Behav Ecol Sociobiol (1995) 37: 125–135

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Carbohydrate and calling: Depletion of muscle glycogen and the chorusing dynamics of the neotropical treefrog *Hyla microcephala*

Received: 8 November 1994 / Accepted after revision: 17 April 1995

Abstract Chorusing males of the neotropical treefrog *Hyla microcephala* call in distinct bouts punctuated by periods of silence, a pattern known as unison bout singing. Schwartz (1991) previously tested and refuted the hypotheses that males periodically stop calling either because of a female preference for males that call cyclically, or because high ambient noise levels inhibit vocal activity. Males of *H. microcephala* are vocally responsive to the calls of other males, and during calling bouts their rate of note production can exceed 10,000 per hour. In natural choruses females preferentially pair with males that call at the higher rates. Because females can pair with males over many hours, males may stop calling periodically to save energy so they can continue to call for the entire period that females are available. We directly tested this energy conservation hypothesis by collecting samples of males early in the evening just after chorusing commenced and later when chorusing had ended for the night. Trunk muscles (internal and external oblique), which are responsible for the airflow associated with note production, were dissected, frozen, and their glycogen content measured. Data on calling behavior were obtained for late-evening samples. Individual calling behavior was not correlated with a male's final glycogen level. In addition, many males ended their calling before glycogen reserves were exhausted, indicating that factors other than energy can determine when males finally stop chorusing. However, the biochemical assays supported the energy conservation hypothesis. Unless chorusing was punctuated by pauses, most males would have been unable to sustain high rates of calling for an entire evening without exhausting glycogen reserves in their trunk muscles. Because the time females pair with males is probably unpredictable to males, the ability to call for long periods may improve a male's chances of mating.

Key words Frog · Energetics · Chorusing · Glycogen · Female choice

Introduction

Many species of insects and anurans are renowned for the displays of aggregated males advertising for the attention of female conspecifics. These displays may take the form of spectacular coordinated visual signalling, as in certain fireflies (Buck 1988; Smith 1935), or the loud choruses of frogs, toads, and orthopteran insects. Researchers in bioacoustics and animal behavior have been intrigued by the apparently non-random signalling patterns of grouped males for some time (Alexander 1975; Narins and Zelick 1988; Schwartz 1993; Greenfield 1994), and the fine details of the temporal dynamics of signalling have been a subject of recent mathematical modelling and computer simulation (Brush and Narins 1989; Mirollo and Strogatz 1990; Greenfield and Roizen 1993). Because the energetic requirements of male advertisement can be considerable (Ryan 1988; Pough et al. 1992), researchers have suggested that physiological constraints might modulate components of male courtship activity (Halliday 1987; Wells and Taigen 1986, 1989; Elmberg and Lundberg 1991; Murphy 1992; Bailey et al. 1993; Lee and Loher 1993).

Males of the neotropical treefrog, *Hyla microcephala*, are typically found in dense aggregations (Schwartz and Wells 1983), and many aspects of the vocal behavior of individuals only become apparent in

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the chorus environment. For example, when well isolated from other calling males, a male calls at a lower rate and gives calls with fewer secondary notes than when acoustically stimulated (Schwartz and Wells 1985). Males respond to changes in calling by other individuals in order to remain competitive for females, which discriminate in favor of stimuli with higher rates of note delivery in two-choice experiments (Schwartz 1986).

In addition to increasing call rate and complexity, males in a chorus modify the timing of their calls on both fine and gross time scales. On a fine time scale, males increase the delay between their notes when interrupted by the notes of their nearest neighbor(s) in the chorus (Schwartz 1991, 1993). This behavior reduces acoustic interference and enhances a male's ability to attract females (Schwartz 1987, 1993). On a gross time scale, choruses of *H. microcephala* engage in "unison bout singing" (Schwartz 1991; Schwartz and Wells 1983). This is the collective calling by groups of males punctuated by variable periods of relative quiet (Greenfield 1994). Similar patterns of cyclical chorusing have been reported in other species of anurans (Rosen and Lemon 1974; Whitney and Krebs 1975) as well as in chorusing insects (Alexander 1975; Otte 1977; Greenfield and Shaw 1983).

The question of why males periodically stop calling rather than call without pausing for the entire evening has been addressed at both the proximate and ultimate level. The hypothesis that males of some species of anurans call in bouts because of elevated muscle lactate is no longer viable (Whitney and Krebs 1975; Ryan et al. 1983; Pough and Gatton 1984; Taigen and Wells 1985; Prestwich et al. 1989; Grafe et al. 1992; Pough et al. 1992). Schwartz (1991) recently tested the hypotheses that males of *H. microcephala* periodically stop calling because: (1) of a preference by females for males that do so, and (2) high ambient noise levels in the chorus inhibit calling. A two-stimulus female-choice experiment refuted the first hypothesis, and broadcasts of simulated chorus-noise refuted the second hypothesis. However, Schwartz's data were consistent with the idea that males periodically stop calling to conserve energy and, by so doing, are able to call throughout the evening.

The energy conservation hypothesis is plausible for *H. microcephala* for several reasons. Males of *H. microcephala* typically commence calling at 1930–2100 hours, and chorus activity often terminates by midnight. During chorusing bouts, males can produce in excess of 6000 notes/h. The reported aerobic metabolic rate for one such animal is higher than that known for any other ectothermic vertebrate (Wells and Taigen 1989). If males of *H. microcephala* failed to punctuate their calling with periodic quiet, note rates of many individuals would exceed the peak rate recorded by Wells and Taigen (1989) (Schwartz 1991). Because females prefer higher rates of note delivery to slower rates, calling in bouts could allow males to remain attractive when their neighbors vocalize, while simultaneously guaranteeing that males can continue to produce signals for the entire period during which receptive females arrive at the breeding site (Schwartz 1991). Thus, the energy conservation hypothesis addresses an ultimate-level explanation for the cyclical pattern of calling.

We tested the energy conservation hypothesis by measuring glycogen levels in the trunk muscles of males of *H. microcephala.* These muscles are responsible for driving air across the vocal cords of a frog during call production (Pough et al. 1992). Possibly because a supply of glycogen may be necessary to provide a source of oxaloacetate for the Kreb's cycle (see Conlee 1987, p. 20), exhaustion of glycogen causes muscle fatigue (Holloszy and Booth 1976; Hochachka and Somero 1984; Conlee 1987; Hoppler and Billeter 1991), even if lipid is also used as a fuel (Karlsson and Saltin 1971; Hochachka and Somero 1984; Holloszy et al. 1986; Conlee et al. 1990). Moreover, recent data demonstrate that this carbohydrate is utilized during calling activity of male frogs (Walker 1989; Bevier 1995; Wells et al. 1995; T. U. Grafe, unpublished work). By monitoring the calling behavior of males and measuring the levels of muscle glycogen we were able to determine: (1) the extent to which glycogen levels are depleted over the period of calling activity, (2) whether differences in glycogen level reflect differences in the calling behavior of individual males, and (3) the cost in muscle glycogen of producing a note. This estimate was used to test the energy conservation hypothesis. This hypothesis predicts many males lack sufficient energy reserves to support calling at the high rates characteristic of bouts for 3–5 h, the duration of many choruses of *H. microcephala* (mean calling period per male = 3.8 h, see Bevier 1995; J. J. Schwartz personal observations). In addition, we acquired data on the time over which females paired at night and corroborated the importance of note rate as a criterion used by females during mate choice. Such information is necessary to determine whether calling periodically at high rate over a long period of time can improve males' chances of mating.

