

IS WALKING COSTLY FOR ANURANS? THE ENERGETIC COST OF WALKING IN THE NORTHERN TOAD *BUFO BOREAS* *HALOPHILUS*

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Summary

Locomotor mode and the maximal capacity for aerobic metabolism are thought to be co-adapted in anuran amphibians. Species that rely heavily on walking often have high capacities for aerobic metabolism relative to species that rely primarily on saltation. We tested the hypothesis of co-adaptation of gait and aerobic metabolism by investigating the locomotor energetics of *Bufo boreas halophilus*, a toad that walks, but does not hop. Rates of oxygen consumption during locomotion were measured in an enclosed variable-speed treadmill. The steady-state rate of oxygen consumption ($\dot{V}_{O_{2ss}}$) increased linearly within a range of sustainable speeds [$\dot{V}_{O_{2ss}}$ (ml O₂ g⁻¹ h⁻¹) = 0.93 × speed (km h⁻¹) + 0.28]. The minimum cost of transport, C_{min} (the slope of this relationship), varied significantly among individual toads. When expressed in units of oxygen consumed per distance travelled (ml O₂ km⁻¹), C_{min} scaled isometrically with body mass: $C_{min} = 0.69\text{mass}^{1.07}$. Consequently, mass-specific C_{min} (ml O₂ g⁻¹ km⁻¹) was uncorrelated with body mass. Variation in C_{min} was also unrelated to experimental temperature. Mass-specific C_{min} estimates were similar to previous allometric predictions for terrestrial animals of similar size, which contrasts with previous findings for another toad species. Maximum rates of oxygen consumption measured in closed, rotating respirometers were significantly higher than the maximum rates achieved on the treadmill, but lower than those measured previously in other *Bufo* species. Our results indicate that walking is not necessarily a costly gait for toads and that high maximum rates of oxygen consumption are not associated with reliance on walking within the genus *Bufo*.

Introduction

The ‘aerobic capacity’ model (so termed by Taigen, 1983) is an influential hypothesis for the evolution of activity capacity based upon the proposition that aerobic metabolism

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evolved as a correlated response to natural selection on sustained activity. Natural selection favoring enhanced abilities to sustain high levels of activity is thought to bring about an increase in the maximal rate of aerobic metabolism to support that activity (Bennett and Ruben, 1979). Comparative studies of anuran amphibians provide compelling evidence of co-adaptation of behavior and metabolism that is consistent with the model. Differences among anuran taxa in maximal rates of oxygen consumption ($\dot{V}_{O_2\max}$) often show positive correlations with differences in locomotor stamina and activity levels (Taigen *et al.* 1982; Pough *et al.* 1992). Bufonids, for example, have an exceptionally high $\dot{V}_{O_2\max}$ in comparison with other anuran taxa (Walton, 1988a; Gatten *et al.* 1992), which is generally thought to reflect the active foraging behavior and hop-walk locomotor mode typical of this group (Taigen *et al.* 1982; Pough *et al.* 1992). Active foraging and walking are associated with high $\dot{V}_{O_2\max}$ in other anuran taxa as well (Taigen *et al.* 1982).

Several factors, however, cloud the interpretation of these comparative results. First, most previous measurements of $\dot{V}_{O_2\max}$ in anurans were obtained using techniques to elicit locomotor exercise (e.g. electric shocks or rotation of the metabolic chamber) that may activate muscles other than those used during normal locomotion, impair oxygen transport or, generally, induce abnormal locomotor behavior (reviewed by Gatten *et al.* 1992). Second, locomotor economy, or the quotient of the rate of aerobic metabolism and the speed of locomotion, is just as important as aerobic capacity ($\dot{V}_{O_2\max}$) for determining locomotor stamina (Taylor *et al.* 1970; Walton, 1993). Locomotor economy has been investigated previously in only six anuran species (Walton and Anderson, 1988; Anderson *et al.* 1991; Walton, 1993), whereas $\dot{V}_{O_2\max}$ has been measured in at least 42 species to date (see review by Gatten *et al.* 1992). Third, few data are available concerning the energetic cost of routine locomotor behaviors. Hence, previous laboratory measurements of aerobic metabolism may not reflect the true functional constraints on free-ranging anurans (Walton and Anderson, 1988; Pough *et al.* 1992).

