is the rate of airflow being pulled out of the mask, $F_{I_{O_2}}$ is the fractional concentration of oxygen entering the mask, and $F_{E_{O_2}}$ is the fractional concentration of oxygen leaving the mask.

Oxygen consumption during song was related to pre-song activity. However, baseline oxygen consumption was difficult to determine in some cases. Movement, as well as respiratory

Fig. 3. The mask system provided enough temporal resolution to see small oscillations in oxygen consumption rate (\dot{V}_{O_2}) that corresponded to individual breath cycles, measured as air sac pressure (P). The horizontal line represents ambient pressure; air sac pressure above and below the line correspond to expiration and inspiration, respectively. Respiratory changes caused by defecation, presentation of the female and song have rapid responses in the oxygen consumption of the bird. Defecation (marked by first arrow) has a distinct pressurization in the air sacs with simultaneous closure of the airways (airflow data are not displayed). It is accompanied by a pronounced decrease in oxygen consumption. Note that the defecation event is followed by two calls (substantially higher pressure peaks). Elevated respiratory rate caused by female presentation (second arrow) is accompanied by increased oxygen consumption, as is subsequent song (third arrow marks the first of two song bouts).



changes due to excitement caused by presentation of the female, led to increases in oxygen consumption similar in magnitude to those found for song (Fig. 3). In these cases, the segment of the oxygen trace that corresponded to the song bout could not be identified unambiguously. Oxygen baseline determinations were therefore made only for those recordings that had a stable period within 5–10 s before song. For an individual song bout, the volume of consumed oxygen (song O_2 -volume) was determined by integrating the corresponding segment of the oxygen consumption trace above pre-song baseline. This O_2 -volume was then divided by body mass to calculate the mass-specific O_2 -volume.

There was a delay between the occurrence of respiratory events and the time that they were registered by the oxygen analyzer. This delay was determined for each bird and each recording session using defecation events. Defecation causes distinct pressurization in the air sacs but the airways are simultaneously closed, preventing exhalation (Fig. 3; airflow data not displayed). This interruption of normal breathing (500–900 ms) causes a sharp decrease in O_2 consumption. The time from the peak in air sac pressure to the lowest point in the oxygen trace was used to determine the time delay between the pressure and oxygen recordings.

The total duration of the song bout was measured using the air sac pressure recordings because the mask system made acoustic measurements variable in quality. Song duration was calculated by subtracting the inter-song intervals from the total bout length. The relationship between oxygen consumption and the respiratory pattern was investigated by determining the respiratory volume and duration of each expiration and inspiration. Segments of quiet respiration for each recording were used to determine the ambient pressure level, which marks the switching points between respiratory phases. Points where the pressure trace crossed the zero line were used to determine the duration of each phase. Respiratory volume of expirations and inspirations was calculated by integrating each area above and beneath the ambient pressure line, respectively. After a translation along the time axis (rotation function in SIGNAL) of the oxygen trace to correct for the time delay, a

corresponding average oxygen consumption level was determined for each segment in the pressure trace for comparison.