On nights when the vocal activity of males in a chorus is high, we suspected that member males might call until glycogen reserves of trunk muscles were nearly depleted. Therefore, as a final experiment with animals to be assayed for carbohydrate, we tested this idea by acoustically stimulating males to induce them to call at high rate and to continue vocal activity after the rest of the chorus had stopped calling for the night.

Methods

Study area

Field work was conducted during the rainy seasons of 1991, 1992 and 1993 in Gamboa, Panama. The primary field site was a flooded meadow with scattered pools of water and ditches. Stands of vegetation in and adjacent to water provide calling sites for male frogs (see Schwartz and Wells 1984 for further details). Temperatures during field work, which was conducted between 1930 and 2400 hours, ranged from 25 to 27° C.

Male vocal activity

We recorded the vocal activity of males of *H. microcephala* in three groups using a Commodore Amiga 500 (or 600 HD in 1993) computer and an interface board designed and built by the first author. The interface board accepted up to eight microphone inputs and the output of the board was sent to the computer via the parallel port. The board had eight parallel channels, each consisting of an amplifier section, a voltage comparator, and a retriggerable monostable multivibrator (SK4098B). The output for each channel of the board underwent a voltage transition, of preset duration, in response to above threshold input from a microphone. This threshold level was set manually using separate gain controls for each channel and each of eight LEDs. In the field, the temporal resolution of the circuit was adjusted so that each call note of a male triggered an individual output pulse on that male's channel. The outputs of the board's eight channels were sampled at a rate of either 50 or 200 Hz from the eight data lines of the computer's parallel port. The 200 Hz value was used for the second group of males (18 July 1991). Those data contributed to a study of note timing behavior in *H. microcephala* for which greater temporal resolution was necessary than for this study (see Schwartz 1993 for additional technical details).

In the field, an Azden unidirectional microphone (ECZ-660) mounted on a tripod was positioned directly in front of each frog and then plugged into the interface board. The gain controls were adjusted so that the calling of each frog triggered only one channel. The computer was programmed to store the data automatically on a floppy disk either every 5 min (200 Hz sampling rate) or 30 min (50 Hz sampling rate), at which time acquisition of data ceased for approximately 25 s. Before these recording sessions, we attempted to remove any additional males near the experimental subjects.

We both monitored (n = 19) and collected (n = 12) males from three aggregations on the nights of 8 July 1991, 18 July 1991, and 1 August 1993. Calling was monitored in these aggregations from 2115 h to 2350 hours, 2135 h to 2310 hours, and 2100 to 2230 hours, respectively. Because of the time required to locate a group of calling males and set up the monitoring equipment, actual calling times of males were greater than these time periods. During chorusing, we noted which males went into amplexus and the time they did so. After chorusing ended for the night, we collected males (late evening sample) and transported them within 5 min to a nearby laboratory. During this brief period, the animals did not call, and so were unlikely to utilize additional glycogen in their trunk muscles. In addition, glycogen repletion should be trivial during this short time interval (Gleeson 1991). Males were sacrificed by decapitation, and their trunk muscles (external and internal oblique) were expeditiously (< 45 s) dissected on ice. Dissected tissues were wrapped in aluminum foil, immediately frozen in liquid nitrogen, and subsequently stored in a freezer at −80° C until transport to the United States.

Six additional males were collected between 2230 and 2300 hours in August 1991 for our "late male" sample of 18 individuals. A total of 21 "early males" was collected just as chorusing commenced (between 1915 and 2030 hours) on evenings in July and August 1991 and August 1993. All males were collected within 1.0 km of the primary study site. Because these animals were caught in different spots and different dates in 2 years, it is likely that they provide a representative sample of muscle glycogen levels near the start of chorus activity.

We acoustically stimulated six males (three on 16 September, 2.5 h after chorusing commenced from 2215 to 2345 hours, three on 4 November 1992, 1.5 h after chorusing commenced from 2030 to 2230 hours) to call with a recording of calls of *H. microcephala*. Stimulation continued until males no longer responded vocally. They were then collected and their trunk muscles removed within 2 min. Six "early males" were also collected on these nights (before 1920 hours).

The acoustic stimulus was prepared by first digitizing (sampling rate = 20 kHz) a recorded call (Marantz PMD 201 cassette tape recorder, Realistic 33-1062 directional microphone) using a Commodore Amiga 2000 computer equipped with an Applied Visions FutureSound (TM) 8-bit sound digitizer. A 4-note and 5-note call and their timing relationships were created using the sound-editing software supplied with the sound digitizer. The two calls were repeated in sequence for 11 s followed by 3 s of silence. This stimulus was output from the computer continuously and recorded on tape with a Marantz 201 cassette tape recorder. The stimulus was broadcast to males from a distance of 1 m via an SME-SC-A9 wide-range field speaker (Mineroff Electronics). The SPL of the stimulus was adjusted to 86 dB (relative to 20 µPa) and checked both prior to and during playback, with a Realistic sound-level meter (Radio Shack 1992 catalog number: 33-2050) set for C-weighted fast RMS response.

Female pairing times

During nights of chorus activity in 1993, the study site was searched approximately every 30 min for paired females and the time they were found was recorded. We acquired additional data on mating times during observations of the behavior of males in 1991 and 1993. These data were acquired on 22 nights and so should provide a relatively unbiased estimate, subject to the limited temporal resolution of the search period and the problem of pairs missed due to low visibility of quiet individuals.

Glycogen assays

Frozen tissue samples were packed in dry ice, transported to the University of Connecticut, and stored at −80° C until analysis. Samples were weighed (to the nearest 1.0×10^{-5} g), and glycogen assayed according to the method of Marsh and Dawson (1982; see also Keppler and Decker 1974). Briefly, frozen tissue from each male was minced and subsequently homogenized in 0.6 M cold perchloric acid. A portion of the homogenate was hydrolysed with amyloglucosidase, divided in two, and this provided two estimates of total glucose. Free glucose estimates (n = 2) were obtained using the remainder of the homogenate. Glucose concentration was measured spectrophotometrically (450 nm; Gilford model 260 spectrophotometer) according to the glucose oxidase procedure (Procedure No. 510; Sigma Chemical Company, St. Louis, Mo.). Glycogen content of the tissues (mg/g wet mass) was calculated as the mean difference of the free and total glucose estimates. Estimates of glycogen content obtained using individual replicates differed by less than 12% for all males but one (23% difference).

Results

Male vocal activity

Data on the temporal pattern of male calling are presented for the choruses monitored in 1991. Because all males in the chorus monitored on 1 August 1993 paired with females and so sequentially ceased vocal activity, data are not very useful in illustrating the typical pattern of calling dynamics (see below).

The distribution of all inter-note intervals is plotted as the logarithm of a reversed cumulative frequency distribution for the two choruses from 1991 in Fig. 1. The shapes of these curves are similar to those obtained previously (Schwartz 1991) and reveal a rapid change in slope at an interval duration of approximately 5 s. Therefore, intervals greater than 5 s probably represent pauses in calling between bouts, whereas intervals less than 5 s probably represent inter-note intervals and inter-call intervals within bouts (Slater and Lester 1982). Using the procedure of Sibly et al. (1990) to estimate the transition value for inter-bout intervals, the time criterion is 5.3 s (8 July) or 4.8 s (18 July). Accordingly, in subsequent analyses of inter-bout intervals, data were restricted to pauses greater than 5 s.