Several recent studies of anurans, however, have employed treadmill respirometers to measure aerobic energy expenditure during sustained locomotion at speeds typical of free-ranging animals, in addition to speeds that elicit $\dot{V}_{O_2\max}$ (Walton and Anderson, 1988; Anderson *et al.* 1991; Walton, 1993). More importantly, this technique can be used to investigate the economy of, as well as the capacity for, sustained locomotion. In most terrestrial organisms, the rate of oxygen consumption increases linearly with speed up to a maximum rate of oxygen consumption ($\dot{V}_{O_2\max}$). The speed at which $\dot{V}_{O_2\max}$ is achieved is the upper limit for aerobically supported, sustained locomotion and is termed the maximal aerobic speed (MAS). Speeds above MAS are increasingly supported by anaerobic metabolism and are generally not sustainable. The slope of the oxygen consumption *versus* speed relationship, the minimum cost of transport (C_{\min}) (Taylor *et al.* 1970), has been used widely to compare locomotor economy among animals that differ in size, taxon, body form, number of legs and gait (e.g. Full, 1989). It is important to note, however, that $\dot{V}_{O_2\max}$ and C_{\min} act in concert to determine locomotor stamina (Taylor *et al.* 1970). For example, ambystomatid salamanders and hylid frogs of similar mass have a similar MAS, even though hylids have a higher $\dot{V}_{O_2\max}$ (Walton, 1993). Ambystomatids, however, have substantially lower C_{\min} than hylids (Full *et al.* 1988; Walton, 1993). Thus, increased locomotor capacity can be achieved by an increase in $\dot{V}_{O_2\max}$, a decrease in C_{\min} or both.

Studies of treadmill locomotion in the toad *Bufo woodhousii fowleri* reported an intriguing pattern of locomotor energetics that is consistent with the hypothesis that a high $\dot{V}_{O_2\max}$ is associated with a walking gait. During natural locomotion, *B. w. fowleri* mix walking and hopping strides, and the number of walking strides decreases with speed until walking accounts for less than 20 % of strides taken at speeds greater than the MAS (Walton and Anderson, 1988; Anderson *et al.* 1991). The C_{\min} of the natural hop-walk gait of *B. w. fowleri* is significantly higher than that predicted for other animals of similar body mass (Walton and Anderson, 1988). Furthermore, the hopping gait is sustained at speeds substantially greater than the MAS determined for walking alone (Anderson *et al.* 1991). During exclusive walking at slow speeds, oxygen consumption is independent of speed and is greater than that of natural, mixed-gait locomotion at similar speeds. Walking is expensive in *B. w. fowleri*, elevating oxygen consumption to 73 % of $\dot{V}_{O_2\max}$ at relatively slow speeds. In contrast, oxygen consumption during exclusive hopping increased with speed and did not exceed the cost of natural, mixed-gait locomotion until speeds exceeded MAS by 33 %. Thus, *B. w. fowleri* apparently switches from a costly walking gait to a more economical, hopping gait at higher speeds. Similar gait changes have previously been associated with energetic economy in kangaroos and wallabies (Dawson and Taylor, 1973; Baudinette *et al.* 1992), horses (Hoyt and Taylor, 1981), mink (Williams, 1983) and ground squirrels (Hoyt and Kenagy, 1988).

Not all toad species, however, use a hopping gait. Some species, such as *B. boreas* and *B. calamita*, walk exclusively during routine, sustained locomotion (Emerson, 1979, 1982) and run, rather than hop, even when moving at high speeds, for example when they are pursued by predators (or persistent herpetologists) (B. M. Walton and C. C. Peterson, personal observation). If walking is, in general, an expensive gait for toads and if locomotor mode and aerobic metabolism are tightly linked in anurans, then species that rely solely on pedestrian locomotion (i.e. walking and running) would be expected to have higher capacities for aerobic metabolism than species that rely solely on saltatory gaits and those species that mix saltatory and pedestrian gaits.

