

November 01, 1985

## The enzymatic basis of high metabolic rates in calling frogs

Theodore L. Taigen

*University of Connecticut - Storrs*

Kentwood D. Wells

*University of Connecticut - Storrs*

Richard L. Marsh

*Northeastern University - Dept. of Biology*

---

### Recommended Citation

Taigen, Theodore L.; Wells, Kentwood D.; and Marsh, Richard L., "The enzymatic basis of high metabolic rates in calling frogs" (1985). *Biology Faculty Publications*. Paper 2. <http://hdl.handle.net/2047/d20000610>

# THE ENZYMATIC BASIS OF HIGH METABOLIC RATES IN CALLING FROGS<sup>1</sup>

THEODORE L. TAIGEN,\* KENTWOOD D. WELLS,\* AND RICHARD L. MARSH†

\*Department of Ecology and Evolutionary Biology, U-42, University of Connecticut, Storrs, Connecticut 06268; †Biology Department, Northeastern University, Boston, Massachusetts 02115

(Accepted 5/7/85)

Oxygen consumption by male spring peepers (*Hyla crucifer*) increased linearly with calling rate, reaching peak values of 1.5–1.7 ml O<sub>2</sub>/(g · h) at the highest calling rates. The intercept of the regression line describing the relationship between metabolism and calling rate does not differ significantly from daytime resting metabolism (0.11 ml O<sub>2</sub>/(g · h)). Metabolic rate during vigorous locomotor exercise at the same temperature (19 C) was only 1.1 ml O<sub>2</sub>/(g · h). We measured activities of mitochondrial enzymes in the trunk muscles (internal and external obliques) involved in sound production and in mixed hind limb muscles of male and female frogs. Male trunk muscles were very large, accounting for 15% of total body mass, whereas female trunk muscle constituted only 3% of total body mass. Citrate synthase (CS) activity in male trunk muscle, indicative of oxidative capacity, was six times the CS activity in leg muscle (86 vs. 14 μmol/[min · g fresh muscle at 20 C]) and 17 times the CS activity in female trunk muscle (5 μmol/[min · g fresh muscle]). The capacity to oxidize fat, as indicated by β-hydroxyacyl-CoA dehydrogenase activity, was five times higher in male trunk muscle than in leg muscle (30 vs. 6 μmol/[min · g fresh muscle]), suggesting that fatty acid oxidation plays an important role in the energetics of vocalization in this species. Phosphofructokinase activity, a key glycolytic marker, was not significantly different in trunk and leg muscle. The capacity of male spring peeper trunk muscles for aerobic metabolism exceeds considerably the highest values yet reported for ectothermic vertebrate muscle tissue and is comparable to highly oxidative muscles of endotherms.

## INTRODUCTION

Investigations of the natural activities of lower vertebrates have revealed that, though the behavior of these animals is often surprisingly complex, the energetic costs of most activities are quite low, especially in comparison with mammalian or avian energetics. For example, the metabolic rates of salamanders engaged in courtship behavior are barely elevated above resting values (Bennett and Houck 1983). Even continuous, active foraging by lizards at high body temperatures entails only mod-

erate rates of oxygen consumption that are less than four times resting metabolism (Bennett and Gleeson 1979). In striking contrast to these results, recent studies of anuran vocal behavior indicate that the energetic cost of calling by male frogs to attract females can be very high, as great as 25 times resting metabolic rates (Bucher, Ryan, and Bartholomew 1982; Taigen and Wells 1985). Among the published data on energetics of natural activities of terrestrial ectothermic vertebrates, metabolic rates during acoustic advertisement by male frogs are by far the highest (Taigen and Wells 1985).

In addition to documenting the high cost of calling, our previous analysis of vocalization energetics in the gray tree frog (*Hyla versicolor*) produced an unexpected and perplexing result: rates of oxygen consumption while calling were considerably higher than those attained during vigorous locomotor exercise (Taigen and Wells 1985). These data are not consistent with many previous studies of anuran exercise physiology in which oxygen consumption during forced locomotion is assumed to be

<sup>1</sup> Our research was supported by grants from the University of Connecticut Research Foundation to T.L.T. and K.D.W. and by grants from the Northeastern University Biomedical Support Grant (Department of Health and Human Services, RR07143) and the Northeastern University Research and Scholarship Development Fund to R.L.M. We thank C. S. Henry and G. C. Packard for their constructive comments on a draft of the manuscript. The assistance of U. Koehn with the statistical analysis is greatly appreciated.

