The metabolic cost, energy output and efficiency (i.e. the ratio of energy output to metabolic cost) of sound production were compared among male grey treefrogs (Hyla versicolor) as a function of body size and temperature. The effects of call length (in notes per call) and dominant frequency (in kHz) were also considered. Cost, determined from the amount of oxygen consumed, averaged 12.1 mJ per note and was dependent only upon body mass. Acoustic energy per note, determined from oscillograms of recorded calls, averaged 0.34 mJ and was dependent only upon temperature. Conventional theory suggests that the efficiency of sound production should be a function of the ratio of the linear size of the radiating structures to the wavelength of the sound generated (i.e. efficiency is assumed to be a function of the product of mass$^{0.33}$ and frequency), but efficiency in H. versicolor was found to be a function of the product of temperature$^{2.1}$ and mass$^{-1.08}$. Adjusting for temperature and body mass, the efficiency of sound production in H. versicolor (average 2.4 %) is greater than the efficiency of other frog species for which data are available. Temperature may affect acoustic energy output because trunk muscle contraction speed increases with temperature, which increases the velocity of airflow across the vocal cords.

Key words: sound production, metabolic cost, efficiency, grey treefrog, Hyla versicolor, temperature, body size.
are both correlated with a number of call variables in *H. versicolor* and other frogs (Zweifel, 1968; Gerhardt, 1978; Gayou, 1984). Body mass does influence the cost of call production (Taigen and Wells, 1985), but the effects of temperature on the cost of call production are not known. However, temperature does affect the contractile strength and the contraction frequency of calling muscles (McLister et al., 1995), both of which are important for determining the amount of positive work done during cyclical muscle activity (Josephson, 1993; Syme and Stevens, 1988; Swoap et al., 1993). Adult male *H. versicolor* may range in body mass from under 4 g to over 10 g and call over a broad range of temperatures (8–33 °C; Gayou, 1984; Grafe, 1997); therefore, any influence of either mass or temperature on the energetics of call production may be biologically important to the species.

A potentially important determinant of the energetics, and particularly the efficiency, of sound production in animals is the wavelength-to-size ratio. The fraction of the energy of a vibrating object that becomes acoustic energy in the surrounding medium depends on the ratio of the linear size of the vibrating object to the wavelength of the sound generated (Bennet-Clark, 1989, 1998; Prestwich, 1994). Therefore, the wavelength-to-size ratio should be a covariate of the efficiency of sound production, and small animals should be able to produce high-frequency sounds more efficiently than low-frequency sounds (Prestwich, 1994). Unfortunately, high-frequency sounds are not very useful for long-range communication because they undergo more attenuation in the environment than do low-frequency sounds (Marten and Marler, 1977; Wiley and Richards, 1978; Wells and Schwartz, 1982). Consequently, it has been argued that the low efficiency of sound production observed among frogs and insects occurs because selection for long-distance communication favours wavelengths that travel effectively through the environment even though such wavelengths are disproportionately large relative to the size of the radiating structures (Ryan, 1985; Prestwich et al., 1989).

To estimate the effects that temperature and body size have on the energetics of note production in *H. versicolor*, data were collected for male *H. versicolor* across a wide range of temperatures and body sizes. Oxygen consumption during call production was used to measure the metabolic cost of sound production, and sound pressure values obtained from field recordings were used to measure the energetic output during call production. To evaluate the importance of wavelength on the energetics of note production, spectral analysis was performed upon recordings of mating calls to measure the dominant frequency (i.e. the carrier frequency with the most sound energy associated with it) of each individual. Because frequency is inversely proportional to wavelength, and because the linear dimensions of the sound radiator should be proportional to mass$^{0.33}$ (i.e. the nominal length), the wavelength-to-size rule predicts that the efficiency of sound production should increase as a function of the product of mass$^{0.33}$ and frequency. Nominal length rather than the length of any specific anatomical structure was chosen as an indicator of relative resonator size because the identities of the radiating structures of frogs and the extent to which they function as radiators are not entirely clear. It is generally assumed that the vocal sac is the principal radiator, but there is little direct evidence to confirm this, and other structures may contribute to sound radiation as well (Purgue, 1997). Whichever anatomical structures do contribute to the radiation of sound in *H. versicolor*, it is reasonable to assume that they scale isometrically with body size and that their linear dimensions therefore scale proportionally to nominal length.

### Materials and methods

#### Specimens

Two different populations of *H. versicolor* Le Conte were used: one near Ashland, Missouri (Boone Co.), and the other at Reis Biological Field Station in Steeleville, Missouri (Crawford Co.). Data were collected over a 3 year period (1996–1998) during the months of May and June when both populations were chorusing.