On 8 July, the durations of inter-bout intervals and note rates varied significantly during the nightly recording period (10-min blocks; intervals: $F = 22.54$, $P < 0.0001$; notes: $F = 52.06$, $P < 0.0001$) as well as among males (intervals: $F = 2.49$, $P = 0.0214$; notes: $F = 43.66$, $P < 0.0001$; two-way ANOVA on ranked durations or ranked notes). Inter-bout intervals tended to be longer both near the start of the recording period and the end of chorusing than they were in the middle of the evening (Fig. 2a). Note rates showed the opposite pattern of inter-bout intervals and were greatest in the middle of the evening (Fig. 3a). On 18 July, inter-bout intervals and note rates also differed significantly at different stages of the evening (Figs. 2b, 3b; intervals: $F = 4.14$, $P < 0.0001$; notes: $F = 3.02$, $P = 0.0017$) and among males (intervals: $F = 10.14$, $P < 0.0001$; notes: $F = 178.08$, $P < 0.0001$). However, no clear early, middle, and late evening pattern to calling pause durations was evident.

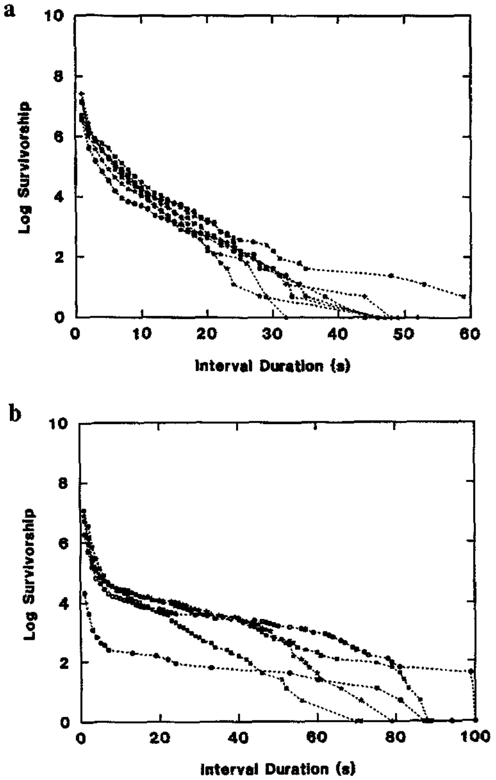

Fig. 1 The cumulative frequency distribution of inter-note intervals of males of *Hyla microcephala* plotted as log survivorship versus interval duration. **a** Chorus of 8 July 1991 (n = 7 males, 74,219 intervals; 3 intervals were greater than 60 s and are not plotted). **b** Chorus of 18 July (n = 5 males, 11,206 intervals)

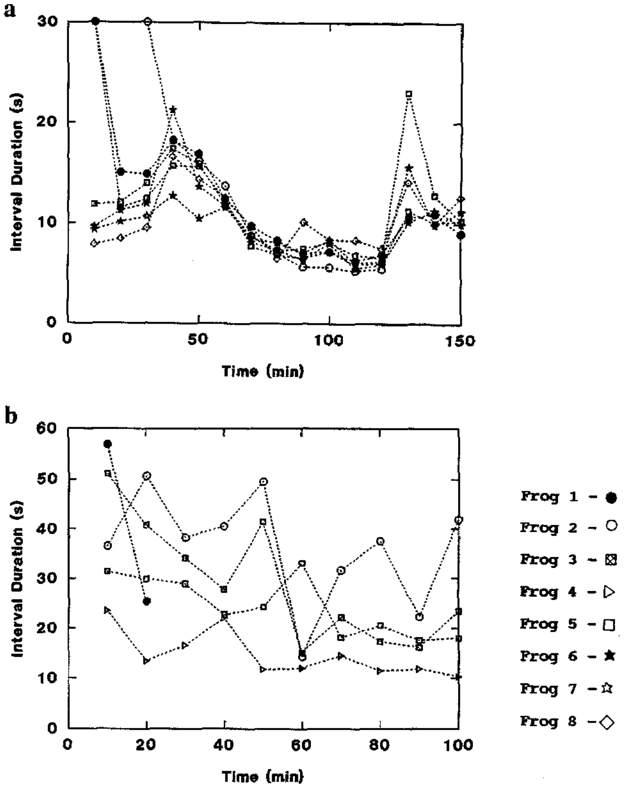

Fig. 2a, b Durations of inter-bout intervals for each male over the duration of the monitoring session of the chorus. Each *point* represents the average of the intervals (> 5 s) recorded for each of successive 10-min periods. **a** Chorus of 8 July (n = 7 males, 1288 intervals; 4 points were greater than 30 s). **b** Chorus of 18 July (n = 5 males, 545 intervals). The *key to individuals* is for both choruses. There is no frog 4 in the chorus of 8 July

Additional data on the calling behavior of the males we monitored are presented in Table 1. These include the total note production of subjects, their calling durations and note rates, and the total time they devoted to inter-bout intervals.

Pairing of females

On 8 July 1991, male 2 in the group of frogs we monitored went into amplexus after approximately 2 h of calling. This individual had the greatest note production in the hour prior to pairing (Fig. 3a). In the chorus of 1 August 1993, all seven males went into amplexus within 91 min after we began to monitor vocal activity. The order of pairing was inversely related to males' note rate for males 7, 1, 2, and 6 (Fig. 4).

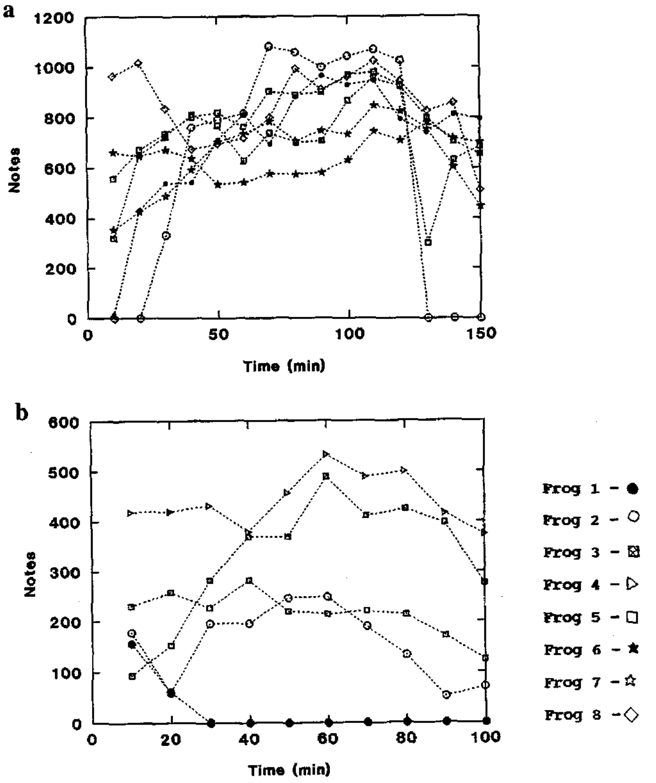

Fig. 3a, b Note rates for each male over the duration of the monitoring session of the chorus. Each *point* represents the number of notes given for each of successive 10-min periods. **a** Chorus of 8 July ($n = 7$ males, 74,225 notes). **b** Chorus of 18 July ($n = 5$ males, 11,214 notes). There is no frog 4 in the chorus of 8 July