*Physiol. Zool.* 58(6):719–726, 1985.

© 1985 by The University of Chicago. All rights reserved. 0031-935X/85/5806-8528\$02.00

the maximum metabolic rate the animals can sustain (Bennett and Licht [1973]; Seymour [1973]; for additional references before 1978, see review of Bennett [1978]; thereafter Carey [1979*a*, 1979*b*]; Hillman and Withers [1979]; Hillman et al. [1979]; Hillman [1980]; Miller and Hutchison [1980]; Taigen and Pough [1981]; Taigen, Emerson, and Pough [1982]; Taigen [1983]; Taigen and Pough [1983]; Withers and Hillman [1983]; Taigen and Beuchat [1984]; and numerous references therein). An explanation suggested by Taigen and Wells (1985) for these unusual data is that vocalization and locomotion involve the use of muscles with different oxidative capacities. Current understanding of anuran vocalization indicates that the internal and external obliques (located in the trunk region and hereafter collectively referred to as "trunk" muscle) are used to create the pressure in the thoracic cavity necessary for sound production (Martin and Gans 1972; Gans 1973). High metabolic rates in calling frogs may be the result of sustained, intense contraction of large trunk muscles with high catabolic enzyme activities. These considerations led us to investigate the energetics of sound production in the spring peeper (*Hyla crucifer*), together with the size and enzyme profiles of muscles used in calling.

## MATERIAL AND METHODS

### METABOLIC MEASUREMENTS

Spring peepers emerge early in the spring and form dense choruses in northeastern Connecticut shortly after ponds are free of ice. We collected calling males in early April from a chorus located at a small pond near Storrs, Connecticut. The animals were taken to a laboratory and maintained individually in small plastic containers (ca. 1 liter volume) in an environmental cabinet at 19 C (12L:12D). Within 2 days of their arrival in the laboratory, the frogs became accustomed to the plastic containers and began calling voluntarily during scotophase in the environmental cabinet. Once the laboratory chorus was formed, we selected a single male and put him in an airtight metabolic chamber constructed from Plexiglas (250 ml volume). The metabolic chamber was placed in the middle of the environmental cabinet, surrounded by 15-

20 calling frogs. When the animal inside the metabolic chamber joined the chorus and began calling, a 15-ml gas sample was drawn into a plastic syringe from the chamber through a short length of Tygon tubing attached to a remotely controlled solenoid valve mounted on the side of the chamber. The solenoid valve allowed us to open and close the metabolic chamber to extract gas samples without opening the environmental cabinet and disturbing the calling frogs. After 20 min of calling, a second gas sample was taken from the chamber. Oxygen consumption by the calling frog ( $\dot{V}O_2$  call) was calculated from the difference in fractional oxygen concentration of the two gas samples, measured with an Applied Electrochemistry S-3A oxygen analyzer. We used a syringe pump (Razel Scientific Instruments, Inc.) to inject the samples through a small glass tube packed with Ascarite and Drierite (to remove  $CO_2$  and  $H_2O$ , respectively) and into the oxygen analyzer. All metabolic measurements of calling frogs were made between 2000 and 2300 hours EST at an air temperature of 19 C, selected to facilitate comparison with data collected at 19 C for *Hyla versicolor* (Taigen and Wells 1985). Temperature inside the plastic containers, monitored with a Wescor thermocouple telethermometer, varied less than 0.5 C throughout the experiment. The mean body mass of the frogs used in these measurements was  $1.22 \text{ g} \pm 0.10 \text{ SD}$ .

Spring peepers produce simple tonelike calls that vary in duration and rate (Rosen and Lemon 1974; Wilczynski, Zakon, and Brenowitz 1984). We recorded the vocal behavior of the frogs in the metabolic chamber through a miniature condenser microphone (Realistic model 33-1056A) mounted in a rubber stopper at one end of the chamber. Calling rate and call duration were later analyzed from these recordings using a Tektronix 5111B storage oscilloscope. We also measured the calling rate of spring peepers in a natural chorus at the time that the experimental animals were collected.

