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Aerobic and Anaerobic Energy Expenditure During Rest and Activity in Montane *Bufo b. boreas* and *Rana pipiens*

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Summary. The relations of standard and active aerobic and anaerobic metabolism and heart rate to body temperature (T_b) were measured in montane groups of *Bufo b. boreas* and *Rana pipiens* maintained under field conditions. These amphibians experience daily variation of T_b over 30° C and 23° C, respectively (Carey, 1978). Standard and active aerobic and anaerobic metabolism, heart rate, aerobic and anaerobic scope are markedly temperature-dependent with no broad plateaus of thermal independence. Heart rate increments provide little augmentation of oxygen transport during activity; increased extraction of oxygen from the blood probably contributes importantly to oxygen supply during activity. Development of extensive aerobic capacities in *Bufo* may be related to aggressive behavior of males during breeding. Standard metabolic rates of both species are more thermally dependent than comparable values for lowland relatives. Thermal sensitivity of physiological functions may have distinct advantages over thermally compensated rates in the short growing season and daily thermal fluctuations of the montane environment.

Introduction

Many ectothermic animals encounter some, if not substantial, daily and seasonal variation in body temperature (T_b) due to behavioral choice (Brett, 1971) or fluctuations in the thermal environment. Since extensive research over the past few decades has established that most physiological rates are partially or totally temperature-dependent (Precht, 1973), basic questions remain unanswered concerning the regulation of physiological processes during change in T_b and the role of fluctuating T_b in the energetics of ectothermic species. On the assumption that fluctuations in T_b could pose substantial problems for the maintenance

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of homeostasis (Hochachka and Somero, 1973), much attention has been directed towards understanding what mechanisms ectothermic animals might possess for thermal compensation of physiological and biochemical rates (see reviews by Bullock, 1955; Prosser, 1958; Fry, 1958; Fry and Hochachaka, 1970; Hazel and Prosser, 1974). The temperature insensitive rates of oxygen consumption in certain intertidal invertebrates (Newell, 1966; Newell and Northcroft, 1967; Newell and Pye, 1970, 1971) have often been cited to suggest that ectotherms encountering broad and rapid variations in T_b may have the most developed capacities for thermal compensation. However, thermal compensation appears to be only one mechanism for dealing with variation in T_b, since other intertidal invertebrates and terrestrial ectotherms lack such broad plateaus of temperature insensivity in physiological rates (Tribe and Bowler, 1968; Mangum and Sassaman, 1969; Coyer and Mangum, 1973; Bennett and Dawson, 1976). Despite the potential benefits of constant T_b and temperature compensation, the possibilities still exist that fluctuating T_b and temperature dependent rates may be advantageous in certain habitats.

The purpose of this study was to determine the relation between T_b and resting and active aerobic and anaerobic metabolism, heart rate, and the contribution of heart rate in support of activity in two terrestrial ectotherms. These physiological rates were chosen since they are important processes which vary with T_b and degree of activity (Moberly, 1968; Bennett, 1972; Brett and Glass, 1973; Seymour, 1973; Gatten, 1974a). Montane populations of *Bufo b. boreas* and *Rana pipiens* were chosen for the study because T_b of these *Bufo* can fluctuate over a 30° C span in a 24-h period (Carey, 1978) and T_b of *Rana* vary up to 23° C daily (Carey, unpubl. data). These variations in T_b are among the widest and most rapid recorded for ectothermic species. The intent of the study was to determine the physiological responses of these groups to natural variation of T_b in the field. Therefore, they were maintained under natural conditions of temperature and photoperiod. The physiological responses of *Bufo* to acclimation to constant and fluctuating T_b in the laboratory will be presented in a subsequent paper (Carey, 1979a).

Materials and Methods

Capture and Maintenance of Animals

Fifty adult *Bufo b. boreas* (mean mass = 40.2, SE = 5.6, range = 27.1–58.4 g) were captured at altitudes between 3,000 and 3,355 m in the East River valley near the Rocky Mountain Biological Laboratory (RMBL), Gothic, Gunnison County, Colorado. Thirty-seven *Rana pipiens* (mean mass = 34.8, SE = 3.2, range = 22.3–47.3 g) were collected at Allen's Pond (2,654 m) near Curecanti Creek, Gunnison County, Colorado. Toads and frogs were maintained at RMBL in $1 \times 0.3 \times 0.15$ m plastic containers with screen tops. *Tenebrio* larvae and grasshoppers were provided several times weekly. The cages were placed on the ground near a building, where the animals had access to shade at all times and to sunlight on clear days. Body temperatures of captive toads, measured orally with a thermistor used in conjunction with a YSI telethermometer, fell within the same daily ranges documented for toads in the field (Carey, 1978).

The effects of body temperature on standard and active rates of aerobic and anaerobic metabolism and heart beat were tested at RMBL at an altitude of 2,900 m in June and July, 1973 and

1974. Each animal was used only once and was in captivity no more than a week before testing. Forty-two of the *Bufo* were males. The number of females was insufficient to determine whether any variance in the data is attributable to sex. The sexes of the *Rana* were undetermined.