Tracheal flow measurements

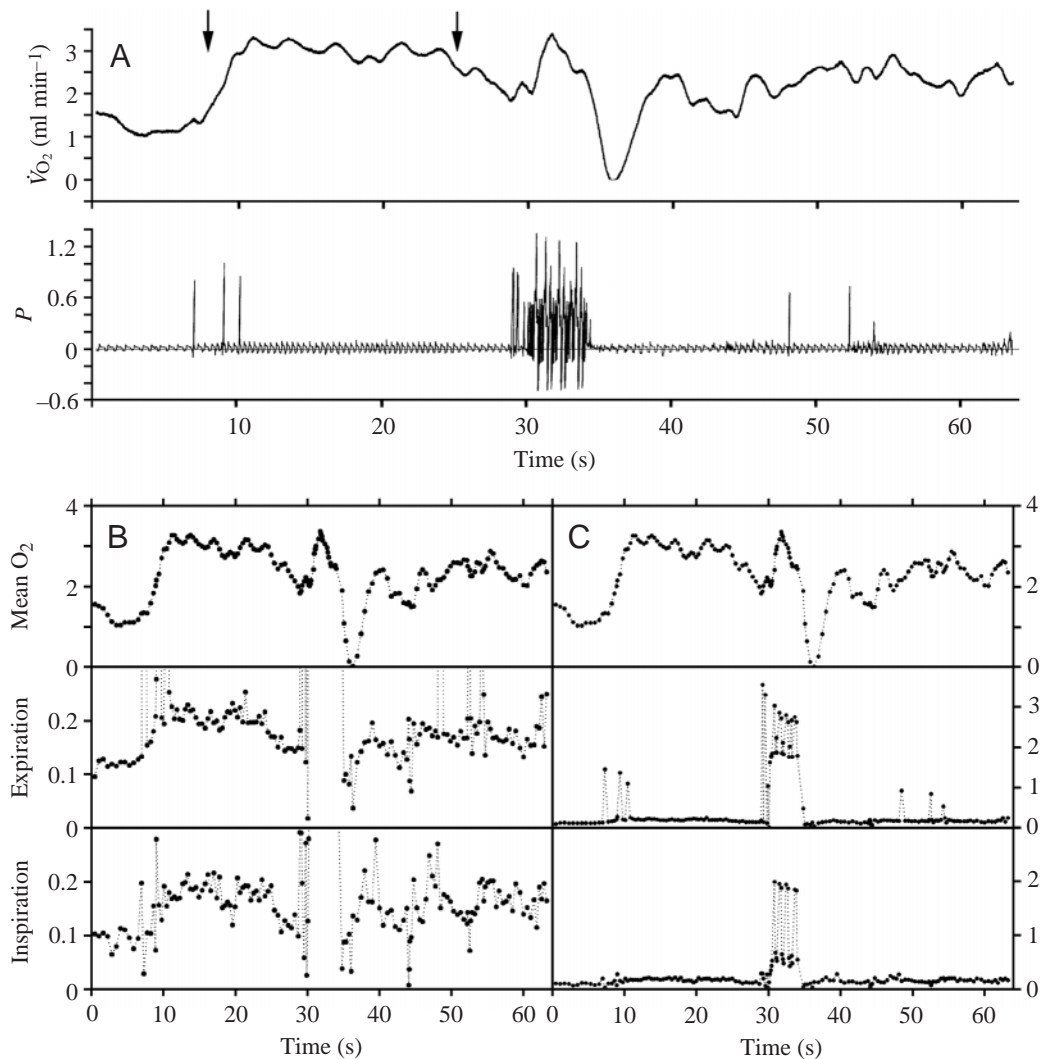
In three male zebra finches, tracheal airflow was measured simultaneously with air sac pressure. Flow was monitored with a microbead thermistor (BB05JA202, Thermometrics, Edison, NJ, USA), which was surgically implanted into the lumen of the base of the trachea (more detailed descriptions of the procedures are presented in Hartley and Suthers, 1989; Suthers et al., 1994). A feedback circuit (Hector Engineering, Ellettsville, IN, USA) supplied current to heat the thermistor to a constant temperature (approximately 60°C). The voltage needed to maintain this temperature was proportional to the airflow past the thermistor bead. After song was recorded, birds were euthanized and air was supplied with a known flow rate through the air sac cannula to calibrate the non-linear voltage output of the thermistor. With this technique, calibrations were only obtained for expiratory airflow. Airflow data were linearized with calibration values to calculate approximate volumes of air for single expiratory pulses during quiet respiration and song. Absolute values are only an approximation, because during calibration the possibility of slight positioning differences of the bead compared with the position during song cannot be excluded.

All experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Utah.

Results

We collected data on oxygen consumption and air sac pressure during song in six zebra finches. The temporal response of the oxygen measurements was sufficient to show small oscillations when the bird switched between the respiratory phases (Fig. 3). Typical breath rates in zebra finches during quiet respiration are in the range of

Fig. 4. Respiratory changes cause fluctuations in oxygen consumption. (A) The pattern of oxygen consumption ($\dot{V}O_2$) and respiration (air sac pressure, P) can vary substantially and rapidly. The horizontal line in the pressure trace represents ambient pressure. (B,C) The volume and duration of each expiratory and inspiratory pressure pulse were calculated along with the mean oxygen consumption rate ($\dot{V}O_2$) for that segment. Data points for expiration and inspiration are values calculated by dividing the volume of each pulse by its corresponding duration, giving an estimate of respiratory rate and effort. Whereas in B the axes for expiratory and inspiratory effort are scaled to illustrate small fluctuations unrelated to song, C shows these data at full scale. These traces illustrate that oxygen consumption reflects even small fluctuations in respiratory activity. As respiratory activity increases (marked by first arrow in A), demonstrated by shorter air sac pressure pulses of higher amplitude, $\dot{V}O_2$ also increases. Shortly before song (second arrow in A) there is a small decrease in oxygen consumption that corresponds to a decrease in respiratory rate (long duration and small amplitude pressure pulses). Respiration increases during song followed by a distinct peak in $\dot{V}O_2$. After song there is a significant decrease in respiration (pulses of short amplitude and duration) and a large decrease in oxygen consumption. Note that vocalizations are characterized by markedly increased amplitude of air sac pressure, as illustrated by the song bout, but also by several calls around the 10 s and 50 s mark (A). Because the syringeal resistance changes for vocalization and airflow patterns change, this altered pressure is not accompanied by a correspondingly large change in oxygen consumption.



2–3 breaths s⁻¹, indicating that switching events of 150–200 ms duration can be partially resolved by our system. Substantial and rapid fluctuations in oxygen consumption, unrelated to song, were present in most of the 60 s long data files. These fluctuations correlate well with changes in respiratory depth and frequency, as indicated by the analysis of air sac pressure recordings (Fig. 4).

Oxygen consumption during song

Song was accompanied by characteristic profiles in oxygen consumption. At the beginning of song, oxygen consumption initially decreased relative to pre-song levels. During song, there was a consistent increase followed by a post-song decrease in O₂ consumption. When longer bouts were sung, the oxygen trace showed motif-by-motif oscillations (Fig. 5).

However, oxygen consumption decreased from the first motif to later motifs of the bout. During long bouts, the decline in the oxygen peak associated with each motif levelled off towards the end of the bout. Sometimes the distinct peak during song was masked by other activities, such as movements shortly before or during song. Because it was impossible to determine how much such activity contributed to the oxygen consumption during song, these song bouts were not used for analysis.