#### Metabolic energy measurements

Sound production in most species of frogs occurs in conjunction with the oscillatory flow of air through the larynx, back and forth, between the lungs and the vocal sac, with sound typically being produced during the forward phase of each airflow cycle (Martin and Gans, 1972; Dudley and Rand, 1991). Each airflow cycle is driven by the contraction of the trunk muscles, which constitute up to 15% of a male’s body mass (Marsh and Taigen, 1987; Prestwich et al., 1989). Given the large mass of the trunk muscles, most of the metabolic cost of sound production in frogs is presumably consumed in conjunction with the production of airflow cycles. Therefore, the cost per airflow cycle is a reasonable basis of comparison among frogs and the ‘note’, which refers to the total amount of sound energy generated during a single airflow cycle (McLister et al., 1995), is an appropriate acoustic unit to use when comparing the energetics of sound production in frogs. The mating call of *H. versicolor* consists of several amplitude modulations delivered in rapid succession, with each amplitude modulation corresponding to a single airflow cycle and, therefore, to a single note (Girgenrath and Marsh, 1997; see Fig. 1A).

The calling muscles of frogs are capable of very high rates of aerobic metabolism. Consequently, even though the cost of call production may be very high, calling frogs do not generally develop a large ‘oxygen debt’, and the recovery costs associated with sound production are negligible (Wells and Taigen, 1992). Therefore, it is valid to estimate the metabolic cost of sound production entirely from the amount of oxygen consumed during sound production.

Male frogs were collected from an active chorus between 21:00 h and 02:00 h and allowed to amplex with a female that had been collected on a previous night. The amplexed pairs were then taken back to the laboratory or to a location in the field that was away from the chorus to prevent variation in
chorus intensity from influencing male calling activity (Wells and Taigen, 1986). After at least 30 min of amplexus, the males were removed from the backs of the females and placed inside an airtight, 11 chamber for 25 min. Allowing the male to amplex prior to placing him in the container greatly increased the likelihood that he would call while in the chamber. A General Electric model 3-5301B tape recorder was placed beside the container to record every call that the male produced during the 25 min interval. At the end of the 25 min interval, a 50 ml air sample was removed from the chamber through a stop valve in the lid. The ambient temperature was recorded. The next day, males were placed in an airtight 11 chamber for 25 min in the laboratory. The chambers were placed in water baths to maintain an air temperature within the chamber that was consistent with the temperatures at which the males were calling the previous night. At the end of the 25 min rest period, a 50 ml air sample was removed from the container. The body mass of each male was then measured. Whenever frogs were to be placed inside a metabolic chamber, the metabolic chamber was first rinsed with water at ambient temperature to provide the frog with a moist, humid environment.

The fractional volume of oxygen in each of the air samples collected was determined by running the air samples, in an open-loop mode, through an Applied Electrochemistry S-3A oxygen analyser and oxygen sensor after first passing it through a column of Drierite and Ascarite to remove any water vapour and CO2, respectively. The analyser measured the fractional volume of oxygen to four decimal places. Total oxygen consumption per 25 min interval (VO2) while calling and while at rest could then be calculated using Vleck’s (1987) equation:

\[ V_{O_2} = (V_{total} - V_{H_2O})(F_{O_2} - F_{E_{O_2}})/(1 - F_{E_{O_2}}), \]

where \( V_{total} \) is the volume of air in the metabolic chamber, \( V_{H_2O} \) is the volume of water vapour inside the metabolic chamber determined by multiplying \( V_{total} \) by the temperature-specific fractional volume of water in saturated air (Hogman et al., 1959), \( F_{O_2} \) is the fractional volume of oxygen in the chamber at the time it was sealed (assumed to be 0.2095), and \( F_{E_{O_2}} \) is the fractional volume of \( O_2 \) in the chamber at the end of the 25 min interval as determined from the analysed air sample. Final \( V_{O_2} \) measurements were converted to standard temperature and pressure values. The total metabolic cost of calling for each frog was determined by subtracting the volume of oxygen consumed during resting from the volume consumed while calling and multiplying the difference by 20.1 J ml\(^{-1}\)O2. The conversion factor of 20.1 J ml\(^{-1}\)O2 was used because the respiratory quotient (RQ) for \( H. \) versicolor during call production is 0.81 (Grafe, 1997).

The tape recordings made while the males were calling inside the containers were played back to count the total number of calls produced. Fifty calls from each recording were examined with a Nicolet 3091 digital oscilloscope to determine the average call length (in notes per call). The product of the average call length and the total number of calls was used to determine the total number of notes each frog produced during the 25 min interval. The metabolic cost, \( E_{Met} \), per note was calculated as the metabolic cost of calling divided by the total number of notes produced. The number of notes produced by each frog during the 25 min interval ranged from 0 to over 7000. Frogs that produced fewer than 1000 notes were excluded from the analysis since frogs that called infrequently when inside the containers were often observed to be physically active, which would lead to an overestimate of the cost per note. Frogs that called frequently tended to remain stationary.