Resting metabolic rates ( $\dot{V}O_2$  rest) were measured between 1300 and 1500 hours EST, when animals in the field are normally inactive. We placed both male and female frogs individually in Plexiglas metabolic chambers at 0700 hours EST and left them

undisturbed for 6–8 h before starting our measurements. Values of  $\dot{V}O_2$  rest were calculated from fractional oxygen concentrations of static gas samples drawn from the chambers before and after a 1-h period of quiescence. Immediately following these measurements, we determined oxygen consumption during forced locomotor exercise ( $\dot{V}O_2$  ex), again using a closed respirometer system based on the analysis of static gas samples. Gas samples were taken from the chambers before and after a 4-min bout of intense exercise, stimulated by mechanical rotation of the cylindrical chambers using an electric motor (see Seymour [1973] for details). Rotation rates were precisely controlled with an electric motor speed controller (G. K. Heller Corp., model T2) to elicit maximum activity. By the end of the exercise period, the animals exhibited clear signs of fatigue, including reduced control over the actions of the hind limbs and slow righting response. Both resting and activity metabolism were measured at  $19 \pm 0.25$  C. The male frogs on which these measurements were made were the same individuals used in our investigation of calling metabolism. The female frogs were collected in the field at the same time as the males and maintained in the laboratory under the same conditions. All the females had laid their eggs before they were included in our experiment.

#### ENZYME ACTIVITIES

We measured catabolic enzyme activities in mixed hind limb muscles and in the internal and external oblique muscles of male and female frogs used in the metabolic experiments described above. Muscles were quickly dissected from pithed male and female frogs and weighed immediately on an electronic balance ( $\pm 0.001$  g) to determine fresh mass of the combined hind limb muscles and the trunk muscles. We assayed for three enzymes: (1) phosphofructokinase (PFK), an indicator of glycolytic capacity (Crabtree and Newsholme 1972); (2) citrate synthase (CS), an indicator of capacity for aerobic ATP production (Marsh 1981); and (3)  $\beta$ -hydroxyacyl-CoA dehydrogenase (HOAD), an indicator of capacity for fatty acid oxidation (Marsh 1981). Trunk muscle tissue and mixed leg muscle tissue were divided in half, with one-half used for the de-

termination of PFK activity and the other half for measurement of CS and HOAD activity. Catalytic activities of PFK, CS, and HOAD were measured in crude muscle homogenates at 20 C using spectrophotometric assays with saturating levels of substrates, as described in Marsh (1981). We used a Gilford model 260 UV/VIS spectrophotometer with a computerized data acquisition system (Apple II+ computer with Interactive Microware, Inc., Adalab System) in these measurements. The PFK and CS assays were run at pH 8.2, the HOAD assays at pH 7.0. Duplicates were made for all assays, and enzyme activities are reported as  $\mu\text{mol}/(\text{min} \cdot \text{g fresh muscle mass})$ . We also measured total protein content of the muscles using the method of Lowry et al. (1951) with bovine serum albumin as a standard.

#### RESULTS

Oxygen consumption of calling frogs increased linearly with calling rate, reaching peak values of 1.5–1.7 ml  $O_2/(\text{g} \cdot \text{h})$  at the highest calling rates (fig. 1). The intercept of the least-squares regression line describing the relationship between metabolism and calling rate is not significantly different from daytime resting metabolism. Call duration did not vary significantly among males (mean = 0.109 s/call, range 0.098–0.127) and consequently was not a source of variation in  $\dot{V}O_2$  call. We used the results of a least-squares regression analysis (fig. 1) to estimate rates of oxygen consumption of free-ranging frogs calling in a natural chorus. Calling rates of animals in the field averaged 4,500 calls/h (range 3,200–5,700,  $n = 12$ ) at 16 C. The predicted metabolism of spring peepers calling at this rate is 1.51 ml  $O_2/(\text{g} \cdot \text{h})$ . This value is 38% higher than the metabolic rate of male frogs during vigorous locomotor exercise (table 1), a difference that is highly significant ( $t_{13} = 4.1$ ,  $P < .005$ ). The statistical comparison of  $\dot{V}O_2$  call in a natural chorus with  $\dot{V}O_2$  ex is complicated because the variance in our estimate of  $\dot{V}O_2$  call actually has two components: (1) variance stemming from variation in calling rate among males at the pond, and (2) variance associated with the regression analysis used to predict  $\dot{V}O_2$  call from calling rate. These components were calculated using standard methods (Hogg