Table 1 Data on calling behavior of males of *Hyla microcephala* in choruses monitored in July 1991 and August 1993. [*Notes* total notes given, *time* total time that calling was monitored (min), *intervals* total duration of all inter-bout intervals (s), *rate 1* note production rate (notes/h), *rate 2* = note production rate with inter-bout intervals excluded]

Frog	Notes	Time	Intervals	Rate 1	Rate 2
8 July					
1[a]	10601	135	2784	4712	7179
2[a]	8974	90	2569	5983	11410
3[a]	10947	150	2197	4379	5793
4[a]	11525	150	3030	4610	6950
5[a]	9707	150	1433	3882	4618
6[a]	9746	150	1906	3898	4946
7[a]	12725	150	2102	5090	6641
18 July					
1	218	20	575	654	1256
2	1534	95	3240	969	2245
3[a]	3128	95	3170	1976	4451
4	4236	95	2440	2675	4677
5[a]	2098	95	3192	1325	3012
1 August					
1	4031	51	998	4742	7038
2	3925	53	1178	4443	7058
3[a]	5185	90	1221	3457	4467
4[a]	6747	90	970	4498	5483
5[a]	6282	90	1649	4188	6029
6	4943	60	992	4943	6823
7	1903	21	399	5437	7957

[a] Male collected for glycogen assay

This was true when note rate for all males not in amplexus was calculated for periods of 5, 10, 15, and 20 min prior to the time each male paired. The remaining three males paired as data in computer RAM were being filed to floppy disk after 90 min of monitoring. The note rates of these frogs were nearly identical.

We located 71 paired females at times ranging from 1010 to 0030 hours on 22 nights (including 8 July 1991 and 1 August 1993). The distribution of these times, using a 30-min bin width, is shown in Fig. 5.

Glycogen levels

Trunk muscle glycogen levels for the early ($\bar{x} = 11.91$, median = 11.39 mg/g muscle, $n = 21$) differed significantly from the late ($\bar{x} = 3.84$, median = 3.99 mg/g, $n = 18$) evening sample of males (Fig. 6; Wilcoxon two-sample test, $Z = -5.00$, $P < 0.001$). Glycogen levels in trunk muscles of the six males we acoustically stimulated along with data from six individuals collected early in the evening are shown in Table 2. The stimulated males failed to prolong their vocal activity beyond the time when other members of the chorus ceased calling (2345 hours on 16 September and 2245 hours on 4 November). Substrate levels for "early males" were comparable ($\bar{x} = 10.24$, median = 10.31) to those obtained for early males on nights in 1991 and 1993. On 16 September two of the three males which we exposed to recorded calls had nearly exhausted their glycogen reserves while the remaining male's level was below the mean value for late males collected in 1991. However, on 4 November, glycogen levels were well above zero when calling subsided, and one individual had a trunk muscle glycogen level greater than that of any of the early males collected that night. This male was the last *H. microcephala* to cease calling on that night.

Neither the total note production or note rate of the late evening males was correlated with the glycogen

Table 2 Glycogen levels (mg/g) in trunk muscles of 12 males of *H. microcephala* on two nights when frogs were stimulated with playback of recorded calls (early: levels for males collected as chorusing commenced, late levels for stimulated males collected at termination of calling)

	Early	Late
16 September	11.12	0.66
	11.75	0.86
	10.25	2.27
4 November	9.10	11.60
	8.83	5.18
	10.37	5.27

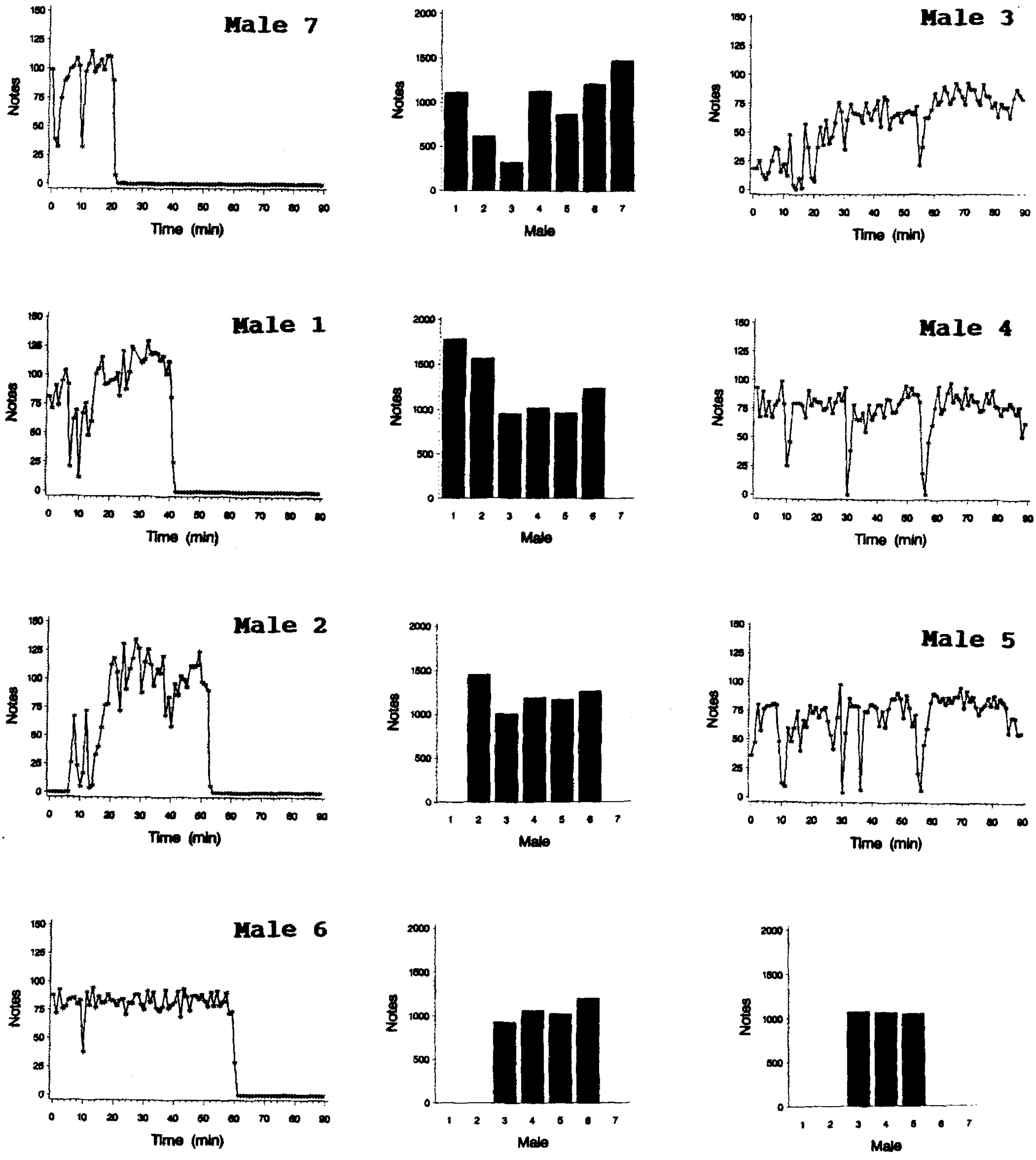

Fig. 4 Plots of the notes produced per min by each male in the chorus of 1 August 1993. For *males 7, 1, 2, and 6*, females paired sequentially with the male with the highest note rate (sequence illustrated: *top to bottom*). The note production for unpaired males in the 15 min prior to amplexus of each male is illustrated in the *histograms* to the right of the plots. The remaining males paired between time = 90 and time = 91 min. The totals of their note production during their final 15 min of calling is shown in the *chart* at the bottom of the third column

level of their trunk muscles (8 July: Spearman's $r = 0.071$, $P = 0.879$; $r = -0.179$, $P = 0.702$; pooled dates: $r = -0.042$, $P = 0.897$; $r = -0.126$, $P = 0.697$). Males with the lowest glycogen levels did not stop calling sooner; on 8 July, male 2 stopped calling before the other males because he went into amplexus.