**Acoustic energy measurements**

The intensity \( I \) (W m\(^{-2}\)) of an acoustic signal can be represented by the equation

\[ I = p^2/pc, \]

where \( p \) is the pressure amplitude, \( \rho \) is the density of air, and \( c \) is the speed of sound in air (Alexander, 1983). Since the product of \( \rho \) and \( c \) changes little across a wide range of humidity and temperature values (Hogman et al., 1959), it can be treated as a constant. Sound pressure level (SPL; in dB) is determined by the equation

\[ SPL = 10\log_{10}(I/10^{-12}). \]

Assuming that a microphone generates a voltage amplitude proportional to \( p \) (Alexander, 1983), the \( \log_{10} \) of the average squared voltage of a tape-recorded signal should be proportional to the root mean square (rms) SPL of the original acoustic signal.

A PC with a National Instruments board (AT-M10-16E-10) and LabView software was used to sample tape recordings at 40 kHz to produce an array of voltage values that could be displayed as a digital oscillosgram of the recorded signal. Subarrays of voltage values corresponding to specific portions of the oscillogram were isolated, each individual voltage value \( (v_i) \) within the subarray was squared, and the average \( v_i^2 \) for the subarray was calculated as \( \Sigma(v_i^2)/n \), where \( n \) is the number of elements within the subarray. A digital oscillogram 1 s in duration, for example, would contain a total of 40,000 sampled voltage values, and a subarray of the digital oscillogram that was 50 ms in duration would have an \( n \) of 2000.

To determine the appropriate conversion equation for transforming values of \( \log_{10}[\Sigma(v_i^2)/n] \) into values of rms SPL for the original acoustic signal, a series of seven pure tones (2 kHz) of known intensity (72, 77, 82, 87, 92, 97 and 102 dB), was recorded with a Sony Professional Walkman tape recorder and a Sony ECM-909A microphone with the tape recorder set at recording level 6 and the microphone held 50 cm from the sound source. These tones were generated with a Dynoscan 3010 function generator and a push–pull emitter follower (Horowitz and Hill, 1989) to amplify the signal. The rms SPL of these tones was determined during recording by a Radio Shack SPL meter placed alongside the microphone. A frequency of 2 kHz was selected because it approximates the dominant carrier frequency of the mating calls of \( H. \) versicolor. The conversion equation was determined by the linear regression of rms SPL of the pure tones as a function of
log₁₀[Σ(vᵢ²/n)] calculated for the tape recordings of the pure tones.

The same tape recorder and microphone were used to record the calls of male _H. versicolor_ in the field. All recorded males were calling from tree branches elevated between 1 and 2 m above the ground, and an effort was made to locate males calling from thin branches with minimal surrounding vegetation. The microphone was held either to the left or right of the frog, and it was kept in the same relative position during recording by using the tips of twigs or leaves in the environment as reference points. After the calls had been recorded, the frogs were collected and weighed. The distance between the perch site and the microphone position was measured, as was the temperature at the perch site.

To measure the acoustic energy output per airflow cycle of a calling _H. versicolor_, individual note cycles were isolated from digital oscillograms of entire calls. A single note cycle consists not only of one note but also of the silent interval between one note and the next such that note cycle duration is equal to the inverse of the note repetition rate (NRR). The rms SPL for the entire note cycle was calculated from log₁₀[Σ(vᵢ²/n)], and rms SPL was converted into average intensity, _I_ave, using equation 3. Assuming that sound radiated outwards from a calling treefrog in a spherical pattern (Gerhardt, 1975; Prestwich et al., 1989), _I_ave was converted into units of acoustic energy, _E_Ac_, using the equation:

\[ E_{Ac} = 4\pi d^2 NRR^{-1} I_{ave}, \]

where _d_ is the distance between the calling frog and the microphone.

Power spectra were generated for each note using the LabView signal analysis function ‘power spectrum.vi’. The frequency yielding the highest power value from each LabView-generated power spectrum was taken as the dominant frequency for each note (Fig. 1B).

The average _E_Ac_ and the average dominant frequency for each frog were calculated from average values for the sixth, seventh and eighth notes of three different calls, for a total sample size of nine for each individual frog. Since there is some variability among notes, particularly at the very beginning of each call, it was important to compare notes of equivalent position within each call. Oscillograms of calls (Fig. 1A) suggest that, by the sixth note of a call, note production has reached a reasonably stable equilibrium and there is little progressive change in note dynamics between the sixth and last notes of a call. Therefore, any note between the sixth and the last note of a call should be representative of a typical note. Measurements of note repetition rate and call length were also taken from the oscillograms of field-recorded calls.