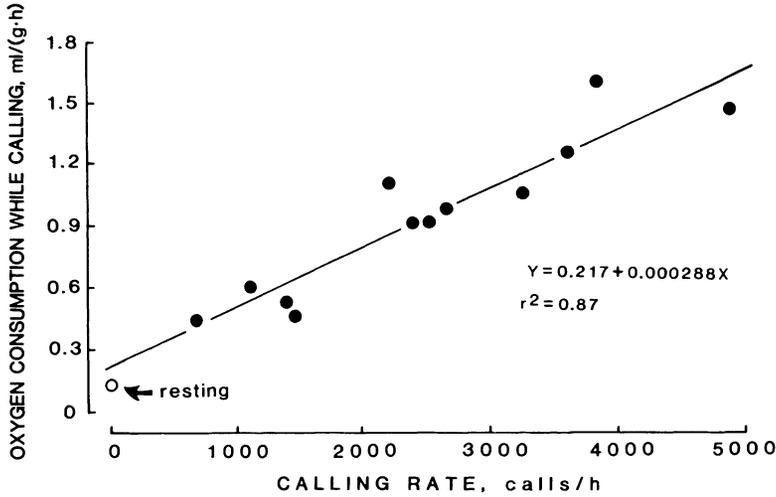


FIG. 1.—Oxygen consumption by male spring peepers as a function of calling rate. The solid line represents the results of a least-squares regression analysis, and the open circle is the metabolic rate of male frogs at rest.

and Craig 1978, pp. 176–178) and account for 26% and 74%, respectively, of the total variance. The standard error given in table 1 incorporates both components of variance. The difference between  $\dot{V}O_2$  call in a natural chorus and  $\dot{V}O_2$  ex was analyzed with a two-sample *t*-test for means with unequal variance (Brownlee 1960).

The metabolic rates of exercising females were significantly lower ( $t_{22} = 3.4$ ,  $P < .01$ ) than those of exercising males (table 1). Males and females did not differ in  $\dot{V}O_2$  rest (table 1).

The trunk muscles used to produce sound in male spring peepers accounted for an average of 14.8% of the total body mass of these animals and were nearly as large as the combined mass of all the muscles in both legs (table 2). In comparison, females

had much smaller trunk muscles, constituting only 3% of body mass. The striking hypertrophy of male trunk muscles appears not to be the direct consequence of exercise. We have collected males at the beginning of the breeding season with fully developed trunk muscles.

In addition to being very large, male trunk muscles also had an extraordinary capacity for aerobic metabolism. The CS activity of male trunk muscle was six times that of male leg muscle and 17 times that of female trunk muscle (table 3). Males also differed significantly from females in the activity of citrate synthase in leg muscle (table 3,  $t_{14} = 3.5$ ,  $P < .01$ ). Total leg CS activity of males, calculated as the product of leg muscle mass and mass-specific CS activity, was 34% greater than that of fe-

TABLE 1

OXYGEN CONSUMPTION BY SPRING PEEPERS AT REST, DURING FORCED LOCOMOTOR EXERCISE, AND WHILE CALLING IN A NATURAL CHORUS

	$\dot{V}O_2$ rest (ml/(g·h))	$\dot{V}O_2$ ex (ml/(g·h))	$\dot{V}O_2$ call (ml/(g·h))
Male . . . . .	.108 ± .018 (10)	1.10 ± .03 (12)	1.51 ± .10 (12)
Female . . . . .	.112 ± .007 (10)	.91 ± .05 (10)	. . .

NOTE.— $\dot{V}O_2$  rest and  $\dot{V}O_2$  ex were measured in a laboratory at 19 C.  $\dot{V}O_2$  call at 16 C was estimated from the calling rates of 12 randomly selected males in a natural chorus, using the regression analysis presented in fig. 1 as described in the text. Values are means ± SE. Numbers in parentheses are sample sizes.

TABLE 2

BODY SIZE AND MUSCLE MASS OF MALE AND FEMALE SPRING PEEPERS

	n	BODY LENGTH (cm)	BODY MASS (g)	TRUNK MUSCLE MASS		LEG MUSCLE MASS	
				(g)	(%)	(g)	(%)
Males	6	2.61 ± .02	1.25 ± .05	.185 ± .012	14.8 ± .7	.205 ± .013	16.6 ± 1.1
Females	6	2.72 ± .09	1.05 ± .12	.035 ± .006	3.3 ± .3	.200 ± .029	18.7 ± .7

NOTE.—Females were weighed after they laid their eggs. Values are means ± SE.

males, a difference that parallels closely the difference between males and females in  $\dot{V}O_2$  ex (fig. 2). The capacity of male trunk muscles for fatty acid oxidation, as evidenced by HOAD activity, was also very high, five times that of male leg muscle and 12 times that of female trunk muscle. PFK activity, a key glycolytic marker, did not differ significantly in the muscles we assayed.