Metabolic cost of singing

We measured the cost of song using different analysis techniques for comparative purposes. Measurements of the volume of oxygen consumed in excess of the pre-song baseline level (song O₂-volume) yielded a mean cost of singing of

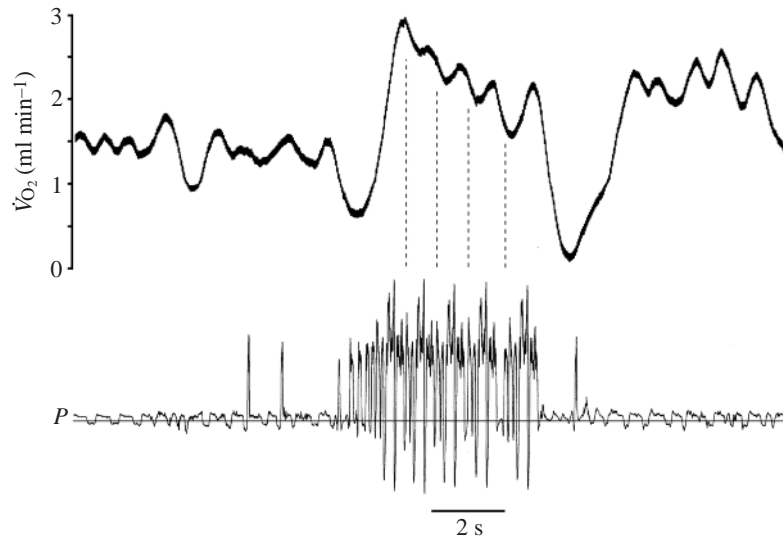


Fig. 5. Oxygen consumption fluctuates during a song bout. Longer song bouts showed oxygen consumption rate (\dot{V}_{O_2}) oscillations (top) that corresponded to motif repetitions in the pressure pattern (P). The first of the five motifs has the highest peak in \dot{V}_{O_2} . The level of the \dot{V}_{O_2} peak lowers for each subsequent motif until stabilizing at the end. Broken vertical lines represent the beginning of subsequent motifs.

song duration, which enters the calculation of song O_2 -volume rate (Table 1).

Respiratory patterns of song

Song was elicited by presenting a female to the male in a separate cage. Presentation of the female typically resulted in an increase of respiration and oxygen consumption 5–20 s before the song bout occurred (Figs 3, 4). This increase in \dot{V}_{O_2} could be greater than 2-fold and was accompanied by faster respiratory rate and deeper breaths. The increase prior to song was shorter and of smaller amplitude when the cage with the female was sitting in front of the male's cage for a longer period of time.

A song bout was consistently accompanied by a short, but marked, decrease in \dot{V}_{O_2} shortly before the peak. In four of the birds, the decrease before song coincided with the introductory notes of the first motif. However, no significant correlation was found between the number and duration of introductory note series and the decrease in \dot{V}_{O_2} . In the other two birds (R31 and B1), the decrease in \dot{V}_{O_2} began before the introductory notes. For these birds, a change in quiet respiration before song corresponded to the decrease in \dot{V}_{O_2} . The amplitude of respiratory pressure decreased and respiratory rate declined, resulting in lower values of calculated respiratory activity (Fig. 4). Although these values do not take possible changes in syringeal resistance into consideration, tracheal airflow data in other individuals show that no marked changes in syringeal resistance are noticeable a few seconds before song is initiated.

This decrease in \dot{V}_{O_2} shortly before song probably contributes to the metabolic peak that is associated with the first motif. \dot{V}_{O_2} decreases slightly with each motif until a constant level is reached after 4–5 motifs (e.g. Fig. 5). Individual motifs can be clearly distinguished in most birds by