To verify the accuracy of the approach used in this study for measuring acoustic energy from the recorded calls of frogs, a series of electronically synthesised calls of known rms SPL were recorded and analysed with the same equipment and procedures used for frogs calling in the field. The synthetic calls mimic real _H. versicolor_ calls both in the pattern of amplitude modulation and in harmonic structure. The method of producing these synthesised calls and their acoustic structure has been described elsewhere (Gerhardt et al., 1996). Synthetic calls with rms SPLs of 75, 80, 85, 90, 95 and 99 dB were recorded at level 6.

**Results**

**Reliability of analysis**

If field recordings are a reliable means of measuring the _E_Ac_ of frog calls, a clear and consistent relationship must exist between the fast rms SPL values of the sounds recorded and log₁₀[Σ(vᵢ²/n)] measured from the digitised oscillograms of the tape recordings. For the recording equipment used in the present study, a strong correlation did exist between the sound intensity measured directly from pure tones and the value of log₁₀[Σ(vᵢ²/n)] determined from tape recordings of the same pure tones (_r^2=0.989; P<0.001_) with a best-fit linear relationship between the two variables of:

\[ \text{rms SPL} = 0.068 \log_{10}[\Sigma(v_i^2/n)] - 7.98 \]
Efficiency of sound production in treefrogs

for a recording level of 6 and a playback volume level of 5. Altering either the recording level during recording or the volume level during playback resulted in different regression equations but had little effect on the strength of the correlation.

The accuracy of equation 5 in converting the voltage readings of complex tape-recorded acoustic signals into values of rms SPL was confirmed by the high correlation between the known rms SPL of the notes of synthetic calls and the rms SPL calculated from the tape recordings of the same synthetic calls ($r^2 = 0.995; P < 0.001$).

Metabolic and acoustic energy per note

In total, 75 male *H. versicolor* were used in this study. Measurements of $E_{Ac}$ were made for 51 individuals in the field and measurements of $E_{Met}$ were made for 43 individuals either in the laboratory or under controlled conditions in the field. In total, 19 males were used for both $E_{Ac}$ and $E_{Met}$ measurements. Average call and energetic variables did not differ between the Ashland and the Reis populations (Table 1), so the data were pooled. Overall, mean $E_{Ac}$ per note was 0.340 mJ and mean $E_{Met}$ per note was 12.1 mJ. Among the 19 males for which both $E_{Ac}$ and $E_{Met}$ measurements were available, the average efficiency was 2.44% (Table 1). Call lengths produced by males during the $E_{Ac}$ measurements in the field tend to be longer than the those produced by males during the $E_{Met}$ measurements in the laboratory, presumably because of the complex social environment in the field (Wells and Taigen, 1986).

The accuracy of the measurement of $E_{Met}$ per note depends on the assumption that resting metabolic rates for *H. versicolor* are the same under laboratory conditions during the day as they are under chorusing conditions in the evening. Daytime resting metabolic rate was measured directly for each individual, but the average night-time resting metabolic rate can be approximated by performing a linear regression analysis of the mass-specific rate of oxygen consumption of calling frogs (in $\mu$LO$_2$g$^{-1}$h$^{-1}$) versus the number of notes produced per hour.

The resulting $y$ intercept from this analysis approximates the resting metabolic rate under chorusing conditions because it represents the metabolic rate when no notes are produced. The mean mass-specific resting metabolic rate measured for male *H. versicolor* under laboratory conditions during the day is 0.259±0.014 mL O$_2$g$^{-1}$h$^{-1}$ (mean ± s.e.m., N=43), which falls within the 95% confidence limits of the y intercept of oxygen consumption regressed against the number of notes produced per hour (Fig. 2). Earlier work also failed to find a significant difference between daytime and night-time resting metabolic rates in *H. versicolor* (Taigen and Wells, 1985).

Table 1. A comparison between data collected for *Hyla versicolor* from two different populations in Missouri