We tested the hypothesis of co-adaptation of aerobic capacity and walking gait through an investigation of locomotor energetics in the northern toad *B. boreas halophilus*. We examined the overall pattern of metabolic response to exercise to determine whether *B. b. halophilus* shows a linear increase in $\dot{V}_{O_2\max}$ with locomotor speed, as is typical for terrestrial animals, or whether $\dot{V}_{O_2\max}$ is independent of speed, as was observed previously in walking *B. w. fowleri*. We calculated C_{\min} to determine whether the locomotor costs of *B. b. halophilus* are indeed high in comparison with those of other animals of similar body mass (particularly other anuran species). We also measured $\dot{V}_{O_2\max}$ using both a treadmill and rotating closed respirometers to compare the $\dot{V}_{O_2\max}$ estimates obtained using these two methods and to determine whether *B. b. halophilus* has a high $\dot{V}_{O_2\max}$ compared with those of congeneric species.

Materials and methods

Study animals

Toads (*Bufo boreas halophilus* Baird & Girard) were captured during the summer of

1993 in Lassen County, California, primarily from the Lassen National Forest, and transported to a nearby field laboratory at the Eagle Lake Biological Field Station, Susanville, CA. The animals were held in glass terraria at a natural photoperiod and were fed frequently with crickets, earthworms and a variety of insects available locally. All animals used in the study were in good health.

Endurance

Endurance was measured in several toads to determine the appropriate range of sustainable speeds over which to calculate C_{\min} (if speeds that elicit fatigue and anaerobiosis are included in this range, C_{\min} will be underestimated). To minimize the potential effect of anaerobiosis, only speeds that were sustainable for at least 1 h were used in the calculation of C_{\min} . Endurance was measured by exercising toads on the motorized treadmill at a constant speed. Endurance trials were concluded following any indication of locomotor distress that persisted for more than 1 min (e.g. repeated stumbling, refractory behavior). Trials were terminated if an animal locomoted continuously for 1 h. Endurance trials were run at 0.2, 0.3 and 0.4 km h⁻¹. Additional information on endurance was obtained during the oxygen consumption trials in which several toads maintained pace with the treadmill for 1 h or more at speeds greater than 0.4 km h⁻¹.

Oxygen consumption during treadmill locomotion

Measurements of oxygen consumption before and during sustained locomotion were made using open-flow respirometry in a clear acrylic respirometer containing a variable-speed treadmill (as described by Walton and Anderson, 1988; Anderson *et al.* 1991). We measured the fractional oxygen concentration of incurrent and excurrent air-streams with an Ametek S-3A/II oxygen analyzer. Before analysis, the air-streams passed through columns of absorbents, Drierite and Ascarite, to remove water and CO₂, respectively. An air pump drew dry, CO₂-free air into the analyzer at flow rates of 200 or 300 ml min⁻¹, measured by an electronic flowmeter (Omega, model FMA-5606) placed downstream from the absorbent columns.

Toads were placed in the treadmill and left undisturbed in dim light for 30 min to 1 h before exercise. Pre-exercise rates of oxygen consumption were measured during the last 5–10 min of the rest period. The treadmill was then activated at a slow speed (≤ 0.18 km h⁻¹). After an animal had exhibited satisfactory even-paced locomotion, oxygen consumption was measured until at least 10 min of steady-state oxygen consumption had been recorded (some records were as long as 1 h at a single speed). The treadmill speed was then increased by approximately 0.05 km h⁻¹, until a speed was reached at which toads could no longer keep pace long enough to measure 10 min of steady-state oxygen consumption. The treadmill was timed with a stopwatch every few minutes to verify the speed. All trials consisted of satisfactory measurements at three or more speeds. Steady-state rates of oxygen consumption were calculated according to Withers (1977).

Experimental temperature was usually 23–26 °C, but in some cases temperature in the field laboratory increased to 30 °C. The temperature of the treadmill interior during

measurements of steady-state oxygen consumption was measured with an electronic thermocouple thermometer (Omega, model HH23) and its effects were assessed by regression analysis.