Standard Metabolism and Heart Rate

Oxygen consumption was measured in a closed-circuit system similar to that described by Gatten (1974a). Preliminary experiments indicated that rates of oxygen consumption declined continuously for 8 to 10 h after an animal was placed in the chamber and that following a 10-h equilibration period, values of oxygen consumption were lower between 0300 and 0600 than at any other time of day or night. Therefore, the toads and frogs were placed in the metabolism chamber between 1700 and 1800 h so that the values of oxygen consumption recorded during 0300 and 0600 h the next morning represented true standard metabolic rates (SMR).

Toads and frogs were fasted 3 days before use. Each animal was weighed to the nearest 0.2 g on an Ohaus balance, accurate to 0.01 g. It was then placed in a metabolism chamber consisting of a Plexiglas cylinder 15 cm long, 16 cm in diameter, and 45 cm in circumference. A saturated sponge provided water in the chamber for the animal. Mass loss was limited to 2–4 percent of the initial value during the 16–18 h test period. The chamber was placed in a cabinet in which air temperature could be controlled within 0.5° C between 5 and 35° C.

A peristaltic pump circulated air via tygon tubing through the closed system at a rate of approximately 120–160 cm^3/min. The air leaving the metabolism chamber was channelled sequentially through a drying column filled with Drierite (anhydrous $CaSo_4$), a flowmeter, a Beckman F3 paramagnetic oxygen analyzer, and then back to the chamber. Room air was sampled from air supplied by a Little Giant pump (Gelman Industries) 5 min every hour. The oxygen content of the air in the system never dropped below 20% during the tests. The fractional oxygen concentration of the air was recorded by a Honeywell potentiometric recorder every 15 s between 2200 and 0900 h. The oxygen consumption of the animal was calculated for each hour period between 0300 and 0600 h and the two lowest hourly values were averaged as the estimate of SMR. The results were discarded if any activity, evidenced by an abrupt decline in oxygen concentration of the system, occurred between 0300 and 0600 h.

Heart rate was measured at various body temperatures during tests of SMR in *Bufo b. boreas*. Three small gold-plated safety pins were inserted through the skin and musculature of both forelimbs and a hindlimb of each toad before it was placed in the metabolism chamber. These pins were connected by alligator clamps to flexible leads that left the metabolism chamber through an air-tight port. Electrocardiograms, from which heart rates were determined, were recorded automatically for 1 min intervals each hour between 2200 and 0900 h by a Model 126 Sanborn recorder used in conjunction with a Grass P5C preamplifier. The rates coinciding with the periods of minimal oxygen consumption were designated the standard heart rates (SHR).

The animal was removed from the chamber at 0900 h and its oral body temperature was taken immediately with a small animal thermistor used in conjuction with a YSI telethermometer.

Metabolism and Heart Rate During Activity

The animal was returned to the metabolism chamber following measurement of standard metabolism and body temperature. The EKG leads were disconnected from the safety-pin electrodes. Air was circulated very slowly for approximately an hour while the animal came to thermal equilibrium with its surroundings. The air flow was then increased for 5 min to flush the chamber thoroughly. A sample of the excurrent air was collected in a balloon which was attached immediately to a drying train filled with Drierite. The air passed from the balloon through the drying train into the Beckman F3 oxygen analyzer at a rate of 120–160 cm^3/min. Meanwhile, the incurrent and excurrent air lines were disconnected, and the ports were sealed. The air-tight metabolism chamber was then rotated by a small motor at approximately 5–7 rev/min. The speed of rotation was increased until the animal could remain upright only by using exceedingly rapid and vigorous running and hopping movements. Once the speed of rotation was adjusted, the door of the controlled temperature cabinet was closed. Two horizontal bars on the inside of the chamber prevented

the animal from sliding as the chamber rotated. Some animals fell onto their backs and struggled to right themselves. *Rana pipiens* fatigued rapidly and handling of the frogs after the 5 min exercise period failed to induce struggling. *Bufo* continued moving in the chamber for more than 1 h without visible signs of fatigue. They showed vigorous escape responses after removal from the chamber. No significant differences existed between the rates of oxygen consumption (per unit time) of individual toads when tested over 10, 20, or 30 min. Therefore, oxygen consumption of active toads was routinely measured after 10 min of exercise.

At the termination of the activity period, the rotation of the chamber was stopped, the excurrent air port was unclamped, and a second balloon was attached. An air line was connected via a tube to the third balloon located in the chamber. As the balloon in the chamber inflated, the volume of the chamber rapidly decreased. This forced the air out the excurrent air port into the second balloon. It was assumed that the air in the chamber was well mixed because the animal was constantly moving around during rotation. The balloon attached to the excurrent air port was then disconnected and the air it contained directed through the drying train into the oxygen analyzer. Meanwhile, the animal quickly was removed from the chamber. A thermistor was inserted into the esophagus to measure body temperature. The animal was then weighed to the nearest 0.1 g. The active metabolic rate was calculated from the decrement of oxygen concentration in the chamber over the 5- or 10-min period of activity. All gas concentrations were corrected to STPD.