<table>
<thead>
<tr>
<th>Variable</th>
<th>Ashland</th>
<th>Mean</th>
<th>Reis</th>
<th>Mean</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Call length$_{lab}$ (notes per call)</td>
<td>33</td>
<td>9.94±0.35</td>
<td>10</td>
<td>10.45±0.60</td>
<td>0.48</td>
</tr>
<tr>
<td>Call length$_{field}$ (notes per call)</td>
<td>40</td>
<td>15.42±0.56</td>
<td>11</td>
<td>17.04±1.36</td>
<td>0.21</td>
</tr>
<tr>
<td>Dominant frequency (kHz)</td>
<td>40</td>
<td>2.22±0.024</td>
<td>11</td>
<td>2.23±0.021</td>
<td>0.84</td>
</tr>
<tr>
<td>rms SPL at 1 m (dB)</td>
<td>40</td>
<td>86.3±0.54</td>
<td>11</td>
<td>84.3±1.06</td>
<td>0.10</td>
</tr>
<tr>
<td>$E_{Ac}$ per note (mJ)</td>
<td>40</td>
<td>0.366±0.039</td>
<td>11</td>
<td>0.245±0.055</td>
<td>0.14</td>
</tr>
<tr>
<td>$E_{Met}$ per note (mJ)</td>
<td>33</td>
<td>11.9±0.75</td>
<td>10</td>
<td>13.1±2.77</td>
<td>0.33</td>
</tr>
<tr>
<td>Efficiency (%)</td>
<td>17</td>
<td>2.5±0.43</td>
<td>2</td>
<td>2.0±1.34</td>
<td>0.67</td>
</tr>
</tbody>
</table>

Two different values of call length are given, one measured from isolated frogs during oxygen consumption measurements (call length$_{lab}$) and the other measured from frogs calling in a chorus during sound energy measurements (call length$_{field}$).

The P values represent the probability that the means of each variable are the same for both populations.

Values are means ± s.e.m.

rms SPL, root-mean-squared sound pressure level; $E_{Ac}$, acoustic energy; $E_{Met}$, metabolic cost.

![Fig. 2. The relationship between mass-specific rate of oxygen consumption and the number of notes produced per hour for 43 male *Hyla versicolor*. The solid line represents the best-fit linear regression line ($y = 0.41 + 0.000076x; P < 0.001$), and the dotted lines represent the 95% confidence limits of the regression. The mean measured resting metabolic rate for the same set of individuals is shown as an open circle.](attachment:image.png)
The relationships between $E_{Ac}$ and $E_{Met}$ and each of the four variables, temperature, mass, call length and dominant frequency, are shown in Figs 3 and 4. Multiple linear regression was carried out on the log$_{10}$-transformed data to determine the extent to which variation in $E_{Ac}$ or $E_{Met}$ is attributable to variation in body size, temperature, call length and dominant frequency (Table 2). For log$_{10}$-transformed values of $E_{Ac}$, only log$_{10}$-transformed temperature values were found to have a significant regression coefficient. For log$_{10}$-transformed values of $E_{Met}$, only the log$_{10}$-transformed values of body mass were found to have a significant regression coefficient. Log$_{10}$-transformed values of call length and dominant frequency were found not to contribute significantly to the variation in the log$_{10}$-transformed values of either $E_{Ac}$ (mJ) or $E_{Met}$ (mJ). A second regression analysis for log$_{10}$ $E_{Ac}$ was carried out using only log$_{10}$ temperature as the independent variable, which, after an antilog$_{10}$ conversion, yielded the equation:

$$E_{Ac} = 0.00051T^{2.1}$$  \( (r^2 = 0.26; \ P < 0.001) \)

where $T$ is a unitless parameter equivalent in value to temperature in °C. For log$_{10}$ $E_{Met}$, regression analysis was carried out using only log$_{10}$ mass as the independent variable, which, after an antilog$_{10}$ conversion, yielded the equation:

$$E_{Met} = 0.0075 + 1.02M$$  \( (r^2 = 0.04; \ P = 0.025) \)

Fig. 3. Mean values for the acoustic energy ($E_{Ac}$) generated during single note cycles by 51 male *Hyla versicolor* plotted against temperature, mass, average call length and dominant frequency. The significant regression line and its 95% confidence limits are shown. The $r^2$ value and its corresponding $P$ value indicate the strength of each correlation.

Table 2. The extent to which acoustic energy output ($E_{Ac}$) and metabolic energy input ($E_{Met}$) per note are dependent upon temperature (in °C), body mass (in g), call length (in notes per call) and carrier frequency (in kHz)

<table>
<thead>
<tr>
<th>Regression coefficient</th>
<th>log(temperature)</th>
<th>log(mass)</th>
<th>log(call length)</th>
<th>log(frequency)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Versus log$E_{Ac}$ (N=51)</td>
<td>2.11±0.58</td>
<td>0.11±0.57</td>
<td>0.58±0.37</td>
<td>−1.26±2.11</td>
</tr>
<tr>
<td>(P&lt;0.001)</td>
<td>(P=0.85)</td>
<td>(P=0.12)</td>
<td>(P=0.55)</td>
<td></td>
</tr>
<tr>
<td>Versus log$E_{Met}$ (N=43)</td>
<td>0.0075±0.32</td>
<td>1.02±0.44</td>
<td>0.47±0.37</td>
<td>−0.22±1.79a</td>
</tr>
<tr>
<td>(P=0.98)</td>
<td>(P=0.025)</td>
<td>(P=0.22)</td>
<td>(P=0.90)</td>
<td></td>
</tr>
<tr>
<td>Versus log(efficiency) (N=19)</td>
<td>2.10±0.95</td>
<td>−1.72±1.03</td>
<td>0.79±0.69</td>
<td>−0.29±2.39</td>
</tr>
<tr>
<td>(P=0.044)</td>
<td>(P=0.115)</td>
<td>(P=0.27)</td>
<td>(P=0.91)</td>
<td></td>
</tr>
</tbody>
</table>