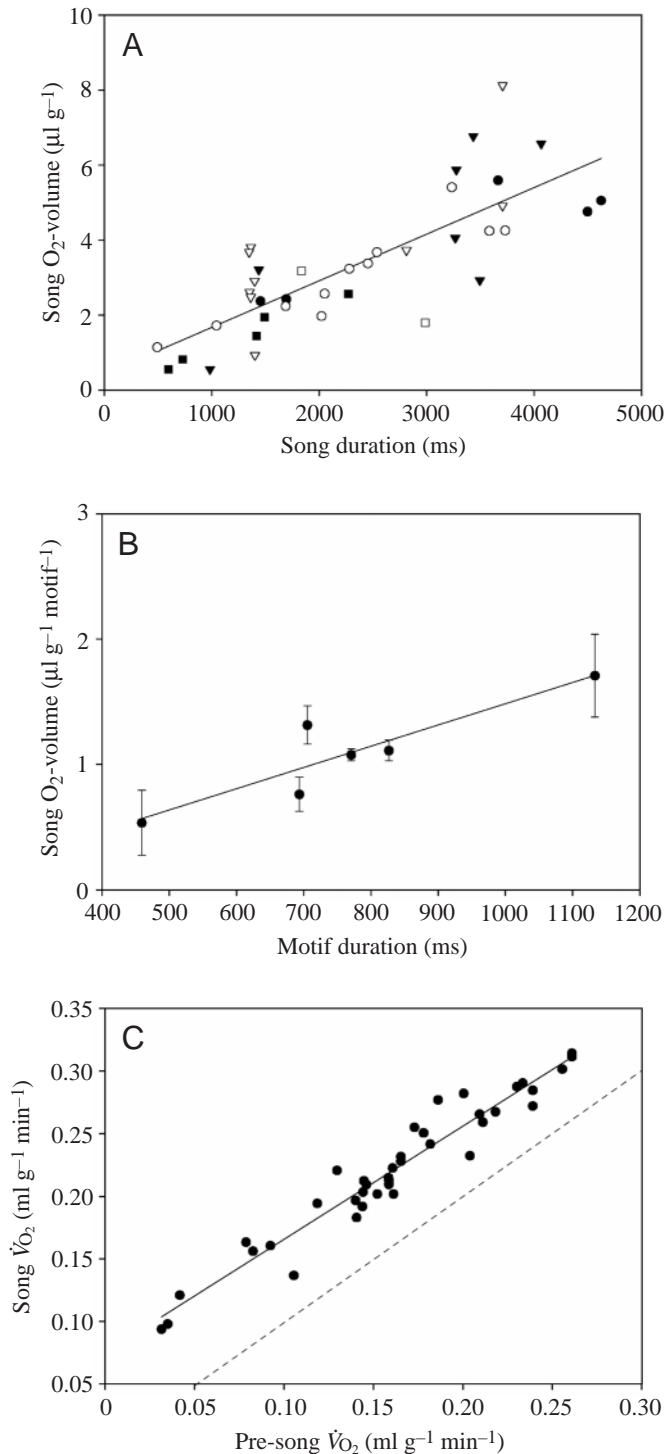
85.7 $\mu\text{l O}_2 \text{g}^{-1} \text{min}^{-1}$ (Table 1). Song O_2 -volumes increased with increasing song duration (Fig. 6A). The slope of this relationship indicates a decreasing cost of singing with increasing song duration. However, this trend can be attributed to the observation that oxygen consumption was highest for the first motif and then slowly decreased to a constant level for subsequent motifs (Fig. 5). The initial high level for the first motif is related to the decrease in oxygen consumption before the song (see below).

Interindividual differences in song O_2 -volume per motif can be largely explained by motif duration. The average song O_2 -volume per song motif increased with motif duration (Fig. 6B) for the six individuals. Song duration measurements do not include the variable number of introductory notes.

Metabolic cost was also measured as mean oxygen consumption during song (Table 1). Song \dot{V}_{O_2} depended strongly on pre-song \dot{V}_{O_2} (Fig. 6C), which varied substantially between different song bouts within and among individuals (Table 1; Fig. 6C). If the difference between song \dot{V}_{O_2} and pre-song \dot{V}_{O_2} is calculated to estimate the metabolic cost of song, values are consistently lower than those resulting from the data on song O_2 -volume. This difference can be largely explained by the longer duration of the oxygen peak, which is used to calculate mean \dot{V}_{O_2} , compared with

Table 1. Oxygen consumption measurements

Bird	<i>N</i>	Mass (g)	O ₂ consumption (ml g ⁻¹ min ⁻¹)			O ₂ -Volume per song (μl g ⁻¹)		Song duration (ms)
			Pre-song (mean±S.E.M.)	Song	Song-pre-song (μl g ⁻¹ min ⁻¹)	(μl g ⁻¹)	(μl g ⁻¹ min ⁻¹)	
B8	5	12.7	0.054±0.011	0.127±0.014	73	4.048±0.683	76.22	3186.76
B1	11	12.1	0.154±0.003	0.209±0.005	55	3.084±0.386	81.04	2283.27
P31	7	13.7	0.184±0.019	0.244±0.015	60	4.283±0.856	90.10	2852.06
R31	9	13.5	0.191±0.010	0.255±0.007	64	3.693±0.666	108.04	2050.84
P27	5	13.8	0.233±0.020	0.286±0.019	53	1.465±0.366	67.57	1300.86
P28	2	14.1	0.099±0.007	0.149±0.012	50	2.486±0.690	61.69	2410.85
Mean (<i>N</i> =39)			0.162±0.010	0.222±0.009	60	3.325±0.287	85.69	2328.15



small fluctuations in \dot{V}_{O_2} throughout the bout. This indicates that the release of expired air during the song motif varies or that O₂-content of expired gas is not constant.

To address this issue, we estimated volumes of exchanged air during quiet respiration and song. Typically, the volume of air exchanged during introductory notes and short syllables of the motif was lower than or equal to that during quiet respiration. The volume of long syllables of the motif was substantially higher than during quiet respiration in two of the

Fig. 6. (A) The volume of oxygen consumed during song (V) increases with duration (D) of song bouts (regression equation: $V=0.437+0.00124D$; $r=0.797$, $F=64.409$, $P<0.0001$). Different symbols represent data points from different individuals (N as in Table 1). (B) Individuals with a longer motif duration (d) consumed a larger volume of oxygen (v) per motif (regression equation: $v=-0.210+0.0017d$; $r=0.9024$, $F=17.54$, $P=0.014$). (C) Song oxygen consumption rate (s) is closely related to pre-song oxygen consumption rate levels (p) (regression equation: $s=0.075+0.904p$; $r=0.967$, $F=529.02$, $P<0.0001$). The broken line indicates equal values on the x - and y -axes.

birds and not much higher in one individual (Table 2). This indicates that there is individual variability in air exchange during the song motif and that the volume of air exchanged during different syllables of the motif may be quite variable.