Maximum rates of oxygen consumption in closed respirometers

Measurements of the maximum rate of oxygen consumption were made in a closed system using methods described by Walton (1988*b*). Toads were placed in cylindrical, air-tight plastic respirometry chambers. An initial 30 ml air sample was extracted from the chamber with a gas-tight syringe, the chamber was then sealed, and toads were forced to exercise for 5 min by mechanically rotating the chambers at rotational speeds of 1.5 body lengths s^{-1} . This procedure caused the toads to scramble, flail and right themselves within the chamber. After 5 min of exercise, a second 30 ml air sample was extracted from the chamber. Oxygen content of the gas samples was determined by injecting them into the oxygen analyzer at a rate of 5 ml min^{-1} through columns packed with Drierite and Ascarite. Rates of oxygen consumption were calculated according to Vleck (1987). All reported rates of gas exchange have been adjusted to STPD.

Results

Locomotor behavior and endurance

The locomotor behavior of *B. b. halophilus* on the treadmill resembled natural locomotion, which is intermittent and consists of a few strides followed by brief stationary periods (Walton, 1988*b*; B. M. Walton, personal observation). None of the toads hopped at any speed during many hours of observation in either oxygen consumption or endurance trials. Toads walked a few strides forward, rode the treadmill to the back of the chamber and then walked forward again. During the period between strides, toads maintained a walking posture. Typically, strides were initiated when the trailing foot touched the back of the treadmill chamber. Many toads, however, quickly became accustomed to the treadmill and strode forward before reaching the back of the treadmill (particularly at slow speeds).

At speeds of 0.35 $km\ h^{-1}$ or below, toads moved steadily without apparent fatigue. Three individuals moving at 0.3 $km\ h^{-1}$ in the endurance tests matched the treadmill speed for 1 h. A few toads did not provide satisfactory steady-state oxygen consumption values at very low speeds ($<0.2\ km\ h^{-1}$) because of erratic escape behavior; data from those periods were excluded from subsequent analyses. These same animals, however, moved steadily forward at faster speeds. Many toads experienced difficulty keeping pace with the treadmill at speeds of 0.35–0.45 $km\ h^{-1}$. At these speeds, some toads occasionally lost their footing, stumbled and became briefly refractory. These brief periods of poor locomotor performance, however, were often followed by long periods of vigorous, steady locomotion. Thus, we hesitate to say that toads were fatigued at these speeds because poor performance appeared to be due to motivation. Endurance at 0.41 $km\ h^{-1}$ was measured in five toads, all of which moved steadily for more than 50 min of the 1 h test period. All five, however, stumbled occasionally and one toad refused to move after 57 min. During the oxygen consumption measurements, several toads did move steadily without difficulty at

Table 1. *Linear regression statistics for the relationship between speed and oxygen consumption for 13 individual toads Bufo boreas*

Mass (g)	Speed range (km h ⁻¹)	N	Slope, C _{min} (ml O ₂ g ⁻¹ km ⁻¹)	y-intercept (ml O ₂ g ⁻¹ km ⁻¹)	r	Pre-	y-intercept/
						exercise V̇O ₂	pre-exercise V̇O ₂
40.8	0.26–0.43	4	0.852±0.067	0.424±0.009	0.98*	–	–
54.0	0.02–0.25	4	1.009±0.184	0.293±0.031	0.94	0.089	3.29
84.5	0.08–0.49	7	1.159±0.099	0.403±0.031	0.98**	0.229	1.77
87.3	0.20–0.41	3	0.791±0.030	0.178±0.005	0.99*	0.089	1.99
90.7	0.13–0.35	3	0.748±0.008	0.306±0.001	0.99*	0.094	3.24
95.6	0.09–0.31	3	0.950±0.177	0.213±0.029	0.97	0.073	2.91
101.8	0.14–0.31	4	1.233±0.420	0.355±0.053	0.81	0.135	2.63
117.0	0.24–0.33	3	1.935±1.108	0.078±0.074	0.75	0.404	0.19
118.9	0.08–0.30	3	0.776±0.008	0.112±0.001	0.99*	0.063	1.77
120.5	0.08–0.30	3	0.918±0.008	0.065±0.001	0.99*	0.025	2.64
124.1	0.08–0.41	4	0.995±0.056	0.138±0.013	0.99**	0.090	1.54
132.1	0.14–0.61	7	0.702±0.061	0.358±0.061	0.96**	0.137	2.61
140.2	0.8–0.30	5	1.016±0.269	0.084±0.046	0.83	0.051	1.64
Mean values							
100.6			1.006	0.231	0.94	0.123±0.030	2.18