*a Carrier frequency data were collected only from field recordings. Since field recordings were not available for all the males for which $E_{Met}$ per note was calculated, a smaller sample size (N=19) was used to derive this value than was used to determine the other regression coefficients. Regression coefficients (±s.e.m.) are derived from a multiple linear regression analysis. $P$ values represent the probability that the regression coefficient for each dependent variable is not different from zero.
Efficiency of sound production in treefrogs

Independent variable, which, after an antilog 10 conversion, yielded the equation:

\[ E_{\text{Met}} = 1.49 M^{1.08} \]  

\((r^2 = 0.14; P = 0.013)\), where \( M \) is a unitless parameter equivalent in value to body mass in g. The value of the exponent in equation 7 has a standard error of 0.42 and is not significantly different from 1.0.

As an additional confirmation that different variables affect \( E_{\text{Ac}} \) and \( E_{\text{Met}} \), no significant correlation was found between \( E_{\text{Ac}} \) and \( E_{\text{Met}} \) among the 19 frogs for which both variables were measured \((r^2 = 0.04; P = 0.41)\). However, for the same set of 19 frogs, temperature-specific \( E_{\text{Ac}} \) (mJ °C\(^{-1}\)) and mass-specific \( E_{\text{Met}} \) (mJ g\(^{-1}\)) showed a much stronger correlation \((r^2 = 0.20; P = 0.06)\).

Efficiency of note production

The relationship between efficiency and each of the four variables, temperature, mass, call length and dominant frequency is shown in Fig. 5. As expected, given the results of the \( E_{\text{Met}} \) and \( E_{\text{Ac}} \) analyses, a positive correlation was found between efficiency and temperature while a negative correlation was found between efficiency and mass. Efficiency differences among individual males were not dependent upon either call length or dominant frequency.

There are two ways to derive a model equation for efficiency with the available data. One way is to perform a multiple regression analysis of log-transformed values, as for equations 6 and 7. The alternative is to divide equation 6 by equation 7. Multiple regression analysis on the efficiency values showed temperature to be the only variable with a significant correlation coefficient (Table 2). The failure to identify mass as a significant covariate of efficiency may be due to the relatively small sample size available for the multiple regression analysis. Dividing equation 6 by equation 7 yields:

\[ \text{Efficiency} = 0.0003 T^{2.1} M^{-1.08} \]  

\((8)\)

The exponents for \( T \) and \( M \) in equation 8 are similar to those calculated from the multiple regression of log efficiency values. The observed efficiency values measured directly for individual males are significantly correlated with the expected efficiency values obtained from equation 8 \((r^2 = 0.47; P < 0.001; \text{Fig. 6B})\), confirming that equation 8 is a good predictor of the efficiency of note production in \( H. \) versicolor. Contrary to the predictions of the wavelength-to-size rule, the product of mass\(^{0.33}\) and dominant frequency is not significantly correlated with the efficiency of note production (Fig. 6A).

Interspecific comparisons

Data on the average efficiency and the \( E_{\text{Met}} \) of note production are available for four other species of hylid frog, \( H. \) cinerea, \( H. \) gratiosa, \( H. \) squirrella and \( Pseudacris \) crucifer (Prestwich et al., 1989). Since the calls of all four of these species consist of a single note, the \( E_{\text{Met}} \) per call is equivalent to the \( E_{\text{Met}} \) per note. Average \( E_{\text{Ac}} \) per note can be derived for these four species by multiplying \( E_{\text{Met}} \) by the efficiency values reported by Prestwich et al. (1989). Analysis of covariance (ANCOVA) comparing \( E_{\text{Ac}} \) per note, \( E_{\text{Met}} \) per note and efficiency between \( H. \) versicolor and the other hylids shows...
the variation in the energetics of note production within *H. versicolor* and among the four other species to be affected by temperature and body mass in the same fundamental way (i.e. no significant differences among slopes; Fig. 7). The adjusted mean cost per note is significantly lower and the adjusted mean efficiency of note production is significantly greater in *H. versicolor* than in the other hylid species. The calculated value of the efficiency of sound production derived for *H. versicolor* by Prestwich et al. (1989) falls within the values of efficiency calculated for *H. versicolor* in the present study (Fig. 7).