At the end of the song bout, a marked decrease in \dot{V}_{O_2} occurred consistently. This decrease below the pre-song baseline level lasted from 0.5 s to several seconds and varied from a few µl of O₂ to several ml. In some instances, oxygen consumption was reduced to zero (Fig. 7A). The decrease in \dot{V}_{O_2} coincided with a decrease in respiratory activity (Figs 4, 5, 7), which ranged from a reduced rate and amplitude to complete apnea (Fig. 7A,B). The reduction in respiratory activity after song bouts predicted the reduction in \dot{V}_{O_2} (Fig. 8) but varied among individuals. Within individuals, the duration of apnea was generally positively correlated to song duration. In the three individuals, regression coefficients were significant ($r=0.71-0.97$, $P=0.021-0.026$), and in two individuals there was a positive but non-significant trend.

Of the three males in which tracheal flow was recorded, one (R44) showed no marked changes in respiration after song, whereas two (R42 and R35) showed a pronounced decline. Airflow during the song motif was not much higher than that during quiet respiration for R44 and was substantially higher in the other two individuals (Table 2), suggesting that the volume of air exchanged during the song motif is related to the amount of reduction in respiration.

This pronounced reduction in post-song respiratory activity was followed by a subsequent increase in respiration and \dot{V}_{O_2} in comparison with pre-song levels. It was difficult to measure the exact volume of the increase because it declined gradually to new levels and was often masked by post-song locomotor activity. It appears, however, that the increase correlated with the duration and amplitude of the post-song reduction in respiration and \dot{V}_{O_2} .

Discussion

In this study, we analyze gas exchange and respiratory patterns during song in zebra finches using a mask system with high temporal resolution. We assess the metabolic cost of singing and discuss methods of calculating the cost of singing. In addition, detailed measurements on respiratory parameters give insight into gas exchange during the drastically altered ventilation patterns of song.

Table 2. Average flow measurements

Bird	Quiet respiration (ml s ⁻¹)	Before song (ml s ⁻¹)	Introductory notes (ml s ⁻¹)	Song (ml s ⁻¹)	After song (ml s ⁻¹)	Factorial increase over quiet respiration			
						Before	Intro	Song	After
R44	1.689	1.686	1.492	1.950	1.432	0.998	0.883	1.155	0.848
R42	0.989	0.900	1.643	1.910	0.692	0.910	1.661	1.931	0.700
R35	0.755	0.635	0.953	1.308	0.207	0.841	1.262	1.732	0.274

Metabolic cost of singing

The metabolic cost of avian vocalization has been studied in some detail (e.g. Horn et al., 1995; McCarty, 1996; Jurisevic et al., 1999; Bachmann and Chappell, 1998; Chappell et al., 1995). Song production in songbirds has received less experimental attention (Eberhardt, 1994; Oberweger and Goller, 2001) but has been subject to extensive discussion (Gaunt et al., 1996; Eberhardt, 1996). While metabolic rate during song was found to be high in Carolina wrens (maximum increase was up to 9-fold over resting metabolic rate; Eberhardt, 1994), oxygen consumption of three other species increased much less during song (1.77–3.41-fold over RMR;

Oberweger and Goller, 2001). Oberweger and Goller suggest that this discrepancy might be attributable to differences in how the energetic cost of song was assessed. The present study confirms that reporting the metabolic cost of song as an increase over basal metabolic rate (BMR) or RMR can be misleading. In male zebra finches, mean song \dot{V}_{O_2} varies substantially within and among individuals, but this variation can be largely explained by variations in pre-song \dot{V}_{O_2} (Fig. 6C). Overall, the range of pre-song \dot{V}_{O_2} values (approximately 0.03–0.26 ml g⁻¹ min⁻¹) is at least 4-fold greater than the mean difference between song \dot{V}_{O_2} and pre-song \dot{V}_{O_2} (approximately 0.05 ml g⁻¹ min⁻¹), which results in erroneous estimates of the cost of singing by calculating a factorial increase (song \dot{V}_{O_2} /RMR). Average \dot{V}_{O_2} measurements during song are, therefore, not a reliable estimate of the cost of song production in zebra finches (the present study). A similar conclusion was reached for other species (Oberweger and Goller, 2001) and might explain the unusually high estimates of song metabolic rate in wild-caught Carolina wrens (Eberhardt, 1994).

Song O₂-volume is used here to estimate the cost of singing relative to that of pre-song metabolism. Data obtained with the mask system can be compared with the same estimates made from oxygen consumption measurements in a respirometer chamber. Oberweger and Goller (2001) induced singing in male zebra finches by presenting a female in front of a small transparent window in the opaque chamber. Males perched near this window and sang directly into the air outlet of the respirometer chamber. Estimates for the cost of singing over pre-song levels obtained with this method (1.2–1.36-fold increase) are close to those for the mask system (1.23–1.5-fold increase for five birds; 2.35-fold increase for B8). Although there is a good agreement overall, the interindividual differences again illustrate the limitations of reporting cost as a factorial increase. Variation in pre-song metabolic rate can explain a large degree of variability in the factorial increase for song (Fig. 9).