DISCUSSION

The metabolic data we report here, though discordant with the prevailing view of anuran exercise physiology, nevertheless are consistent with our earlier analysis of vocalization energetics in the gray tree frog (*Hyla versicolor*). In both studies, metabolic rates of calling frogs were significantly higher than metabolic rates during exhaustive locomotor exercise. There are at least two possible explanations for these unexpected results. One is that our technique for eliciting locomotor exercise did not fully stimulate the animals and that higher  $\dot{V}O_2$

ex values than those we measured are possible. However, the results we obtained are comparable to those of other investigators working with related hylid species. Hillman et al. (1979), using an exercise stimulation technique similar to ours, measured metabolic rates of 1.16 and 1.25 ml  $O_2$ /(g · h) for *Hyla regilla* and *H. cadaverina*, respectively, during forced locomotor exercise at 22–24 C. Moreover, many anuran species have substantially lower  $\dot{V}O_2$  ex values than that reported here for *H. crucifer* (Withers and Hillman 1981; Taigen et al. 1982). In general, anurans with high capacities for oxygen consumption during locomotor exercise have been found to possess high activities of oxidative enzymes in leg muscles (Bennett 1974; Putnam and Bennett 1982). Our results (fig. 2) extend this generalization to an intraspecific relationship and suggest that the frogs in our study attained metabolic performances during forced locomotion consistent with the enzymatic characteristics of their limb musculature.

A more likely explanation, strongly indicated by our data on catabolic enzyme

TABLE 3

CATABOLIC ENZYME ACTIVITIES IN TRUNK AND LEG MUSCLES OF SPRING PEEPERS

	ENZYME ACTIVITIES ( $\mu$ mol/[min · g fresh muscle])			TOTAL PROTEIN CONTENT (mg/g)
	Citrate synthase	$\beta$ -Hydroxyacyl-CoA dehydrogenase	Phosphofructokinase	
Males:				
Trunk:	85.8 ± 1.9 (6)	30.1 ± 1.8 (6)	5.2 ± .9 (5)	154 ± 9.7 (6)
Leg:	14.0 ± 1.3 (7)	5.7 ± .5 (7)	7.5 ± 1.1 (6)	197 ± 14.5 (7)
Females:				
Trunk:	5.0 ± .8 (6)	2.6 ± .3 (6)	3.7 ± .6 (6)	113 ± 15.6 (6)
Leg:	9.0 ± .7 (8)	3.3 ± .3 (8)	5.1 ± .2 (8)	128 ± 3.5 (8)

NOTE.—All activities were measured at 20 C. Values are means ± SE. Numbers in parentheses are sample sizes.

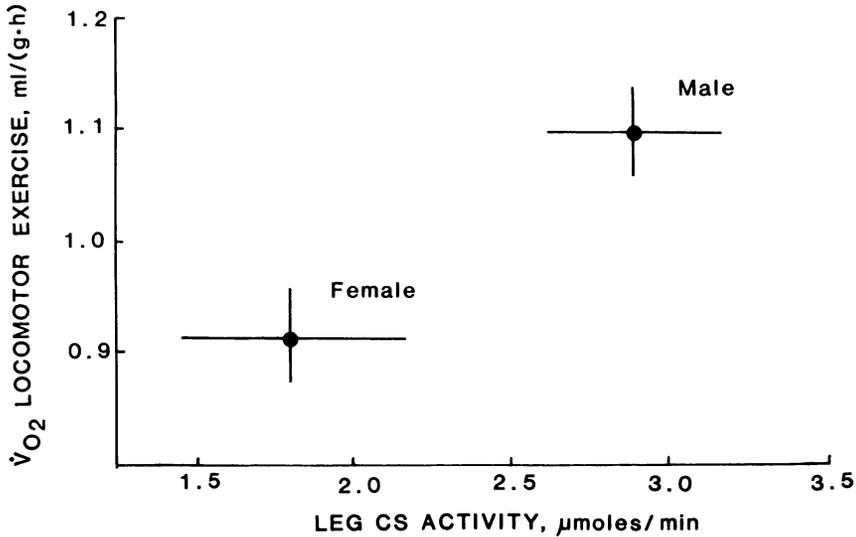


FIG. 2.—Relationship between  $\dot{V}\text{O}_2$  ex and total leg CS activity in male and female spring peepers. Bars represent 95% confidence intervals of the means.