Electrocardiograms of *Bufo b. boreas* subjected to 10 min of intense activity were recorded for 30 s during measurement of body temperature, just after removal of the toads from the metabolism chamber. The rapid attachment of leads to the safety pin electrodes and the short span of the measurement prevented the body temperature from changing appreciably from the level existing within the chamber. It was assumed that immediate post-activity heart rates closely approximated the heart rate during activity.

Standard and Active Anaerobic Metabolism

The contribution of anaerobic energy expenditure to activity at body temperatures from 10 to 30° C was estimated in 31 *Bufo b. boreas* and 29 *Rana pipiens*. Each animal was weighed to the nearest 0.1 g on an Ohaus balance and allowed to rest overnight at the desired test temperature in the metabolism chamber. Sixteen toads and 15 frogs measured after 16 h in the resting state were removed from the chamber quickly at about 0900 h. The animals were sacrificed by concussion and immediately homogenized by a Waring blender in a volume of chilled 0.6 N perchloric acid equalling 4 times their body mass. Fifteen toads and 14 frogs were subjected to 10 min or 5 min activity, respectively, in the rotating chamber after resting overnight at the appropriate temperature. After removal from the chamber, the body temperature of these animals was recorded rapidly prior to sacrifice and homogenization in cold perchloric acid. The maximum interval between removal and homogenization was approximately 20 s.

A portion of the total body homogenate was centrifuged for 30 min in a clinical centrifuge and frozen for later analysis. The supernatant was treated with reagents from a Boehringer-Mannheim lactate test kit and the lactate concentration assayed at 366 nm on a Cary 17 spectrophotometer.

Statistics

In cases where a function was clearly linear when plotted against body temperature on an arithmetic grid (lactate concentration) or on a semi-logarithmic grid (oxygen consumption and heart rate), the regression equations best describing the relation of the function to body temperature were computed by the method of least squares. Computation of the equations for oxygen consumption and heart rate used logarithmically transformed data. In cases where a function was obviously non-linear, least square polynomial regressions of increasing order were computed until no significant improvement in fit was obtained. A one-way analysis of covariance was used to test for equality of slopes and intercepts of linear regressions. Polynomial regressions were not compared statistically owing to an absence of suitable tests.

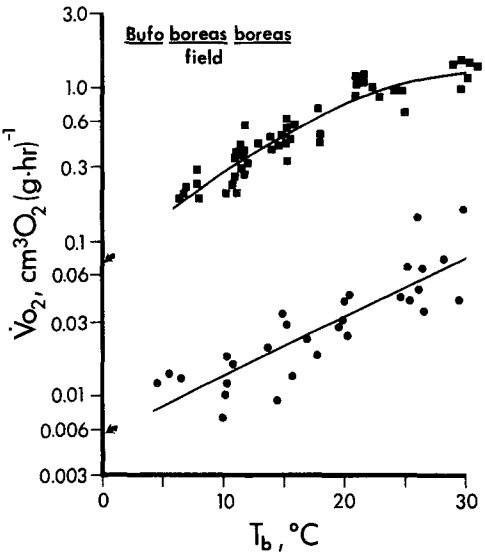

Fig. 1. The relation of oxygen consumption ($\dot{V}_{O_2}$) of *Bufo b. boreas* to body temperature (T_b) under standard conditions (*circles*) and over 10 min of vigorous activity (squares). Regression lines are constructed on the basis of Eq. 1 and 2. Data are plotted on a semilogarithmic grid. Resting values represent the mean of the two lowest hourly records. Active rates represent single measurements. $N=50$. *Arrows* signify the intercept of the regression line with the ordinate

Results

Standard and Active Aerobic Metabolism

Oxygen consumption ($\dot{V}_{O_2}$) of resting *Bufo b. boreas* that had been maintained under field conditions increases directly with body temperature (T_b), with little evidence of pronounced plateaus of thermal independence (Fig. 1). The equation best describing the relation of $\dot{V}_{O_2}$ (as standard metabolic rate) to T_b is:

$$\log \text{SMR} = -2.23 + 0.037\ T_b \qquad (1)$$
$$(n=30,\ r=0.84,\ S_{yx}=0.183,\ S_b=0.004),$$

where oxygen consumption is expressed as $cm^3\ O_2 (g \cdot h)^{-1}$ and T_b is in degrees Celsius (C). The Q_{10} for this relationship is 2.37 between 0 and 30° C. The polynomial equation best describing the relation of active metabolic rate to T_b is:

$$\log \text{AMR} = -1.13 + 0.067\ T_b - 0.0008\ (T_b)^2 \qquad (2)$$
$$(n=49,\ r=0.92,\ S_{yx}=0.094).$$

No fatigue was observed after 10 min of activity. The Q_{10} for this relation decreases continuously from 3.72 in the interval 5–10° C to 1.42 between 25 and 30° C.

Oxygen consumption of resting *Rana pipiens* maintained under field conditions increases with T_b (Fig. 2) according to the relation:

$$\log \text{SMR} = -2.34 + 0.049\ T_b \qquad (3)$$
$$(n=22,\ r=0.94,\ S_{yx}=0.140,\ S_b=0.004).$$

The overall Q_{10} for this relationship between 0 and 30° C is 3.06. Ranges of pronounced thermal independence are not evident in resting rates (Fig. 2).