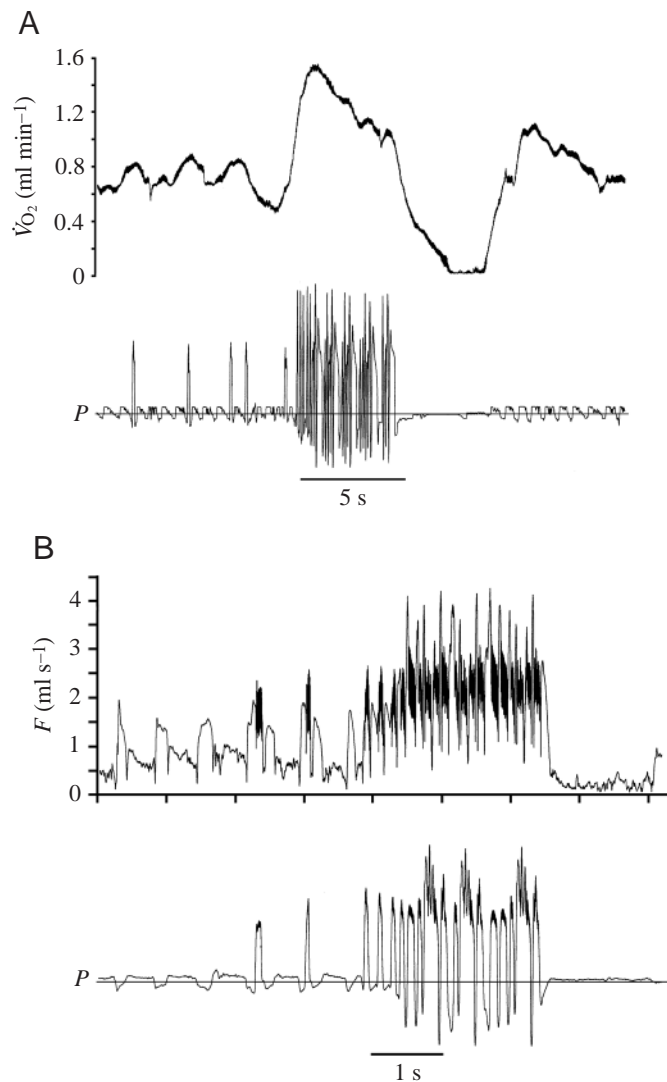


Fig. 7. Significant apnea can occur after song. (A) After a deep inspiration following the song bout, respiratory movements cease for several seconds, as indicated by air sac pressure (P) measurements. Oxygen consumption rate (\dot{V}_{O_2}) also declines and remains at zero for 200 ms during apnea. \dot{V}_{O_2} increases again as normal respiratory movements are resumed. (B) Tracheal airflow (F), recorded simultaneously with air sac pressure in another individual, confirms that apnea is correctly inferred from air sac pressure patterns. Airflow is near zero for more than 1 s following the song bout.

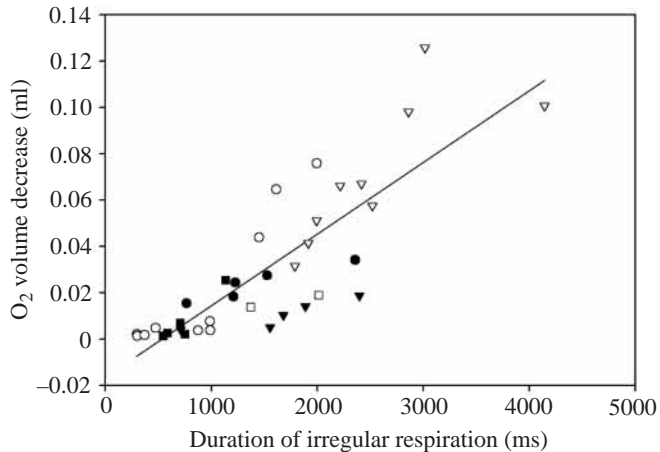


Fig. 8. The duration of irregular respiration (from decreased amplitude and rate of respiration to complete apnea; D) corresponds to the volume by which oxygen consumption declines (V) after song (regression equation: $V = -0.016 + 3.092 \times 10^{-5} D$; $r = 0.832$, $F = 76.616$, $P < 0.0001$). Different symbols represent different birds.

Our measurements also allow comparison between the volumetric measurements (song O_2 -volume) and mean song \dot{V}_{O_2} data. The volumetric data indicate, on average, a 1.369-fold higher cost of singing if calculated as a rate (i.e. per unit time; Table 1). The main reason for this discrepancy is the fact that the duration of increased oxygen consumption during the song bout is somewhat greater (200–900 ms) than the duration of the song bout itself. Because the former is used in the calculation of the mean \dot{V}_{O_2} and the latter in the song O_2 -volume, calculations using the mean \dot{V}_{O_2} values underestimate the cost of song production (Table 1). The small difference between song bout duration and the duration of increased oxygen consumption can probably be attributed to the washout characteristics of the mask system.

In conclusion, we suggest that the absolute cost of song production can be estimated most reliably as the volume of oxygen consumed over pre-song metabolic rate (Fig. 6A,B). Oberweger and Goller (2001) used such estimates for comparisons among different taxa and found these comparisons to be more meaningful than if factorial increases are used. However, changes in ventilation patterns before and after song are tightly linked with the song motor pattern and therefore need to be considered in assessing the effective cost of singing behaviour in zebra finches (see below).