The treatment of *H. cinerea*, *H. gratiosa*, *H. squirrella* and *Pseudacris crucifer* as a distinct group of frogs is justifiable because the four species share the same basic biomechanical call structure (i.e. a single note per call) which differs from the biomechanical call structure of *H. versicolor* (i.e. multiple notes per call). The results shown in Fig. 7 suggest not only that temperature and body mass account for a large amount of the intraspecific variation in the efficiency of anuran sound production, but that they may also account for much of the variation in efficiency among species with similar call structures.

**Discussion**

Prestwich et al. (1989) suggested that oscillograms obtained from recorded calls would be the best way accurately to measure the known rms SPL of synthetic calls but also because the rms SPL values measured for *H. versicolor* in the field are comparable in range with rms SPL values reported elsewhere for this species (Gerhardt, 1975). Some of the variability in the measurements of $E_Ac$ in the present study may arise from the limitations of measuring $E_Ac$ from field recordings, and a substantial amount of the variability may also be attributable to microenvironmental differences among the perch sites of the calling frogs. Under more controlled conditions, better estimates of the effects of temperature, body mass and wavelength on the energy output of calling frogs may be obtained. However, the biological and evolutionary importance of these variables depend upon the extent to which their influences on $E_Ac$ can be detected *in situ* by a receiver (i.e. a female) that monitors the signal from only one position at a time. The failure to detect a significant effect of mass or dominant frequency on $E_Ac$ in this study does not necessarily mean that such effects would not be detectable under more controlled conditions, but it does suggest that mass and dominant frequency may be neither detectable nor important determinants of $E_Ac$ in *H. versicolor* within a natural, complex environment.

The cost per unit time of sound production will increase substantially with temperature in *H. versicolor* because of the increase in note repetition rate. The $E_{Met}$ per note, however, and hence the metabolic cost of a single contraction of the trunk muscles, remains constant across a wide range of body temperatures. Similar results have been found for sound-
producing muscles in the frog *Pseudacris crucifer* (Wells et al., 1996), orthopteran insects (Prestwich and Walker, 1981) and rattlesnakes (Conley and Lindstedt, 1996), in which temperature increases the rate of muscle activity but not the metabolic cost per muscle contraction. In contrast, \( E_{Ac} \) per note increases substantially as a function of temperature, indicating that much more of the metabolic energy consumed by the calling muscles of *H. versicolor* is being transformed into acoustic energy at high temperatures than at low temperatures.

Data relating temperature to acoustic power in other organisms are lacking, but the SPL of at least one other species, the cicada *Tibicen winnebanna*, may also increase with temperature (Sanborn, 1997).

One possible explanation for the positive temperature-dependence of note efficiency in *H. versicolor* is that the increasing note repetition rate associated with elevated temperatures results in higher levels of elastic recoil of the trunk muscles and greater storage of mechanical energy in the system. A similar situation occurs in vertebrate locomotion, for example, where the elastic energy storage of tendons increases as a function of stride frequency (Biewener, 1998). Another explanation may be that the vibrational energy generated by the vocal cords is an exponential function of the velocity of airflow \( (U_{air}) \) across them. Assuming that the volume of air moving through the larynx per note remains relatively constant, \( U_{air} \) through the larynx should be proportional to note repetition rate. Since note repetition rate is proportional to temperature, \( E_{Ac} \) in *H. versicolor* is approximately proportional to \( U_{air}^2 \). In brass and woodwind instruments, acoustic power also increases exponentially as a function of \( U_{air} \) (Bouhuys, 1965) and, in his model of the energetics of
sound production in the crow *Corvus mellori*, Fletcher (1988) predicts that increasing $U_{\text{air}}$ across the syrinx by a factor of 10 will cause acoustic power to increase nearly 100-fold (in a non-linear fashion). Given that air-driven sound-producing systems such as those of frogs, birds and musical instruments generally function at very low efficiencies, a relationship whereby the power output increases as an exponential function of the power input is certainly possible to maintain over a limited range of power inputs.

*The effects of body size*

The independence of body size from $E_{\text{Ac}}$ per note in *H. versicolor* may seem counterintuitive because it is unlikely that that extremely small and extremely large frogs would produce equally loud sounds. Still, it does seem to be generally true that body size in anurans is not an important determinant of sound amplitude. There is one species of frog, *Bufo americanus*, in which a positive correlation between body size and rms SPL has been reported (Gerhardt, 1975), but among other intraspecific comparisons, correlations between body size and rms SPL are absent (Gerhardt, 1975; Passmore, 1981; Penna and Solis, 1998). Nor do interspecific comparisons yield a relationship between the size and acoustic intensity for frogs. Comparisons among 21 species of frogs from North America (Gerhardt, 1975), 17 species from subtropical Africa (Passmore, 1981) and five species from southern Chile (Penna and Solis, 1998) found no interspecific relationship between mean body size and mean rms SPL.