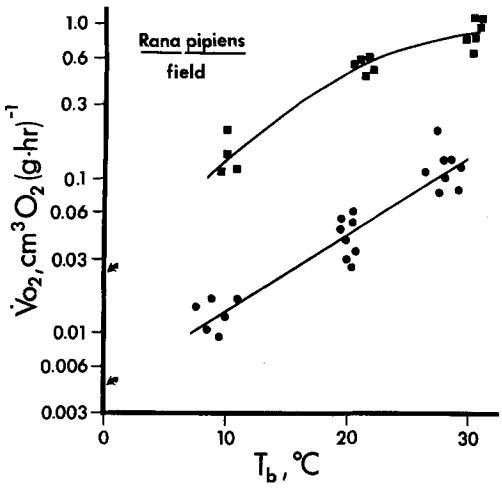

Fig. 2. The relation of oxygen consumption ($\dot{V}_{O_2}$) of *Rana pipiens* to body temperature (T_b) under standard conditions (*circles*) and during 5 min of vigorous activity (*squares*). Regression lines are constructed on the basis of Eq. 3 and 4. Data are plotted on a semilogarithmic grid. Resting values represent the mean of the two lowest hourly measurements. Active rates represent single measurements. $N=37$. *Arrows* signify the intercept of regression line with the ordinate

The polynomial regression best describing the relation of oxygen consumption to T_b for active *Rana* is:

$$\log \text{AMR} = -1.65 + 0.93\ T_b - 0.0013\ (T_b)^2 \quad (4)$$

$(n=15,\ r=0.96,\ S_{yx}=0.96)$.

Frogs were visibly exhausted after 5 min of activity and did not respond to mechanical stimulation. The Q_{10} for this relationship decreases continuously from 4.85 for the interval 5 to 10° C to 1.37 between 25 and 30° C. Analysis of covariance of the equations relating oxygen consumption T_b for resting *Bufo* and *Rana* (Eq. 1 and 3) indicates that the slopes are statistically indistinguishable ($P=0.07$), but that the intercepts differ significantly ($P < 0.01$).

The aerobic scope for activity, i.e., the difference between active and standard rates of oxygen consumption at a given temperature, are listed in Table 1 for *Bufo* and *Rana*. These values are calculated from Eq. 1–4. The aerobic scope of *Bufo* is higher than that of *Rana* at all temperatures. Another parameter allowing the comparison of active and standard metabolic rates is the ratio of AMR to SMR, an indication of relative metabolic expansibility during activ-

Table 1. Aerobic and anaerobic scope of *Bufo boreas boreas* and *Rana pipiens* maintained under field conditions. Values are calculated from equations 1–4 (aerobic) and 5–8 (anaerobic)

T_b	*Bufo*		*Rana*	
°C	Aerobic cm^{3}O$_2$ (g·h)$^{-1}$	Anaerobic mg lactate (g·10 min)$^{-1}$	Aerobic cm^{3}O$_2$ (g·h)$^{-1}$	Anaerobic mg lactate (g·5 min)$^{-1}$
10	0.27	0.50	0.12	0.79
20	0.74	0.59	0.41	1.17
30	1.12	0.70	0.69	1.33

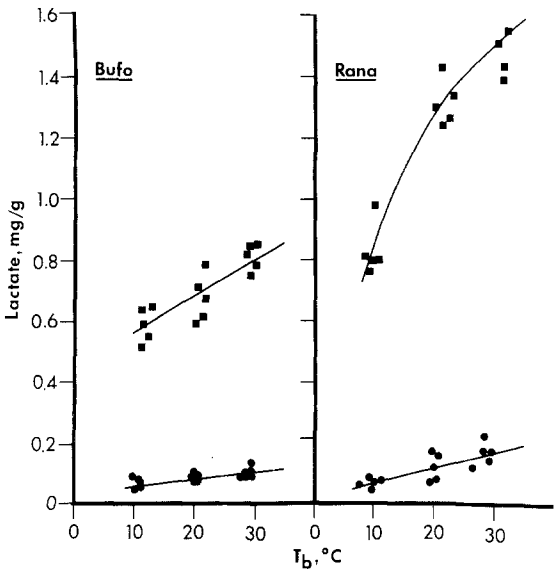

Fig. 3. The relation of lactate concentration to body temperature (T_b) in *Bufo b. boreas* and *Rana pipiens*. Values for resting and active animals are represented by circles and squares, respectively. *Bufo* and *Rana* were subjected to vigorous activity for 10 and 5 min, respectively. Regression lines were constructed on the basis of Eq. 5–8. Each point represents a single measurement. $N=31$ for *Bufo* and 29 for *Rana*

ity. Calculations of ratios over 5° C intervals from 5 to 30° C from Eq. 1–4 reveal that the ratio extends from 16.2 (5° C) to 24.3 (20° C) in *Bufo*. The corresponding range for *Rana* is 6.3 (30° C) to 10.8 (25° C).