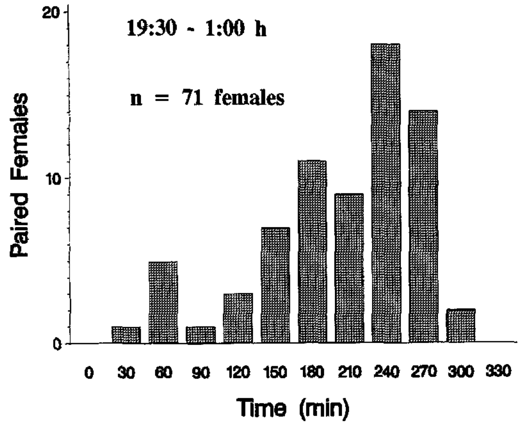

Fig. 5 The distribution of times paired females where captured ($n = 22$ nights). *Time values* shown are midpoints after 19:30 h

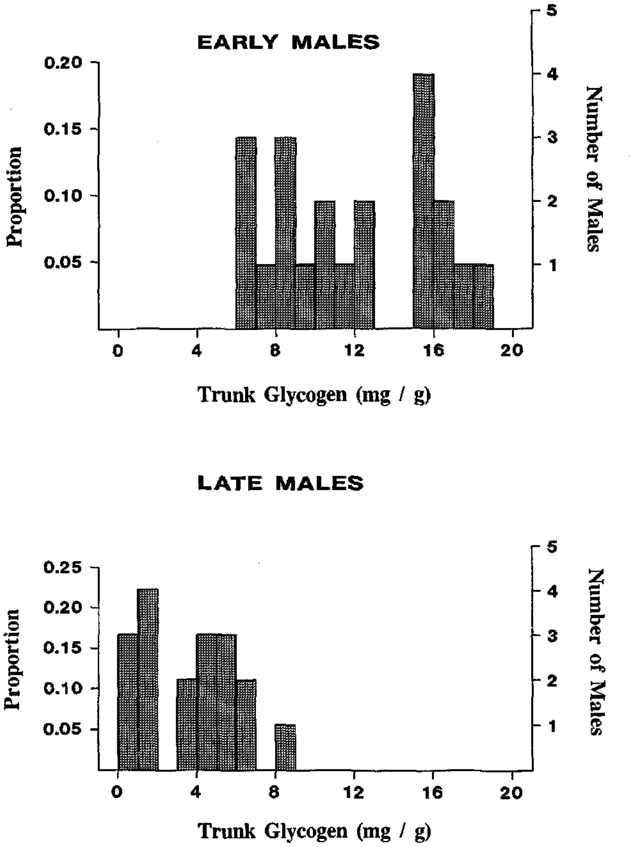

Fig. 6 Histogram of levels of glycogen in the trunk muscles of males collected early in the evening (*top*) and later, as calling activity subsided

We estimated the cost in muscle glycogen of producing a note in two ways. First, we assumed each of the 12 monitored late males that we collected started and ended calling with the mean level of trunk muscle glycogen in our sample of early males ($n = 21$) and late males ($n = 12$), respectively. We divided the total change in glycogen for these late evening males ($12 \times$ mean early level $- 12 \times$ mean late level; mean for monitored late males = 3.95 mg/g, median = 3.99 mg/g) by the total number of notes they produced ($n = 97665$). This calculation gives a first estimate of 0.978 μg of muscle glycogen per gram per note. Therefore an average sized male with a trunk muscle mass of 0.06 g (Pough et al. 1992) would use about 0.06 μg of muscle glycogen per note. Second, we obtained a distribution of possible costs per late evening male by using each of the 21 early-evening glycogen levels as a potential starting value. We divided the difference between each of the 21 early values and a male's late value by the number of notes the male gave to obtain 21 estimates of cost. The distribution of possible costs for all 12 late evening males is shown in Fig. 7 ($\bar{x} = 1.18$ μg of muscle glycogen per gram per note, $n = 252$). In subsequent calculations used to test the energy conservation hypothesis we use the first, lower cost, estimate since this provides a more conservative test of energetic constraints. Our cost estimate is also conservative because we assume no replenishment of muscle glycogen stores during calling. The rate at which this may occur is unknown.

Discussion

Note rates among males varied by a factor of 1.54 in the chorus of 8 July, and by over 4.00 on 18 July (Table 1, rate 1). Previous data on calling behavior of males of *H. microcephala* also revealed considerable variation among males in calling rates (Schwartz 1986, 1991; Schwartz and Wells 1985; Wells and Taigen 1989). In spite of individual variation in call rate, the general pattern of calling during the course of an evening's chorusing appears similar among many of the males in a natural chorus (Figs. 2a, 3a). This was expected because playback experiments and recordings of pairwise interactions demonstrated that males are vocally responsive to the calls of other individuals (Schwartz, 1986; 1993; Schwartz and Wells 1985). Males typically

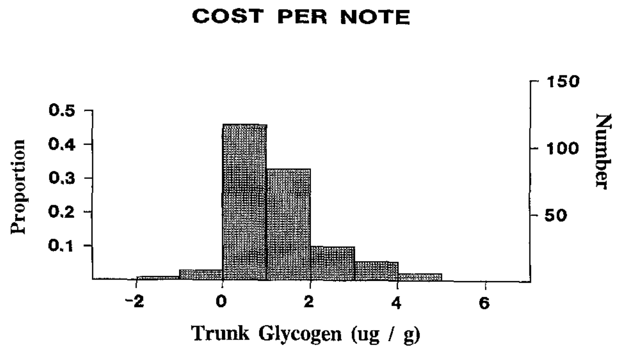

Fig. 7 The distribution of estimates of the cost in trunk muscle glycogen of producing a note for each of the 12 late-evening males whose vocal activity was monitored (see text for explanation)

exhibit relatively low note rates and long inter-bout intervals both early in the evening and at the end of chorusing. Calling rates are higher during the central period of chorus activity. This pattern of vocal activity was apparent in the chorus of 8 July, although it was somewhat less evident on 18 July. A similar pattern was seen in *H. versicolor* (Wells and Taigen 1986).

Considerable variation in trunk muscle glycogen existed among males of *H. microcephala* at both the initiation and termination of chorusing. However, our data indicate that differences in substrate levels are not translated into clear differences in vocal behavior. This would have been expected if males have some way of gauging their fuel reserves and adjusting their note output rate accordingly. Rather, males with low initial trunk muscle glycogen may continue to call at rates comparable to those of their neighbors until glycogen is nearly or entirely exhausted. Such a pattern would be consistent with data from mammals, in which performance does not decline until muscle carbohydrate reserves approach very low levels (Karlsson and Saltin 1971; Holloszy and Booth 1976). Therefore, on nights in which vocal activity is high, some males might be expected to cease calling before other individuals. On other nights, when choruses are relatively quiet, the less energetically well endowed males could continue to call for as long as their neighbors.