Interspecific comparisons among cicadas yield a strong significant correlation between body size and rms SPL (Sanborn and Phillips, 1995), but the minimum and maximum body masses exhibited by cicada species differ by a factor of 100 or more. There is only an approximately twofold difference in body mass between the smallest and largest *H. versicolor* and, if the cicada data of Sanborn and Phillips (1995) are examined across twofold ranges in body mass, no clear relationship between body size and rms SPL stands out. Therefore, unless intraspecific variation in body size is very large, size may not be an important covariant of energy output during sound production. In *H. versicolor*, at least, body size and $E_{\text{Ac}}$ do not covary. Selection on body size and sound intensity can occur independently, and body size is not an important evolutionary constraint upon how noisy *H. versicolor* can be.

In contrast to intensity, the cost and efficiency of sound production are significantly dependent upon body size. Selection for the enhanced efficiency of sound production should favour the evolution of reduced body size, and *vice versa*. Two aspects of anuran natural history provide circumstantial evidence for the idea that the evolution of efficiency and body size are linked. First, since only the males vocalise in most species, selection pressure for increased calling efficiency should impose selection for reduced body size in males but not in females, and this may help explain why males of most species of frog tend to be smaller than females (Shine, 1979). Second, since efficiency decreases at low temperatures, selection should be even stronger for reduced body size among species that call at lower temperatures, such as those that call earlier in the spring or at higher latitudes. Although the relationship between body size and mean calling temperature has not been extensively studied, there are examples among North American frogs where smaller species call earlier and have ranges that extend further north than larger species (e.g. *Rana catesbeiana* compared with *R. sylvatica*; *Hyla* spp. compared with *Pseudacris* spp.; Cook, 1984).

*Wavelength-to-size ratio*

Acoustic theory predicts that, when its radius, $r$, is less than one-third of the wavelength of sound, $\lambda$, the efficiency of a spherical radiator is an exponential function of $r/\lambda$ (Bennet-Clark, 1989, 1999; Prestwich, 1994). For the calls of *H. versicolor*, in which the average dominant frequency is 2.2 kHz, the value of $\lambda$ is approximately 15 cm. The vocal sac and the body wall, which are the largest potential sound radiators in *H. versicolor*, are both approximately spherical and both have radii of substantially less than 5 cm. Regardless of the identity of the resonating structure, the condition of $r$ being less than $\lambda/3$ is met in *H. versicolor*. It is therefore strange that the wavelength-to-size ratio has no observed effect on net efficiency in *H. versicolor*. It could be that the effects of body size and dominant frequency upon other aspects of sound production offset any specific effects of body size and dominant frequency on the efficiency of converting vibrational energy into acoustic energy.

The lack of correlation between dominant frequency and efficiency in *H. versicolor* does not contradict the idea that calls with low dominant frequencies have a selective advantage, because they travel more effectively through the environment than calls with high dominant frequencies. What the data do suggest, however, is that there is no efficiency trade-off associated with the production of low-frequency sounds among small frogs. Dominant frequency can undergo an evolutionary transition in *H. versicolor* without imposing any changes upon the net efficiency of sound production, and the low efficiencies observed for sound production cannot be accounted for by the wavelength-to-size rule.

Sound production in frogs is a complex process, consisting of many steps. The step that is most important in generating the low efficiency of sound production in *H. versicolor* is not the conversion of vibrational energy into acoustic energy, but rather the conversion of metabolic energy into mechanical work. *In vitro* measurements performed at 25 °C have shown that the trunk muscles of *H. versicolor* produce a maximum mechanical power of 57.9 W kg$^{-1}$ when operating at between 18 and 21 Hz. At 24 Hz, a frequency closer to the *in vivo* operating frequency of the trunk muscles at 25 °C, mechanical power drops to around 35 W kg$^{-1}$ (Girgenrath and Marsh, 1999). Total trunk muscle mass is approximately 10% of total body mass (Marsh and Taigen, 1987), which means that the estimated mechanical work generated by the trunk muscles of a 7 g *H. versicolor* at 25 °C, with a note repetition rate of 24 notes s$^{-1}$, is 1 mJ per note. Given an expected $E_{\text{Met}}$ per note
of 12.2 mJ for a 7 g frog (from equation 7), the trunk muscles convert metabolic energy into mechanical energy with an efficiency of only 8%. Conversely, given an expected $E_{Ac}$ per note of 0.44 mJ at 25°C (from equation 6), the net combined efficiency of sound production subsequent to the production of mechanical energy by the trunk muscles, including the conversion of vibrational energy into acoustic energy, is 44% (i.e. the ratio of $E_{Ac}$ to mechanical energy per note).

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