Interindividual differences in metabolic cost

The predominant cost of singing presumably reflects muscle activity generating the fluid dynamic energy (airflow) for sound production. In addition, muscle activity controlling sound characteristics (syringeal muscles) and sound modification (upper vocal tract) must also contribute to the direct cost of singing. These direct costs are accompanied by indirect costs resulting from postural changes and non-specific

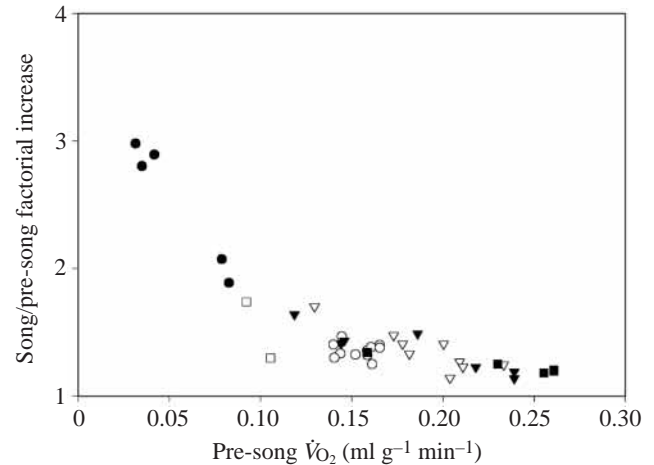


Fig. 9. The factorial increase of song metabolic rate to pre-song metabolic rate decreases with increasing pre-song oxygen consumption rate (\dot{V}_{O_2}). Different symbols identify data points from different individuals.

song-related movements (e.g. courtship dance in zebra finches (Zann, 1996; Williams, 2001).

In zebra finches, song is generated by a series of alternating expiratory and inspiratory pressure pulses. The respiratory rhythm of song differs from that of quiet respiration by its irregularity and rapid switching events between the respiratory phases. The amplitude of expiratory and inspiratory pressure pulses is drastically increased compared with that of quiet respiration. This increase is effected by increased activity of respiratory muscles (Wild et al., 1998) and by increased syringeal resistance, which results from a combination of passive forces (Bernoulli) and valving action by syringeal muscles (Goller and Suthers, 1996b; Goller and Larsen, 2002).

Because the coarse respiratory pattern of song is similar among all individuals used in this study, we expect the major direct cost of song production to be similar for our individuals. However, small differences in the acoustic structure of song, air sac pressure patterns and flow rates during phonation exist among individuals, indicating that some interindividual variability in the metabolic cost to produce song may exist. Song O_2 -volume per motif increased with increasing motif duration (Fig. 6B), confirming that the restructuring of respiratory movements constitutes the major cost of song. Variability in additional costs may be reflected in the limited variation of song O_2 -volume measurements from this linear relationship with motif duration. However, it is likely that resolving such minor differences reliably may be beyond the power of the currently available techniques and equipment.

Respiratory events and oxygen consumption

Song in zebra finches changes the normal pattern of quiet respiration and, consequently, gas exchange in complex ways. These changes are not confined to the motor activity of singing, but consistent alteration of respiration occurs before and after the song bout. Several seconds before the initiation of song,

respiratory rate and amplitude increase followed by an increase in oxygen consumption. The increase is more pronounced if a female is presented in a separate cage to elicit singing but is also present if the cage with the female is sitting near the male's cage for an extended period of time. This respiratory change with increased oxygen consumption suggests that a motivational change takes place up to 20 s before the actual motor action of singing is initiated. It would be interesting to know whether this motivational change (arousal; Zann, 1996) is linked to the motor program for song production (e.g. Margoliash, 1997; Wild, 1997) and how respiratory changes for both events are coordinated.

Male zebra finches sometimes also sing undirected song, where song is not directed to a conspecific (Zann, 1996). The above-described motivational changes may not occur prior to undirected song, which is described as a non-aroused state (Zann, 1996). Because our birds did not sing undirected song during the 2-h periods with the mask, no direct comparison between the metabolic costs can be made. Heart rate measurements during song indicate a smaller increase in the pre-song period for undirected song (M.F. and F.G., unpublished observation), suggesting a lower motivational state.

Shortly before the song bout, respiratory depth and rate decrease briefly, at least in some individuals, causing a reduced rate of oxygen consumption. Neural mechanisms underlying this change are unknown. In other individuals, the decrease in oxygen consumption also coincided with the train of introductory notes preceding the first song motif. This suggests that the rapid alternating between short expiratory pulses and minibreaths during the series of introductory notes either reduces the time air resides in the lung for gas exchange or the exchanged volume is lower than during quiet respiration, leading to lower oxygen consumption. The differences in the ratio of tracheal airflow before song and during introductory notes for the three males (Table 2) indicate that both possibilities may account for the observed reduction in metabolic rate.

The expiratory pressure pulses of the song motif vary in duration and amplitude, and the volume of exhaled air is also variable. Typically, long syllables occur at the end of the motif. This variability between expiratory pulses of the motif is reflected in the oxygen consumption. Each motif of the bout was distinguishable by a small fluctuation in the oxygen consumption trace, such that the long pressure pulses corresponded to rising oxygen consumption. Although flow rate does not differ systematically between expiratory pulses of short and long duration, a long-duration expiration must result in a greater volume of expired air over a period of time during which a number of short syllables and minibreaths might occur. Consequently, increased oxygen consumption will be registered for this time period, even if flow rates and oxygen extraction at the respiratory surfaces remained the same. The methods of the present study are not sensitive enough to allow determination of oxygen extraction efficiency.