Differences in calling rate among males may be associated with physiological constraints (Halliday 1987) and so might reflect differences in such attributes as muscle mass, capillary density, mitochondrial density or even enzyme activity levels (e.g., citrate synthase). These kinds of physical and physiological differences among species of anurans correlate well with interspecific differences in vocal behavior (Pough et al. 1992; Ressel 1993). Unfortunately, at the intraspecific level, few data to test this idea are available. Data from studies in which physical or biochemical measurements were made directly provide either no or only limited support (McKay 1989; Walker 1989; Given and McKay 1990; Lance and Wells 1993; Wells et al. 1995), while results of studies testing the relationship between calling behavior in nature and aerobic capacity during forced exercise in the laboratory have failed to find a significant positive correlation (Wells and Taigen 1984; Sullivan and Walsberg 1985). Additionally, differences in motivational level may contribute to differences in calling behavior.

While contributing to a growing body of data demonstrating that depletion of muscle glycogen accompanies calling activity in anurans, our results also support the energy conservation hypothesis for periodic calling in *H. microcephala*. Using our estimate of the cost in muscle glycogen of producing a note, we calculated the time each male in the three chorus groups could have called, without pausing (rate 2 of Table 1), until its supply of trunk muscle glycogen was exhausted. Three calling durations were estimated, assuming that an individual began vocalizing with the minimum, average, or maximum level of trunk muscle glycogen found in our early evening sample of males (Table 3). The calculations indicate that, with an average starting level of glycogen, most males (16 out of 19) would have exhausted their muscle glycogen reserves in under 3 h [maximum note rate sustainable for 3 h = (11.91 mg glycogen per g per 3 h) × (1/0.000978 mg glycogen per gram per note) = 4059 notes per h]. This is true also for 12 of the 15 males recorded in 1989 during no-stimulus periods discussed in Schwartz (1991; see his Fig. 9). Some males would have run out of muscle glycogen in under 3 h even if they started with the maximum level of 18.34 mg/g. The results are particularly dramatic for the choruses of 8 July and 1 August. If very low or complete depletion of trunk muscle glycogen imposes a physiological constraint on calling, then males of *H. microcephala* appear able to extend their chorusing activity through periodic cessations of calling. Males of *H. microcephala* spend an average of 3.85 nights in the chorus (n = 325; Bevier 1995), and muscle glycogen may be replenished daily from food (Taylor et al. 1993), glycogen stores in the liver, and gluconeogenesis (Weber 1992). The energy reserves conserved by pauses in calling also might allow males to extend the number of nights they spend in the chorus advertising for mates before depleting liver reserves. Thus, the potential enhancement of mating success

Table 3 Estimated durations (min : s) of calling until exhaustion of glycogen reserves for males calling at rates recalculated without pauses (rate 2 of Table 1). With pauses the estimated calling durations could increase by the *percent* shown (*min*, *aver*, and *max* are durations based on starting glycogen levels of 6.2, 11.91, and 18.34 mg/g, respectively)

Frog	Min	Aver	Max	Percent
8 July				
1	52:58	101:44	156:40	52
2	33:20	64:01	98:35	91
3	65:38	126:06	194:10	32
4	54:43	105:00	161:51	51
5	82:20	158:10	243:34	19
6	76:53	147:41	227:25	27
7	57:15	109:59	169:22	30
18 July				
1	302:45	581:34	895:33	92
2	169:23	325:22	501:02	132
3	85:26	164:07	252:43	125
4	81:18	156:11	240:30	75
5	126:15	242:31	373:27	127
1 August				
1	54:02	103:47	159:49	48
2	53:53	103:30	159:22	59
3	85:07	163:31	251:48	29
4	69:21	133:13	205:09	22
5	63:04	121:09	186:34	44
6	55:44	107:03	164:51	38
7	47:47	91:48	141:21	46

accompanying extended chorus tenure may, in part, explain why males engage in unison bout singing even on relatively quiet nights.

We had predicted that males that were stimulated to vocalize by broadcasts of recorded calls would exhibit trunk muscle glycogen levels near zero when they stopped chorusing. This expectation was met for two of the three males tested on 16 September. On 4 November, however, males ended their vocal activity with glycogen stores well above zero. These data demonstrate that additional unknown proximate factors can cause males to finally stop calling. Males might, for example, be able to assess the likelihood of amplexing based on the time of night, recent environmental history at the site (e.g., rainfall pattern), as well as the acoustic milieu. If the costs of continued calling exceed the expected benefits, males would end their vocal activity. Wells et al. (1995) found that most males of *H. versicolor* had considerable trunk muscle glycogen reserves when they were collected near the end of chorusing activity.

Our understanding of the energetics of anuran vocal behavior is far from complete, and there may be considerable heterogeneity among species in the relative importance of different substrates used to fuel calling (Pough et al. 1992). Trunk muscles of males of *H. microcephala* contain large reserves of lipid (Ressel 1993), and our data suggest indirectly that males of *H. microcephala* utilize other substrates in addition to muscle glycogen to support the energetic demands of calling. This is illustrated in the following simple calculation. Our estimated energetic cost per note equals 0.0172 Joules per g trunk muscle [(0.000978 mg glycogen/note × g) × (17.6 J/mg glycogen)]: see Nagy 1983, p. 28]. Therefore the energy contribution made by muscle glycogen for an average-sized male (trunk muscle mass = 0.06 g) calling at a rate of 3800 notes/h should be about 3.9 J/h. Based on measurements of aerobic metabolism, Wells and Taigen (1989) estimated an energy cost of 20 J/h for a male calling at this rate (see their Fig. 2a and calculations on their p. 19). Thus, muscle glycogen supplies, on average, approximately 20% of the energy consumed during calling in *H. microcephala*. These data are consistent with evidence indicating that highly aerobic vertebrates should more heavily rely on lipids than carbohydrates to fuel activity (Weber 1992). Other species of anurans (MacNally 1981; Marsh and Taigen 1987; McKay 1989; Walker 1989; Grafe et al. 1992; T. U. Grafe, unpublished work; Wells et al. 1995) also appear to oxidize fat as well as glycogen during chorusing, perhaps indicating that their trunk muscles are unable to store quantities of glycogen sufficient to maintain high levels of prolonged vocal activity. Measurements of respiratory quotients (RQ: ratio of CO_2 released to O_2 consumed) of calling frogs, which differ for metabolic activity fueled by fat and carbohydrate (Schmidt-Nielson 1990), are essential to gain a more thorough understanding of the pattern and time course of substrate utilization in these animals (see Grafe et al. 1992; T. U. Grafe, unpublished work, for RQ estimates for *Hyperolius marmoratus broadleyi* and the gray treefrog, *Hyla. versicolor*, respectively) as well as the role of energetic constraints on male advertisement. For example, if males of *H. microcephala* increase their reliance on lipid as glycogen is depleted, calling could be extended longer than we have estimated.