Standard and Active Anaerobic Metabolism

Total body concentrations of lactate for resting and active *Bufo b. boreas* and *Rana pipiens* are presented in Fig. 3. Lactate concentrations of both resting and active *Bufo* and *Rana* rise with increasing T_b according to the relations best described by the regression equations listed in Table 2. Statistical comparison of the equations for active toads and frogs are not feasible, but it is clear that lactate concentrations increase much more dramatically with T_b in frogs than in toads. Analysis of covariance of the equations for lactate concentrations

Table 2. Regression equations for body lactate concentrations of resting and active *Bufo b. boreas* and *Rana pipiens* maintained under field conditions. Lactate concentrations are in mg/g body mass and body temperature (T_b) in degrees Celsius

Resting *Bufo*	$\text{Lactate}=0.039+0.0022\ T_b$ $n=16,\ r=0.82,\ S_{yx}=0.012,\ S_b=0.004$	(5)
Active *Bufo*	$\text{Lactate}=0.44+0.012\ T_b$ $n=15,\ r=0.82,\ S_{yx}=0.063,\ S_b=0.002$	(6)
Resting *Rana*	$\text{Lactate}=0.022+0.0051\ T_b$ $n=15,\ r=0.81,\ S_{yx}=0.031,\ S_b=0.001$	(7)
Active *Rana*	$\text{Lactate}=0.21+0.076\ T_b-0.0011\ (T_b)^2$ $n=14,\ r=0.95,\ S_{yx}=0.092$	(8)

in resting frogs and toads (Eq. 5 and 7) indicates that both the slopes and intercepts differ significantly ($P < 0.01$). Again, the effect of temperature is more marked in *Rana* than in *Bufo*.

The amounts of lactate produced per g body mass during 5 and 10 min of vigorous activity in *Rana* and *Bufo*, respectively, are presented in Table 1. These values, defined here as the difference between resting and active amounts, or anaerobic scope, were estimated on the basis of Eq. 5–8. At all temperatures the anaerobic scope of Rana during only 5 min of activity is higher than that of *Bufo* after 10 min of activity.

Standard and Active Heart Rates

Heart rates of resting and active *Bufo b. boreas* maintained under field conditions are strongly dependent on temperature with no pronounced plateaus of temperature independence (Fig. 4). The equation best describing the relation of standard heart rate (SHR) in beats/min to body temperature (T_b) in degrees Celsius (C) is:

$$\log \text{SHR} = 0.96 + 0.032\ T_b \quad (9)$$

$$(n = 23,\ r = 0.95,\ S_{yx} = 0.077,\ S_b = 0.002).$$

The Q_{10} for this relation between 0 and 30° C is 2.09. The equation best describing the relation of active heart rate (AHR) to body temperature (T_b) is

$$\log \text{AHR} = 1.198 + 0.037\ T_b - 0.0003\ (T_b)^2 \quad (10)$$

$$(n = 50,\ r = 0.95,\ S_{yx} = 0.059).$$

The Q_{10} for this relationship decreases continuously from 2.18 between 5 and 10° C to 1.57 between 25 and 30° C. The heart rate increment, i.e., the difference between active and standard rates, is 15 beats/min at 10° C, 25 beats/min at 20° C, and 21 beats/min at 30° C. These values are estimated from Eq. 9 and 10. Heart rates during activity increased approximately 1.8, 1.6, and 1.2 times resting values at 10, 20, and 30° C, respectively.

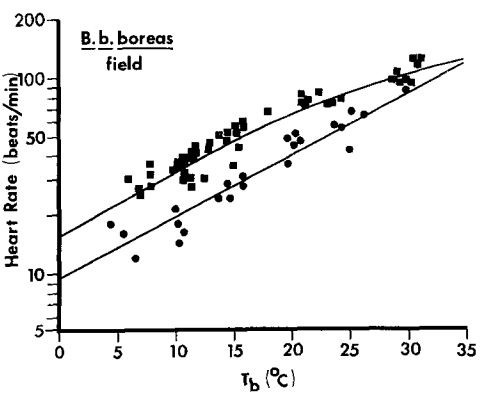

Fig. 4. The relations of standard (*circles*) and active (*squares*) heart rates of *Bufo b. boreas* to body temperature (T_b). Regression lines are constructed on the basis of Eq. 9 and 10. Animals were subjected to vigorous activity for 10 min. Data are plotted on a semi-logarithmic grid. $N = 50$

Discussion

Standard Metabolism

The SMR of montane *Bufo* and *Rana* are directly dependent on temperature and exhibit no pronounced plateaus of thermal independence over the range of T_b they experience daily in the field (Figs. 1 and 2). The Q_{10} values for SMR of montane toads and frogs generally exceed those reported for similar lowland forms (Table 3). Additionally, *Thamnophis elegans vagrans* living near the upper altitudinal limit of their distribution (2,900 m) exhibit a highly temperature-dependent SMR and a higher Q_{10} than those of lowland *Thamnophis* (Carey, unpubl. data). The higher Q_{10} in montane ectotherms may represent methodological differences among studies, since the animals in other studies may not have been fully resting. Levels of activity above resting often involve different relations of metabolism to temperature than those characterizing standard conditions (Brett and Glass, 1973; Newell and Bayne, 1973; Bayne et al., 1973; Dmi'el and Rappeport, 1976). Similarly, the data collected for this report were obtained in the dark between 0300 and 0600 h when SMR was minimal compared to other parts of the daily cycle. Other studies measured SMR during the day or at unreported times. The presence of light during measurement or the time during the diel cycle in which measurements are made may also affect the thermal sensitivity of SMR (Turney and Hutchison, 1974). The differences may also result from the observation that toads maintained under fluctuating thermal conditions exhibit a more thermally sensitive SMR than those acclimated to constant temperatures (Carey, 1979a). However, the differences