The slope and y-intercept estimates are shown ± S.E.M.

*Significant at $P \leq 0.05$; **significant at $P \leq 0.01$.

speeds above 0.4 km h⁻¹ for periods exceeding 1 h. The values for these animals were included in the calculation of C_{min}. One 132 g toad had exceptionally high stamina; it matched the treadmill speed for as long as 1 h at speeds up to 0.49 km h⁻¹ and provided 10 min of steady-state oxygen consumption at 0.61 km h⁻¹ (see Table 1).

On the basis of these observations, the relationship between sustainable speed and oxygen consumption was analyzed for speeds ranging from 0.02 to 0.49 km h⁻¹. Speeds below 0.35 km h⁻¹ were considered indefinitely sustainable and oxygen consumption data are based upon 10 min of steady-state recordings. Above 0.35 km h⁻¹, only those oxygen consumption records in which animals moved steadily for 1 h or longer were considered to be sustainable and were included in the C_{min} analysis.

Locomotor energetics

Analysis of oxygen consumption data pooled for all toads indicated that steady-state oxygen consumption ($\dot{V}_{O_{2ss}}$, ml O₂ g⁻¹ h⁻¹) increased significantly with speed between 0.02 and 0.49 km h⁻¹ (Fig. 1). The linear regression equation relating speed to $\dot{V}_{O_{2ss}}$ for 0.02–0.49 km h⁻¹ was $\dot{V}_{O_{2ss}}$ (ml O₂ g⁻¹ h⁻¹) = 0.93 × speed (km h⁻¹) + 0.28 ($r=0.62$). This relationship was significantly linear; a quadratic term accounted for no significant additional variation [ANOVA: $F_{\text{speed}(1,50)}=31.6$, $P=0.0001$; $F_{\text{speed}^2(1,50)}=1.1$, $P=0.30$]. Oxygen consumption at non-sustainable speeds (i.e. when less than 10 min of $\dot{V}_{O_{2ss}}$ could be measured) was 0.69±0.08 ml O₂ g⁻¹ h⁻¹ (S.E.M.) and was 5.6-fold greater than the