activities, is that high metabolic rates in calling frogs result from the sustained activity of muscles with unusual enzymatic features. The citrate synthase activity we measured in male trunk muscle is by far the highest yet recorded for an ectothermic vertebrate and is comparable to highly oxidative muscles of birds and mammals, including avian flight muscle and mammalian cardiac muscle (table 4). The HOAD activity in male trunk muscle is also very high, suggesting that fatty acid oxidation plays an important role in mediating the

metabolic demands of vocalization in this species. This may reflect the fact that spring peepers call at a time of year when few prey items are available and must rely heavily on lipid stores to sustain their calling activity. Anurans with access to insect prey during the breeding season may rely less on stored lipids and possess a different complement of substrate-oxidizing enzymes in the trunk muscles. In contrast to CS and HOAD activities, PFK activity in trunk muscle is low relative to other vertebrate skeletal muscles (Crabtree and Newsholme

TABLE 4

CITRATE SYNTHASE ACTIVITY IN VERTEBRATE MUSCLES

Animal	Muscle	CS Activity $\mu\text{mol}/$ $(\text{min}\cdot\text{g}$ fresh muscle)	Reference
Ranid frog	Sartorius	10	Alp et al. 1976
Spring peeper (male)	Mixed leg	14	Present study
Ranid frog	Heart	40	Alp et al. 1976
Rainbow trout	Red trunk	50	Alp et al. 1976
Spring peeper (male)	Trunk	86	Present study
Laboratory rat	Heart	96	Alp et al. 1976
Pigeon	Pectoral	115	Alp et al. 1976
Gray catbird	Pectoral	200	Marsh 1981

NOTE.—CS activities of male spring peeper leg and trunk muscles were measured at 20 C. All other values were measured at 25 C. Except for temperature, the CS assay procedures used in the studies referenced in this table were identical.

1972). Low PFK activities in trunk muscle are consistent with observations of little or no lactic acid production in calling frogs and support the hypothesis that the metabolic demands of calling are met entirely by aerobic ATP production (Whitney and Krebs 1975; Ryan, Bartholomew, and Rand 1983; Taigen and Wells 1985).

The functional significance of high activities of enzymes associated with oxidative pathways in the trunk muscles of spring peepers may lie in the sustained use to which the muscles are put in highly variable thermal environments. Spring peepers maintain very active calling schedules for 6–8 wk in the early spring when climatic conditions are frequently harsh. An individual male frog may produce hundreds of thousands of calls during this period, entailing an equal number of full contractions of the trunk muscles, while encountering body temperatures ranging from 5 to 25 C (Rosen and Lemon 1974; Lemon and Struger 1980). Sustained muscle activity and high metabolic rates at body temperatures as low as 5 C presumably require very high concentrations of oxidative enzymes in the functioning muscles. Frogs that call under less extreme thermal conditions, or for shorter periods of time, may have lower trunk CS activities. This interpretation is consistent with studies of mammalian exercise physiology in which endurance and stamina are coupled to skeletal muscle mitochondrial function (Davies et al. 1982, 1984).

It seems unlikely that enzymatic features of muscles involved in sound production limit the metabolic performance of calling frogs under the conditions of our study. In comparison with leg muscle, male trunk muscle is six times higher in CS activity, five times higher in HOAD activity, and nearly identical in total mass. Yet, the metabolic rate during sustained calling, presumably derived largely from the activity of trunk and laryngeal muscles, is only 38% higher than the metabolic rate of these animals during locomotor exercise, when

metabolic performance is presumably derived from the activity of the limb muscles. Thus, the remarkable biochemical differences between leg and trunk muscle are not fully reflected in our measurements of metabolism, and it would appear that calling frogs could achieve even higher metabolic rates than those that occur in a sustained chorus. Circumstantial evidence in support of this interpretation is found in the field behavior of *H. versicolor*. Male frogs increase substantially their calling activity, and presumably their metabolic rate, when they detect females nearby (Fellers 1979). These observations are in agreement with the prediction of Taigen and Beuchat (1984) that sustained activities in anuran amphibians entail rates of oxygen consumption considerably less than the maximum rate possible.