The results of this study raise important questions on the evolution of unison bout singing. For example, given that there is an energetic limitation on calling, what advantage is it to a male of *H. microcephala* to call for 3–5 h with periodic pauses rather than to call steadily for a shorter period? Considerable data are available that demonstrate that male mating success in frogs is positively correlated with male tenure in the chorus on a time scale of days (e.g., Greer and Wells 1980; Arak 1983; Jacobson 1985; Ryan 1985; Halliday and Verrell 1986; Gerhardt et al. 1987; Given 1988; Ritke and Semlitsch 1991; Murphy 1992), and a similar relationship is probable on a time scale of hours. Therefore, if the time at which females pair with males is unpredictable, then those males that can call for extended periods may be at an advantage relative to those that cease calling earlier. Modelling of male mating success using information on female arrival times is necessary to test this idea.

We found that females paired with males over an approximately 4.5-h period. Neither the amount of time that females sit in the chorus before pairing nor the time period during which they compare potential mates is known. Moreover, some females that we found in amplexus may not have been seen during earlier searches of the study site. Accordingly, the actual time window during which vocal advertisement could contribute to a male's chances of mating may be slightly wider than that shown in Fig. 5 and the true distribution of pairing times somewhat flatter. Nevertheless, it seems unlikely that males of *H. microcephala* could optimize their calling effort so as to coincide precisely with the presence of females in their vicinity. How the actual shape of the distribution of female pairing times may have shaped the evolution of unison bout is something that needs to be explored.

A modelling effort could also help answer the question of why males clump their calls together during the same hours rather than calling at different times of night (see Partridge and Krebs 1978 for any early model of frog chorusing). However, the short-term clumping of calling activity within bouts by males may be related to the very strong preference females have for males with higher rates of note production. Recent data (Schwartz 1994) demonstrate that this preference, previously shown in two-stimulus female choice tests, is exercised in natural choruses. Schwartz found that females of *H. microcephala* most often selected the caller with the highest note rate. The chorus we monitored on 1 August 1993 provides a particularly impressive demonstration of how this component of male vocal behavior can influence mate choice. In order to compete effectively for females, males must be vocally responsive to the calls of other individuals (Schwartz and Wells 1985; Schwartz 1986). Therefore, a strategy of waiting until rivals are quiet until calling may not be an effective option because a male's vocalizations would rapidly elicit calling by his rivals. Moreover, a male which adopted such a strategy would himself have to be refractory to the acoustic stimulation of other chorus members.

Another question that remains unanswered is what proximate cues, if any, determine when a male should end a bout of calling. It is unlikely that a physiological constraint is responsible since there was considerable variation in the number of notes produced per bout per frog (8 July, median CV = 85%, median range = 283; 18 July, median CV = 91%, median range = 59). In addition, males in the more active chorus of July 8, produced more notes per bout than males in the chorus of 18 July ($\bar{x}$ = 56.6, n = 1288; $\bar{x}$ = 18.1, n = 545; Wilcoxon two-sample test, $Z = -22.12$, $P < 0.0001$). When males of *H. microcephala* were acoustically stimulated by call-triggered, computer-generated calls simulating those from a large chorus, they increased the durations of their bouts of calling by approximately 50% (Schwartz 1991; see his Fig. 4). This suggests that the acoustic environment can be quite important in determining when a male stops calling. The number of males calling and their rate of note production may allow a male to gauge the competitive environment and determine whether to stop calling. Furthermore, as a chorusing bout progresses, the cumulative probability that males will drop out by chance should increase and so reduce acoustic stimulation of the remaining callers. In this way, the complex acoustic dynamics in the chorus may act as a proximate cue influencing call cessation, while the ultimate explanation for pauses in chorusing appears to be related to conservation of energy reserves.

Acknowledgements We thank Alan Bond, Mandy Dyson, Stan Rand, Lee Dugatkin, and Ted Taigen for helpful discussions and Carl Gerhardt, Kit Murphy, Michael Greenfield, and Kentwood Wells who also provided valuable comments on the manuscript. Stan Rand provided access to an Amiga 2000 computer, Gen Rad and Realistic sound level meters, and a Mineroff Electronics speaker-amplifier. Ted Taigen provided access to his laboratory and use of a Gilson Spectrophotometer (with sipper unit). Financial support was provided by Smithsonian Short-Term Visitor Awards to JJS, a Smithsonian Short-Term Fellowship, Sigma Xi Grant-in-Aid of Research and University of Connecticut Summer Research Fellowship to CRB, and a National Science Foundation Graduate Research Training Grant awarded to the Department of Ecology and Evolutionary Biology at the University of Connecticut.

References

Alexander RD (1975) Natural selection and specialized chorusing behavior in acoustical insects. In: Pimental D (ed) Insects, science, and society. Academic Press, New York, pp 35–77

Arak A (1983) Sexual selection by male-male competition in natterjack toad choruses. Nature 306:261–262

Bailey WJ, Withers PC, Endersby M, Gaull K (1993) The energetic costs of calling in the bushcricket *Requena verticalis* (Orthoptera: Tettigoniidae: Listroscelidinae). J Exp Biol 178: 21–37

Bevier CR (1995) Physiological constraints on calling activity in neotropical frogs. Ph.D. dissertation, The University of Connecticut, Storrs

Brush JS, Narins PM (1989) Chorus dynamics of a Neotropical amphibian assemblage: comparison of computer simulation and natural behaviour. Anim Behav 37:33–44

Buck J (1988) Synchronous rhythmic flashing of fireflies, II. Q Rev Biol 63:265–289

Conlee RK (1987) Muscle glycogen and exercise endurance: a twenty-year perspective. Exercise Sports Sci Rev 15:1–28

Conlee RK, Hammer RL, Windner WW, Bracken ML, Nelson AG, Barnett DW (1990) Glycogen repletion and exercise endurance in rats adapted to high fat diet. Metabolism 39:289–294

Elmberg J, Lundberg P (1991) Intraspecific variation in calling, time allocation and energy reserves in breeding male common frogs *Rana temporaria*. Ann Zool Fenn 28:23–29

Gerhardt HC, Daniel RE, Perrill SA, Schramm S (1987) Mating behaviour and male mating success in the green treefrog. Anim Behav 35:1490–1503

Given MF (1988) Territoriality and aggressive interactions in male carpenter frogs, *Rana virgatipes*. Copeia 1988:411–421

Given MF, McKay DM (1990) Variation in citrate synthase activity in calling muscles of carpenter frogs, *Rana virgatipes*. Copeia 1990:863–867

Gleeson TT (1991) Patterns of metabolic recovery from exercise in amphibians and reptiles. J Exp Biol 160:187–207

Grafe TU, Schmuck R, Linsenmair KE (1992) Reproductive energetics of the African reed frogs, *Hyperolius marmoratus*. Physiol Zool 65:153–171

Greenfield MD (1994) Cooperation and conflict in the evolution of signal interactions. Annu Rev Ecol Syst 25:97–126

Greenfield MD, Roizen I (1993) Katydid synchronous chorusing is an evolutionarily stable outcome of female choice. Nature 364:618–620

Greenfield MD, Shaw KC (1983) Adaptive significance of chorusing with special reference to the Orthoptera. In: Morris GK, Gwynne DT (eds) Orthopteran mating systems: sexual competition in a diverse group of insects. Westview, Boulder, Colorado, pp 1–27

Greer BJ, Wells KD (1980) Territorial and reproductive behavior of the tropical American frog, *Centrolenella fleischmanni*. Herpetologica 36:318–326

Halliday T (1987) Physiological constraints on sexual selection. In: Bradbury JW, Andersson MB (eds) Sexual selection: testing the alternatives. Wiley, New York, pp 247–264

Halliday TR, Verrell PA (1986) Sexual selection and body size in amphibians. Herpetol J 1:86–92