Table 3. Q_{10} values for resting oxygen consumption of *Bufo* and *Rana*. Values of other studies may not necessarily have been calculated from standard measurements

Species	Q_{10}	Temperature span	Reference
B. b. boreas	2.37	0–30° C	This study
B. boreas (lowland)	0.98	5–15° C	Hutchison et al., 1968
	1.87	15–25° C	
B. b. boreas (lowland)	1.11	14–24° C	Tashian and Ray, 1957
B. boreas halophilus	1.19	14–24° C	Tashian and Ray, 1957
B. cognatus	2.70	5–15° C	Hutchison et al., 1968
	1.56	15–25° C	
B. cognatus	1.3	10–30° C	Seymour, 1973
B. marinus	1.91	15–25° C	Hutchison et al., 1968
B. americanus	4.5	5–15° C	Hutchison et al., 1968
	1.1	15–25° C	
B. terrestris	2.63	5–15° C	Hutchison et al., 1968
	1.41	15–25° C	
R. pipiens	3.06	0–30° C	This study
R. pipiens	2.7	5–15° C	Hutchison et al., 1968
	2.41	15–25° C	
R. pipiens	1.3	10–30° C	Seymour, 1973
R. clamitans	2.29	14–24° C	Tashian and Ray, 1957
R. sylvatica	3.07	14–24° C	Tashian and Ray, 1957

in Q_{10} between montane and lowland groups could indeed be real, since SMR of montane *Bufo b. boreas* are more thermally sensitive than those of lowland *Bufo boreas halophilus* maintained and measured under identical conditions (Carey, 1979a).

The potential benefit of thermally sensitive rates may relate to the special problems of terrestrial ectotherms in the montane environment. The season for growth, reproduction, and storage of glycogen and lipid for winter use is short (3–4 months) at high elevations. Ambient temperatures (T_a) during the summer fluctuate up to 20° C daily and nighttime T_a fall close to or below freezing. On cloudy days, T_a may not reach 15° C (Carey, 1978). Toads are active at cold T_b at night and on cloudy days, but few prey are active in the cold. Although digestive efficiency is greater at cold T_b, more calories per day can be extracted from the food at warm T_b, provided that the gut is continuously full of food (Carey, unpubl. data). Therefore, the amount of time each summer in which the toads can attain warm T_b and materials from the food for maintenance, growth, and reproduction is quite limited. Any mechanism serving to minimize maintenance costs in the cold and to maximize synthetic processes at warm T_b at which enzyme activities might be more optimal could well be advantageous. A thermally sensitive SMR would lower maintenance costs at cold T_b and perhaps make more energy available to devote to synthetic processes at warm T_b. Elaborating this theory to an extreme interpretation, these amphibians could be using thermally sensitive rates and the thermal variety of their montane habitat to partition the participation of various metabolic pathways to various ranges of T_b.

A similar thermally sensitive SMR with a Q_{10} approaching 400 at T_b below 10° C has been observed in high latitude snakes (Aleksiuk, 1976). The major benefit of such high Q_{10} would be to reduce energy expenditure to an absolute minimum at cold T_b when the snakes are totally inactive (Aleksiuk, 1976). Therefore, thermally sensitive physiological rates appear to be advantageous in certain habitats when time at warm T_b is limited by short growing seasons and daily variation in T_a.

Aerobic Metabolism during Activity

Rates of oxygen consumption of active toads and frogs are also temperature dependent, although rates become less sensitive to temperature at higher T_b (Figs. 1 and 2). The reduced thermal sensitivity of AMR at high T_b may reflect limited abilities of the oxygen uptake and transport processes for sustaining maximal aerobic expenditures (Bennett, 1972).

Maximal rates of oxygen consumption of *Bufo* clearly exceed those of *Rana* at all T_b (Figs. 1 and 2, Seymour, 1973). A more convenient comparison of the aerobic capacities for exercise is the calculation of metabolic scope. This measure, formulated by Fry (1947), can be used as an index of energy available by aerobic means for activity, provided that the animal does not abandon some maintenance function during activity (Moberly, 1968) and provided that oxygen consumption does not increase above activity levels at the termination

of activity. An increase in oxygen consumption following activity has been noted in two amphibians, *Hyla* and *Batrachoseps* (Bennett and Licht, 1973). Since *Rana* show no such increase during recovery after similar conditions of stimulation (Seymour, 1973) and since *Bufo* exhibit little fatigue after prolonged activity, measurements of scope are valid indicators of aerobic capacity of these two species.