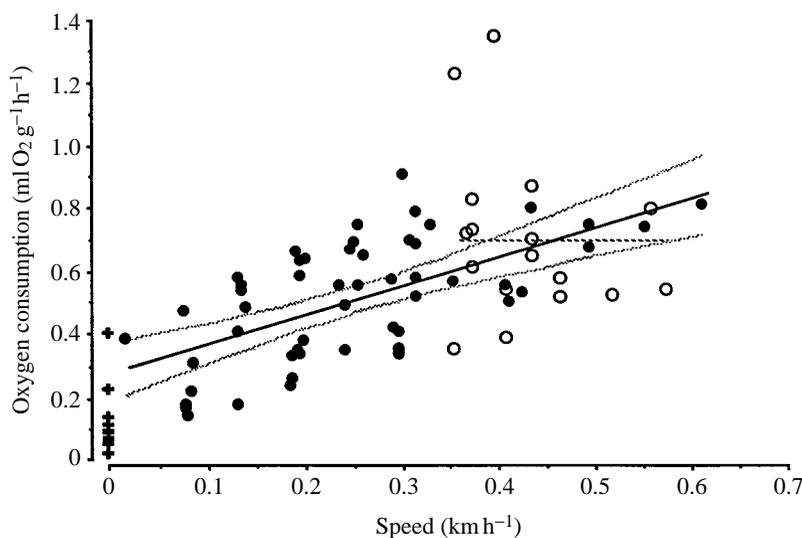


Fig. 1. Rate of oxygen consumption as a function of treadmill speed. Filled circles indicate sustainable speeds, open circles indicate non-sustainable speeds (see text for explanation). Crosses at zero speed indicate pre-exercise rates of oxygen consumption. The minimum cost of transport based upon the pooling of data from all individuals is represented by the slope of the solid line ($0.93 \text{ ml O}_2 \text{ g}^{-1} \text{ km}^{-1}$). Curved lines indicate the 95% confidence limits for the predicted values of oxygen consumption. The horizontal dashed line was drawn through the mean rate of oxygen consumption at non-sustainable speeds.

mean pre-exercise $\dot{V}\text{O}_2$ (Table 1). Non-sustainable rates of oxygen consumption were not related to speed ($P > 0.25$).

Although cost of transport estimates are frequently based on pooled data for several animals (as is the case in Fig. 1), this practice ignores the potential for variability among individual toads (Lighton *et al.* 1993) and, in the current study, may confound analysis of the possible effects of mass and temperature. Table 1 presents the regressions relating $\dot{V}\text{O}_{2\text{SS}}$ to speed for each toad. The mean C_{min} ($\text{ml O}_2 \text{ g}^{-1} \text{ km}^{-1}$) and y-intercept ($\text{ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$) based on the 13 individual analyses were similar to those obtained from pooled data (Table 1). Inter-individual variability in these relationships was examined by analysis of covariance to test for the homogeneity of slopes of the speed– $\dot{V}\text{O}_{2\text{SS}}$ relationships (i.e. equality of C_{min}). Individual toads differed significantly in C_{min} [$F(12,27)=3.27$, $P=0.005$].

The combined effects of temperature and body mass on this inter-individual variability was assessed by multiple regression analysis. It is important to note that, for this analysis, C_{min} was expressed as $\text{ml O}_2 \text{ km}^{-1}$, rather than the more common mass-specific $\text{ml O}_2 \text{ g}^{-1} \text{ km}^{-1}$. When expressed in this way, interindividual differences in $\log_{10} C_{\text{min}}$ co-varied with \log_{10} body mass [$F_{\text{mass}}(1,12)=27.21$, $P=0.0004$], but not with temperature [$F_{\text{temp}}(1,12)=2.17$, $P=0.17$]. C_{min} ($\text{ml O}_2 \text{ km}^{-1}$) scaled isometrically with body mass (g): $C_{\text{min}} = 0.69 \text{ mass}^{1.07}$, $r^2=0.82$, $P=0.0005$. The exponent of this relationship is not significantly different from 1 ($t=0.33$, 11 d.f., $P=0.74$). Consequently, mass-specific C_{min} was unrelated to body mass ($r^2=0.01$, $P=0.74$).

The y -intercept was, on average, twofold greater than pre-exercise rates of oxygen consumption (Table 1). This difference was only marginally significant, however (paired t -test, two-tailed $t=2.10$, 11 d.f., $P=0.06$), largely due to the influence of one 117 g toad with an exceptionally high pre-exercise \dot{V}_{O_2} ($0.404 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$). When this individual was excluded from the analysis, the differential between y -intercepts and pre-exercise \dot{V}_{O_2} became highly significant (paired t -test, two-tailed $t=5.43$, 10 d.f., $P=0.0003$).

Maximum rate of oxygen consumption

Maximum rates of oxygen consumption ($\dot{V}_{O_{2\max}}$) were measured in closed, rotating respirometers in six individual toads of body mass ranging from 58 to 131 g (mean 107.4 ± 11.4 g, s.e.m.). Closed-system measurements were conducted at 25 ± 1 °C, temperatures similar to those on the treadmill (± 1 °C). Maximum, closed-system rates of oxygen consumption ranged from 0.64 to $1.26 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$ (mean $0.97 \pm 0.09 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$, s.e.m.) and were significantly greater than pre-exercise \dot{V}_{O_2} measured in the same individuals by approximately sixfold (paired t -test, two-tailed $t=6.45$, 5 d.f., $P=0.0013$). Closed-system $\dot{V}_{O_{2\max}}$ was also greater than the highest rate of $\dot{V}_{O_{2ss}}$ achieved on the treadmill (paired t -test, two-tailed $t=6.26$, 5 d.f., $P=0.0015$) among the same six toads. Rates measured in the rotating respirometers were, on average, 41 % greater than treadmill rates.