Hochachka PW, Somero GN (1984) Biochemical adaptation. Princeton University Press, Princeton

Holloszy JO, Booth FW (1976) Biochemical adaptations to endurance exercise in muscle. Annu Rev Physiol 38:273–291

Hoppler H, Billeter R(1991) Conditions for oxygen and substrate transport in muscles in exercising mammals. J Exp Biol 160: 263–283

Jacobson SK (1985) Reproductive behavior and male mating success in two species of glass frogs (Centrolenidae). Herpetologica 41:396–404

Karlsson J, Saltin B (1971) Diet, muscle glycogen, and endurance performance. J Appl Phys 31:203–206

Keppler D, Decker K (1974) Glycogen: determination with amyloglucosidase. In: Bergmeyer HU (ed) Methods of enzymatic analysis. Academic Press, New York, pp 1127–1131

Lance SL, Wells KD (1993) Are spring peeper satellite males physiologically inferior to calling males? Copeia 1993:1162–1166

Lee H-J, Loher W (1993) The mating strategy of the male short-tailed cricket *Anurogryllus muticus* de Greer. Ethology 95:327–344

Marsh RL, Dawson WR (1982) Substrate metabolism in seasonally acclimatized American goldfinches. Am J Physiol 242: R563–R569

Marsh RL, Taigen TL (1987) Properties enhancing aerobic capacity of calling muscles in gray treefrogs *Hyla versicolor*. Am J Physiol 252:R786–R793

MacNally RC (1981) On the reproductive energetics of chorusing males: energy depletion profiles, restoration and growth in two sympatric species of *Ranidella* (Anura). Oecologia 51: 181–188

McKay DM (1989) Seasonal variation in calling behavior and muscle biochemistry in spring peepers, *Hyla crucifer*. M. S. Thesis, The University of Connecticut, Storrs

Mirolló R, Strogatz S (1990) Synchronization of pulse-coupled biological oscillators. SIAM J Appl Math 50:1645–1662

Murphy CG (1992) The mating system of the barking treefrog (*Hyla gratiosa*). Ph.D. Dissertation, Cornell University, Ithaca, New York

Nagy KA (1983) Ecological energetics. In: Huey RB, Pianka ER, Schoener TW (eds) Lizard ecology. Harvard University Press, Cambridge, pp 24–54

Narins PM, Zelick R (1988) The effects of noise on auditory processing and behavior in amphibians. In: Fritzsch B, Wilczynski W, Ryan MJ, Hetherington T, Walkowiak W (eds) The evolution of the amphibian auditory system. Wiley, New York, pp 511–536

Otte D (1977) Communication in Orthoptera. In: Sebeok TA (ed) How animals communicate. Indiana University Press, Bloomington, pp 334–361

Partridge B, Krebs JR (1978) Tree frog choruses: a mixed evolutionarily stable strategy? Anim Behav 26:959–963

Pough FH, Gatten RE (1984) The use of anaerobic metabolism by frogs in a breeding chorus. Comp Biochem Physiol 78A: 337–340

Pough FW, Magnusson WE, Ryan MJ, Wells KD, Taigen TL (1992) Behavioral energetics. In: Feder ME, Burggren WW (eds) Environmental physiology of the amphibians. University of Chicago Press, Chicago, pp 395–436

Prestwich KN, Brugger KE, Topping M (1989) Energy and communication in three species of hylid frogs: power input, power output, and efficiency. J Exp Biol 144:53–80

Ressel SJ (1993) A morphometric analysis of anuran skeletal muscle ultrastructure: implications for functional design of muscles in ectotherms. Ph.D. Dissertation, The University of Connecticut, Storrs

Ritke ME, Semlitsch RD (1991) Mating behavior and determinants of male mating success in the gray treefrog, *Hyla chrysoscelis*. Can J Zool 69:246–250

Rosen M, Lemon RE (1974) The vocal behavior of spring peepers, *Hyla crucifer*. Copeia 1974:940–950

Ryan MJ (1985) The tungara frog. University of Chicago Press, Chicago

Ryan MJ (1988) Energy, calling, and selection. Am Zool 28: 885–898

Ryan MJ, Bartholomew GA, Rand S (1983) Energetics of reproduction in a neotropical frog, *Physalaemus pustulosus*. Ecology 64:1456–1462

Schmidt-Nielson K (1990) Animal physiology, 4 edn. Cambridge University Press, Cambridge

Schwartz JJ (1986) Male calling behavior and female choice in the neotropical treefrog *Hyla microcephala*. Ethology 73:116–127

Schwartz JJ (1987) The function of call alternation in anuran amphibians: a test of three hypotheses. Evolution 41:461–471

Schwartz JJ (1991) Why stop calling? A study of unison bout singing in a Neotropical treefrog. Anim Behav 42:565–577

Schwartz JJ (1993) Male calling-behavior, female discrimination and acoustic interference in the Neotropical treefrog *Hyla microcephala* under realistic acoustic conditions. Behav Ecol Sociobiol 32:401–414

Schwartz JJ (1994) Male advertisement and female choice in frogs: new findings and recent approaches to the study of communication in a dynamic acoustic environment. Am Zool 34: 616–624

Schwartz JJ, Wells KD (1983) An experimental study of acoustic interference between two species of neotropical treefrogs. Anim Behav 31:181–190

Schwartz JJ, Wells KD (1985) Intra- and interspecific vocal behavior of the neotropical treefrog *Hyla microcephala*. Copeia 1985:27–38

Sibly RM, Nott HMR, Fletcher DJ (1990) Splitting behaviour into bouts. Anim Behav 39:63–69

Slater PJB, Lester NP (1982) Minimizing errors in splitting behaviour into bouts. Behaviour 79:153–161

Smith HM (1935) Synchronous flashing of fireflies. Science 82:151

Sullivan BK, Walsberg GE (1985) Call rate and aerobic capacity in woodhouses's toad (*Bufo woodhousei*). Herpetologica 41: 404–407

Taigen TL, Wells KD (1985) Energetics of vocalizations by an anuran amphibian. J Comp Physiol A 155:163–170

Taylor R, Price TB, Katz LD, Shulman RG, Shulman GI (1993) Direct measurement of change in muscle glycogen concentration after a mixed meal in normal subjects. Am J Physiol 265:E224–E229

Walker SL (1989) The effect of substrate depletion on the calling behavior of the gray treefrog. M.S. Thesis, The University of Connecticut, Storrs

Weber J-M (1992) Pathways for oxidative fuel provision to working muscles: ecological consequences of maximal supply limitations. Experientia 48:557–564

Wells KD, Taigen TL (1984) Reproductive behavior and aerobic capacities of male American toads (*Bufo americanus*): is behavior constrained by physiology? Herpetologica 40:292–298

Wells KD, Taigen TL (1986) The effect of social interactions on calling energetics in the gray treefrog (*Hyla versicolor*) Behav Ecol Sociobiol 19:9–18

Wells KD, Taigen TL (1989) Calling energetics of the neotropical treefrog *Hyla microcephala*. Behav Ecol Sociobiol 25:13–22

Wells KD, Taigen TL, Rusch SW, Bevier CR (1995) Seasonal and nightly variation in glycogen reserves of calling gray treefrogs (*Hyla versicolor*). Herpetelogica, in press

Whitney CL, Krebs JR (1975) Mate selection in Pacific tree frogs. Nature 255:325–326

Communicated by G.M. Klump