The aerobic scopes of *Bufo* and *Rana* increase continually between 5 and 30° C and the scope of the toads is always greater than that of the frogs (Table 1). The maximal aerobic scope of *Bufo* occurs at 30° C, a temperature 6° C higher than the T_b preferred by fed individuals of that species maintained under field conditions (Carey, 1978). The level of preferred T_b and aerobic scope coincide fairly closely in most reptiles (Bennett and Dawson, 1976). The significance of the disparity in amphibians will be obscure until measurements on more species are completed.

A general pattern of aerobic capacities for activity has emerged indicating that slow, sluggish animals relying on threat postures or a static defense against predators generally have low aerobic scopes and those using rapid escape movements have larger ones (see Bennett, 1978). However, this pattern is reversed in *Rana* and *Bufo*. Bennett and Licht (1974) have postulated that predation has selected for the development of high glycolytic, anaerobic capacities in *Rana* which flee predators rapidly and that *Bufo*, using poison and inflation to discourage predators, have aerobic capacities since intense selection for anaerobiosis has not occurred. However, large aerobic scope and capacity for prolonged exercise without fatigue in *Bufo* are correlated with specialized characteristics of respiratory and cardiovascular systems. Bufonid lungs possess a greater percentage of total respiratory capillaries (Czopek, 1955; Bieniak and Watka, 1962) and extensive septa provide greater surface area for gas exchange (Tenney and Tenney, 1970) than those of *Rana*. Toads have higher blood oxygen capacity due to higher hemoglobin and red blood cell contents (Hillman, 1976; Carey, 1979b). Toads may also have greater tidal volume during activity and more high-oxidative muscle fibers which have a 6–7 fold higher maintenance cost than typical low-oxidative fibers (Gordon, 1968). If these characteristics require more energy for development and maintenance, they most likely evolved in response to positive selection for aerobic capacities rather than lack of selection for extensive anaerobiosis.

The only time in the annual cycle in which vigourous, prolonged activity has been noted in *Bufo b. boreas* is during breeding. Male-male aggression involving mounting, pushing, and rapid swimming occurs frequently, even if no females are present. When males and females are in amplexus before egglaying begins, groups of males attempt to pull the male off the female. Such interactions involve rapid movements continuing for 30–45 min over a wide range of T_b. Clearly, a benefit would be attached to developing extensive aerobic capacities since a male fatiguing in the course of these interactions would be less likely to fertilize the eggs. *Rana*, on the other hand, show few prolonged aggressive movements during breeding (J. Collins, pers. comm.). These observations need to be tested to see if pronounced aerobic metabolism is invariably correlated with aggressive breeding habits in amphibians.

Heart Rate during Rest and Activity

Standard heart rate of *Bufo b. boreas* is thermally sensitive with a Q_{10} of 2.09. This value coincides closely with other Q_{10} obtained during rest in amphibians acclimated to laboratory conditions: 2.00 for *Bufo fowleri* (Stier and Bock, 1966), 2.06 for *Rana pipiens* (Miller and Mizell, 1972), 2.11 for *Rana temporaria* (Harri and Talo, 1975), and 1.89 for *Rana catesbeiana* (Weathers, 1976).

Activity in montane populations of *Bufo b. boreas* has been recorded at T_b ranging from 0.2 to 33.9° C (Carey, 1978). The data presented here for standard and active metabolic and heart rates permit assessment of the contribution of heart rate to active oxygen consumption over such a range of T_b. Elevation of heart rate appears to play a minor role in enhancement of oxygen delivery at any T_b. The heart rate increment (HRI), the difference between standard and active heart rate, ranges from 10.6 beats/min at 5° C to 28.7 beats/min at 25° C (Table 4). The HRI is maximal 2° C above the mean preferred T_b of these toads in the field (Carey, 1978) and does not coincide with the T_b at which maximal aerobic metabolic scope is observed (30° C). Heart rate increments of similar sized reptiles at the same T_b are much higher (Gatten, 1974a).

The percentage contribution of heart rate to increased oxygen transport during activity can be calculated according to the equation of Gatten (1974b):

$$\left[\left(\frac{\text{AHR}-\text{SHR}}{\text{SHR}}\right) \div \left(\frac{\text{AMR}-\text{SMR}}{\text{SMR}}\right)\right] \times 100. \tag{11}$$

The estimated contribution of heart rate to oxygen transport in *Bufo* decreases from 5% at 5° C to 1.7% at 30° C (Table 4). These calculations suggest that stroke volume and/or AV difference must contribute the major amount of oxygen during activity. The contribution of AV difference would be difficult to measure, since oxygenated cutaneous blood drains into the venous flow. However, the variation in contribution of SV and AV difference over a range in T_b can be estimated by calculating the oxygen pulse (oxygen consumption

Table 4. Standard and active oxygen pulse (SOP and AOP), heart rate increment (HRI), oxygen pulse increment (OPinc) and the percentage contribution of heart rate to activity in *Bufo b. boreas* maintained under field conditions. Values were calculated from Eq. 1, 2, 9 and 10. Percentage contribution of heart rate to oxygen consumption during exercise was estimated from Eq. 11. HRI is in beats/min and SOP, AOP, and OPinc are in $cm^3\ O_2 \times 10^{-5}\ (g \cdot beat)^{-1}$