Discussion

Toads moved with a steady, natural walking or running gait on the treadmill in a manner resembling that of unrestrained toads and without changing to a hopping stride at any speed. We calculated the cost of transport from data for speeds at which the toads were moving without indication of difficulty or fatigue. We are therefore confident that our data represent the metabolic rates of normal, sustainable pedestrian locomotion in *B. b. halophilus*.

Metabolic response to locomotor speed

The relationship between \dot{V}_{O_2} and speed in *B. b. halophilus* was similar to that observed in many terrestrial vertebrates: $\dot{V}_{O_{2ss}}$ increased as a linear function of speed within the range of sustainable speeds. The linear increase of \dot{V}_{O_2} with walking speed in *B. b. halophilus* contrasts with the findings of Anderson *et al.* (1991) for walking in *B. w. fowleri*. When forced to locomote on a shortened treadmill, *B. w. fowleri* walked exclusively at speeds at which they would normally hop. Anderson *et al.* (1991) were thereby able to estimate the cost of exclusive walking and found that \dot{V}_{O_2} was independent of walking speed in *B. w. fowleri*. One possible explanation for this unusual pattern is that their method underestimated the slope of the \dot{V}_{O_2} versus speed relationship. Perhaps the shortened treadmill altered the gait in some way either to elevate the cost of walking at slower speeds or (less likely) to depress the cost of walking at higher speeds. Anderson *et al.* (1991), however, reported that stride frequencies and stride lengths in the shortened treadmill were similar to those for periods of walking during unconstrained, mixed-gait locomotion. Another possibility is that energy expenditure during forced walking on the

shortened treadmill increased with speed in *B. w. fowleri*, but walking at higher speeds was supported by anaerobic metabolism. This is also unlikely considering that Anderson *et al.* (1991) reported that forced walking at high speeds did not significantly elevate lactate or depress creatine phosphate levels of limb muscles in comparison with resting levels. Perhaps there are biomechanical differences in the walking gaits of *B. b. halophilus* and *B. w. fowleri*, even though locomotor morphology is quite similar among *Bufo* species (Emerson, 1979, 1982). Alternatively, the range of speeds at which Anderson *et al.* (1991) could obtain sustained walking (0.04–0.14 km h⁻¹) was simply too narrow to detect an increase of \dot{V}_{O_2} with speed. At present we cannot explain the disparity. However, we have shown that independence of \dot{V}_{O_2} with increasing speed is not a general phenomenon within the genus *Bufo*.

Although \dot{V}_{O_2} did reach an apparent maximum on the treadmill (Fig. 1), we hesitate to define an MAS for this species. For several toads, walking at speeds greater than 0.35 km h⁻¹ was of lesser quality than at slower speeds, largely due to occasional ‘stumbles’ and periods when the toads refused to locomote. Among these toads, \dot{V}_{O_2} may have been lower than maximal at higher speeds because they were not working as hard as possible, not because they had reached a physiological maximum. In this case, mechanical constraints may limit the maximum speed of sustained walking. Toads have short, stiff bodies and long hindlimbs relative to their forelimbs. For these reasons, they become dynamically unstable and the degree of body pitching increases as they walk at high speeds (Barclay, 1946).

Minimum cost of transport

Allometric analyses indicate a strong influence of body size on C_{\min} , but surprisingly little variation attributable to other factors (Full, 1989). Consequently, animals with exceptionally high or low C_{\min} are of particular interest because they may be especially useful for elucidating underlying locomotor mechanics.