The volume of air exchanged during the motif is likely to

influence the subsequent respiratory pattern. The airflow data indicate that the volume of exhaled air during the long pressure pulses of the motif varies among individuals, and those with more marked hyperventilation also show more post-song reduction in respiration. Although the airflow data were collected in other individuals than the ones used for the metabolic measurements, we suggest that the degree of hyperventilation also varies among those individuals, accounting for varying degrees of reduced post-song respiration, ranging from only slightly reduced respiratory depth and rate to complete apnea.

This interpretation is supported by physiological evidence collected in singing canaries (Hartley and Suthers, 1989). Although infrequent, apnea up to 0.6 s after song was recorded, while the volumes of expired and inspired air were closely matched during preceding song. Data extrapolated from the tables using the mean tidal volumes and the frequency show variable flow rates (up to 3-fold increase over quiet respiration) for this bird (no. 2) depending on the syllable type. The variability in flow rate for particular syllables and variability in repetition rates of each syllable may explain why apnea was found only infrequently in canaries (Hartley and Suthers, 1989).

The amplitude of respiratory movements is probably mediated by CO₂ receptors in the lung (for a review, see Fedde and Kuhlmann, 1978). Apnea can be produced in birds by passing air, oxygen or hydrogen over the lungs. Peterson and Fedde (1968) demonstrated in the chicken (*Gallus domesticus*) that apnea was caused by lowering the intrapulmonary CO₂ concentration and that CO₂ receptors in the lung have rapid responses to changes in the CO₂ concentration. Below a certain CO₂ concentration, some receptors discharge irregularly and may even cease to fire action potentials. Cessation or experimental interruption of their connections to the brain markedly decreases respiratory rate (Fedde et al., 1963).

The bout of reduced respiration is followed by increased respiratory activity and higher oxygen consumption. Although it is difficult to quantify the duration of the increase, we suggest that it is a consequence of the post-song reduction and not of the song bout itself. Neither reduced respiration nor the following increase was entered into our estimate of the metabolic cost of singing.

In conclusion, singing behaviour in zebra finches includes direct and indirect costs. Whereas we report the cost of generating the song bout with some confidence (see above; direct cost), it is more difficult to assess indirect costs related to motivation and the courtship dance (Williams, 2001) and various changes in respiratory patterns before and after song. Because the experimental situation is likely to have affected the intensity of the courtship behaviour, we did not measure indirect costs associated with singing behaviour here. Zebra finches in our setup directed their song to the female in the adjacent cage but did not perform courtship dances of normal intensity. The tethering procedure and the mask may have physically impeded males or altered their motivational state, preventing normal courtship dancing.

Minibreaths and gas exchange

Minibreaths are short, typically silent inspirations in between expiratory pulses of song. Minibreaths replenish the air expelled during the phonatory expiration (Hartley and Suthers, 1989; Goller and Daley, 2001) but it is unclear whether very short minibreaths allow gas exchange to take place in the lung. In Waterslager canaries, minibreaths can be as short as 15 ms with an inhaled volume that is less than the tracheal deadspace (as low as 0.09 ml; Hartley and Suthers, 1989). The song organization of zebra finches is different to that of canaries and can therefore only give limited insight into this question. The duration of song bouts is typically not as long as song duration in canaries and does not consist of sustained repetitions of individual syllable types (phrases). While some minibreaths of zebra finch song are as short as 15–20 ms, airflow data suggest that the volume exhaled during short syllables is typically greater than the tracheal deadspace (F.G., unpublished data). Oxygen consumption occurred throughout the motif, with fluctuations during different syllables probably reflecting the volume of exhaled air per unit time (see above). It is possible that rapid alternating between expiration and inspiration reduces residence time of inhaled air near the gas exchange surfaces of the lung, which would result in higher oxygen content of exhaled air. Although this possibility cannot be excluded, it is unlikely in zebra finches considering that, during quiet respiration, a specific volume of inhaled air is not exhaled during the same respiratory cycle (Bretz and Schmidt-Nielsen, 1972). However, such a mechanism may be more important in canaries with long phrases of short syllables produced at high minibreath rates (Hartley and Suthers, 1989). Although song in a respirometer chamber did not cause a measurable oxygen debt in canaries (Oberweger and Goller, 2001), it is not clear whether such a debt occurs during particular phrases but is repaid during other phrases of a long-duration song. Oxygen requirements would therefore constrain syllable sequence (i.e. syntax) but do not appear to constrain song duration in canaries or song bout duration in zebra finches.

In summary, respiratory physiology of singing behaviour presents multi-faceted aspects to our thinking about song evolution (Searcy and Anderson, 1986; Searcy and Yasukawa, 1996). It can give insight into how the respiratory aspects of song can provide information about the quality of the singing male and thus affect song evolution. The direct metabolic cost of generating and modifying song is the most obvious aspect. However, the similar nature of respiratory patterns among individuals appears to result in only small variation in the metabolic cost regardless of the acoustic structure of the song. Gas exchange during song may affect song syntax and duration. Considerable differences in air exchange during song exist between different syllables within and among individuals, suggesting that maintaining gas exchange is an important possible constraint. Surprisingly, in the zebra finch, it is hyperventilation and not a lack of oxygen that influences respiratory patterns even seconds after the song. Other species

may face the opposite problem of providing enough oxygen during song, indicating that the need for maintaining gas exchange may pose a severe constraint on respiratory motor patterns for song.

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