	T_b (°C)					
	5	10	15	20	25	30
HRI	10.6	15.5	21.0	25.9	28.7	25.9
SOP	1.14	1.14	1.27	1.34	1.44	1.50
AOP	10.55	14.00	17.18	19.79	20.30	21.99
OPinc	9.41	12.86	15.91	18.45	18.86	24.40
% contribution	5.16	3.82	3.34	2.78	2.49	1.71

divided by heart rate at each T_b). As might be anticipated, oxygen pulse plays an important role in oxygen supply, particularly at high T_b (Table 4). Active values for oxygen pulse (AOP) exceed resting values by 10–20 times. The maximum oxygen pulse for toads, 24.4 cm^3 $O_2 \times 10^{-5}$ $(g \cdot beat)^{-1}$ exceeds all values available for reptiles (Gatten, 1974a). Further studies are necessary to determine if the pattern here is typical of other amphibians.

Anaerobic Metabolism during Activity

Anaerobic scope is the difference between mass-specific, total body resting lactate concentrations and lactate levels following 5 and 10 min of vigorous activity in *Rana* and *Bufo*, respectively. This measurement allows an estimation of the energy provided for activity by anaerobic glycolysis. The high aerobic scope and low anaerobic scope of *Bufo* and the opposite relation for *Rana* (Table 1) at all T_b are consistent with the marked inverse relation between these functions observed previously in amphibians (Bennett and Licht, 1973; Seymour, 1973). As with other functions, anaerobic scope is thermally dependent in both groups.

The contribution of aerobic and anaerobic pathways to the total energy expenditure during activity can be estimated by converting oxygen consumption and lactate generation to ATP production, according to the equations provided by Bennett and Licht (1972): 1.0 mg lactate = 0.0167 mM ATP and 1.0 cm^3 oxygen consumed = 0.290 mM ATP. Aerobic energy production accounts for 60, 78, and 82% of the total energy expenditure by *Bufo* during 10 min of activity at 10, 20, and 30° C, respectively. In contrast, aerobic metabolism accounts for 17, 32, and 43% of the total ATP production during 5 min of activity by *Rana* at similar temperatures (Fig. 5). Total ATP production, estimated in this manner, is a temperature dependent process in both amphibians over the range of 10 to 30° C.

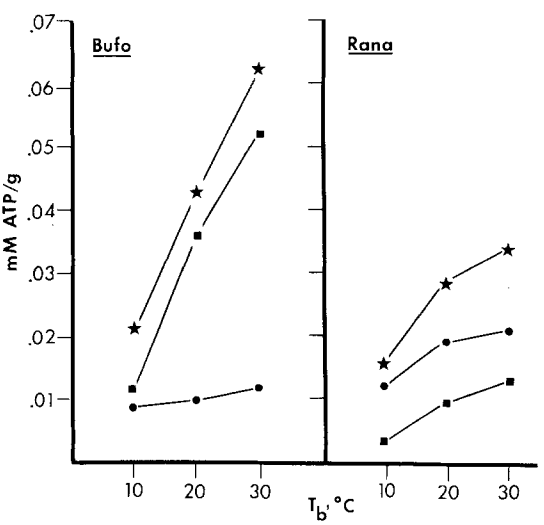

Fig. 5. Estimation of mass-specific production of ATP by *Bufo b. boreas* and *Rana pipiens* during 10 min and 5 min vigorous activity, respectively. Circles represent ATP production from anaerobic metabolism, calculated from lactate concentration. Aerobic ATP production is represented by squares. Stars signify the sum of ATP production by anaerobic and aerobic means. Estimates are based on the data presented in Figs. 1, 2, and 3

Estimation of ATP production during activity using oxygen consumption and lactate production is a commonly accepted practice (see Bennett, 1978) and is based on the following assumptions: 1) a P/O ratio of 3, 2) lactate is the only end product of anaerobic ATP production, 3) lactate is not metabolized during activity, and 4) creatine phosphate is not utilized or resynthesized during activity. The first three assumptions are discussed by Cerretelli et al. (1972) and Bennett (1978), but the latter assumption could lead to an underestimation of the total energy expenditure during an activity bout.

Frog muscle contains about 3 μM ATP/g tissue which could suffice for about 10 contractions (Carlson and Wilkie, 1974). Cellular ATP is in equilibrium with creatine phosphate (PCr) according to the relation: $ATP + Cr \rightleftharpoons ADP + PCr$. Creatine phosphate is present in cells in the approximate concentration of 20 μM/g, a sufficient amount to support 80 to 100 twitches of frog muscle (Carlson and Wilkie, 1974). Lactic acid production during contraction of frog muscle does not commence until the concentration of PCr has dropped to a minimal level (Karpatkin et al., 1964; Cerretelli et al., 1972). Since some initial muscle activity should be supported by PCr, the contribution of PCr to energy expenditure during activity in amphibians deserves further attention.

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