Metabolic rates during locomotion in spring peepers appear not to be limited by central cardiovascular features, since these animals can achieve much higher metabolic rates when calling. Limitations on metabolic performance of spring peepers may reside within the working muscles, perhaps set by factors regulating oxygen transport and availability in the muscle tissue, such as capillary density, or by the muscle enzyme profiles.

In general, vertebrate muscles with very high CS activities are mechanically active for sustained periods (Alp, Newsholme, and Zammit 1976). In the case of spring peeper trunk muscle, the contractile activity is directly related to reproduction, and the elaborate oxidative enzyme system of these animals appears to have arisen as a consequence of sexual selection. Female spring peepers are preferentially attracted to male frogs with high calling rates (Forester and Czarnowsky 1985), thereby conferring a reproductive advantage on males with trunk muscles that can sustain the highest calling efforts. It is presumably this selective process that has given rise to a biochemical sexual dimorphism as dramatic as any in morphology or behavior.

#### LITERATURE CITED

- ALP, P. R., E. A. NEWSHOLME, and V. A. ZAMMIT. 1976. Activities of citrate synthase and NAD<sup>+</sup>-linked and NADP<sup>+</sup>-linked isocitrate dehydrogenase in muscle from vertebrates and invertebrates. *Biochem. J.* 154:689–700.
- BENNETT, A. F. 1974. Enzymatic correlates of activity metabolism in anuran amphibians. *Am. J. Physiol.* 226:1149–1151.
- . 1978. Activity metabolism of the lower vertebrates. *Annu. Rev. Physiol.* 40:447–469.

- BENNETT, A. F., and T. T. GLEESON. 1979. Metabolic expenditure and the cost of foraging in the lizard *Cnemidophorus murinus*. *Copeia* **1979**:573-577.
- BENNETT, A. F., and L. D. HOUCK. 1983. The energetic cost of courtship and aggression in a plethodontid salamander. *Ecology* **64**:979-983.
- BENNETT, A. F., and P. LICHT. 1973. Relative contributions of anaerobic and aerobic energy production during activity in amphibia. *J. Comp. Physiol.* **87**:351-360.
- BROWNEE, K. A. 1960. Statistical theory and methodology in science and engineering. Wiley, New York. 570 pp.
- BUCHER, T. L., M. J. RYAN, and G. A. BARTHOLOMEW. 1982. Oxygen consumption during resting, calling, and nest building in the frog *Physalaemus pustulosus*. *Physiol. Zool.* **55**:10-22.
- CAREY, C. 1979a. Effect of constant and fluctuating temperatures on resting and active oxygen consumption of toads, *Bufo boreas*. *Oecologia* **39**:201-212.
- . 1979b. Aerobic and anaerobic energy expenditure during rest and activity in montane *Bufo b. boreas* and *Rana pipiens*. *Oecologia* **39**:213-228.
- CRABTREE, B., and E. A. NEWSHOLME. 1972. The activities of phosphorylase, hexokinase, phosphofructokinase, lactate dehydrogenase, and the glycerol-3-phosphate dehydrogenases in muscles from vertebrates and invertebrates. *Biochem. J.* **126**:49-58.
- DAVIES, K. J. A., C. M. DONOVAN, C. J. REFINO, G. A. BROOKS, L. PACKER, and P. R. DALLMAN. 1984. Distinguishing effects of anemia and muscle iron deficiency on exercise bioenergetics in the rat. *Am. J. Physiol.* **246**:E535-E543.
- DAVIES, K. J. A., J. J. MAGUIRE, G. A. BROOKS, P. R. DALLMAN, and L. PACKER. 1982. Muscle mitochondrial bioenergetics, oxygen supply, and work capacity during dietary iron deficiency and repletion. *Am. J. Physiol.* **242**:E418-E427.
- FELLERS, G. M. 1979. Aggression, territoriality, and mating behavior in North American treefrogs. *Anim. Behav.* **27**:107-119.
- FORESTER, D. C., and R. CZARNOWSKY. 1985. Sexual selection in the spring peeper, *Hyla crucifer* (amphibia, anura): role of the advertisement call. *Behaviour* **92**:112-128.
- GANS, C. 1973. Sound production in the Salientia: mechanism and evolution of the emitter. *Am. Zool.* **13**:1179-1194.
- HILLMAN, S. S. 1976. Cardiovascular correlates of maximal oxygen consumption rates in anuran amphibians. *J. Comp. Physiol.* **109**:199-207.
- . 1980. The effect of anemia on metabolic performance in the frog, *Rana pipiens*. *J. Exp. Zool.* **211**:107-111.
- HILLMAN, S. S., V. H. SHOEMAKER, R. PUTNAM, and P. C. WITHERS. 1979. Reassessment of aerobic metabolism in amphibians during activity. *J. Comp. Physiol.* **129**:309-313.
- HILLMAN, S. S., and P. C. WITHERS. 1979. An analysis of respiratory surface area as a limit to activity metabolism in anurans. *Can. J. Zool.* **57**:2100-2105.
- HOGG, R. V., and A. T. CRAIG. 1978. Introduction to mathematical statistics. Macmillan, New York. 438 pp.
- LEMON, R. E., and J. STRUGER. 1980. Acoustic entrainment to randomly generated calls by the frog, *Hyla crucifer*. *J. Acoustic Soc. Am.* **67**:2090-2095.
- LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, and R. J. RANDALL. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265-275.
- MARSH, R. L. 1981. Catabolic enzyme activities in relation to premigratory fattening and muscle hypertrophy in the gray catbird (*Dumetella carolinensis*). *J. Comp. Physiol.* **141**:417-423.
- MARTIN, W. F., and C. GANS. 1972. Muscular control of the vocal tract during release signaling in the toad *Bufo valliceps*. *J. Morphol.* **137**:1-28.
- MILLER, K., and V. H. HUTCHISON. 1980. Aerobic and anaerobic scope for activity in the giant toad, *Bufo marinus*. *Physiol. Zool.* **53**:170-175.
- PUTNAM, R. W., and A. F. BENNETT. 1982. Histological, enzymatic, and contractile properties of skeletal muscles of five anuran amphibians. *Am. J. Physiol.* **244**:R558-R567.
- ROSEN, M., and R. E. LEMON. 1974. The vocal behavior of spring peepers, *Hyla crucifer*. *Copeia* **1974**:940-950.
- RYAN, M. J., G. A. BARTHOLOMEW, and A. S. RAND. 1983. Energetics of reproduction in a neotropical frog, *Physalaemus pustulosus*. *Ecology* **64**:1456-1462.
- SEYMOUR, R. S. 1973. Physiological correlates of forced activity and burrowing in the spadefoot toad, *Scaphiopus hammondi*. *Copeia* **1973**:103-115.
- TAIGEN, T. L. 1983. Activity metabolism of anuran amphibians: implications for the origin of endothermy. *Am. Nat.* **121**:94-109.
- TAIGEN, T. L., and C. A. BEUCHAT. 1984. Anaerobic threshold of anuran amphibians. *Physiol. Zool.* **57**:641-647.
- TAIGEN, T. L., S. B. EMERSON, and F. H. POUGH. 1982. Ecological correlates of anuran exercise physiology. *Oecologia* **52**:49-56.
- TAIGEN, T. L., and F. H. POUGH. 1981. Activity metabolism of the toad (*Bufo americanus*): ecological consequences of ontogenetic change. *J. Comp. Physiol.* **144**:247-252.
- . 1983. Prey preference, foraging behavior, and metabolic characteristics of frogs. *Am. Nat.* **122**:509-520.
- TAIGEN, T. L., and K. D. WELLS. 1985. Energetics of vocalization by an anuran amphibian (*Hyla versicolor*). *J. Comp. Physiol.* **155**:163-170.
- WHITNEY, C. L., and J. R. KREBS. 1975. Mate selection in Pacific tree frogs. *Nature* **225**:325-326.
- WILCZYNSKI, W., H. H. ZAKON, and E. A. BRENOWITZ. 1984. Acoustic communication in spring peepers: call characteristics and neurophysiological aspects. *J. Comp. Physiol.* **155**:577-584.
- WITHERS, P. C., and S. S. HILLMAN. 1981. Oxygen consumption of *Amphiuma means* during forced activity and recovery. *Comp. Biochem. Physiol.* **69A**:141-144.
- . 1983. The effects of hypoxia on pulmonary function and maximal rates of oxygen consumption in two anuran amphibians. *J. Comp. Physiol.* **152**:125-129.