The C_{\min} for locomotion in *B. b. halophilus* was similar, if somewhat lower, than that predicted for other terrestrial animals of similar size (Fig. 2). This result contrasts sharply with that for *B. w. fowleri*, which has a C_{\min} during natural, mixed-gait locomotion that is significantly higher than expected for its body mass (Walton and Anderson, 1988). Gatten *et al.* (1992) provide a general equation relating C_{\min} (J kg⁻¹ m⁻¹) to body mass (kg) for a broad taxonomic sample of terrestrial organisms ($C_{\min}=10.76\text{mass}^{-0.31}$, $r^2=0.84$). The predicted C_{\min} value for a 100 g animal based on this equation is 17.5 % higher than the estimate of C_{\min} for *B. b. halophilus* based upon pooled data (Fig. 1) and is, on average, 8.6 % higher than the C_{\min} estimates for each of the 13 toads (Table 1). Both estimates of toad C_{\min} are within the 95 % confidence intervals of the equation of John-Alder *et al.* (1986) relating C_{\min} (ml O₂ g⁻¹ km⁻¹) to body mass (g) for lizards ($C_{\min}=4.22\text{mass}^{-0.28}$, $r^2=0.87$).

Individual toads differed significantly in C_{\min} , but most values were similar or somewhat lower than those predicted for their mass. One 117 g toad in particular had an exceptionally high C_{\min} (1.935 ml O₂ g⁻¹ km⁻¹) that was elevated 86 % above the general equation of Gatten *et al.* (1992) and 74 % above the lizard equation of John-Alder *et al.* (1986). This toad contributed greatly to total inter-individual variability. The coefficient of

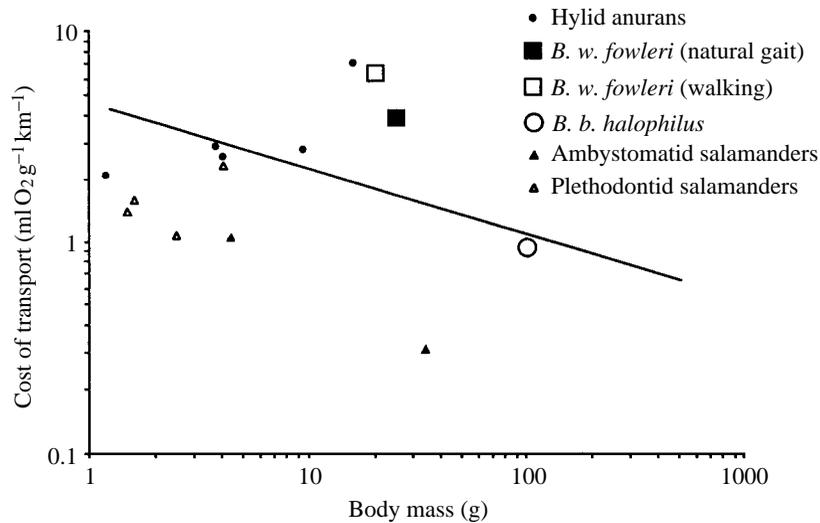


Fig. 2. Cost of transport as a function of body mass for amphibians. Minimum cost of transport measurements, C_{\min} (i.e. slopes of oxygen consumption *versus* speed regressions), are plotted for hylid frogs (Walton, 1993), *B. w. fowleri* using a natural and a mixed gait (Walton and Anderson, 1988) and salamanders (Full *et al.* 1988). Net cost of transport [$(\dot{V}_{O_{2SS}} - \dot{V}_{O_{2rest}})/\text{speed}$] is plotted for *B. w. fowleri* walking at 0.1 km h^{-1} , based on data from Anderson *et al.* (1991). Because $\dot{V}_{O_{2SS}}$ is independent of speed for walking *B. w. fowleri*, minimum cost cannot be calculated in this case. The solid line represents the predicted relationship between body mass and C_{\min} based on the equation of Taylor *et al.* (1982).

variation for the mean C_{\min} averaged across individuals was 31.8% with this individual included, but decreased to 17.7% when this individual was removed from the analysis.

Inter-individual variability in C_{\min} was unrelated to experimental temperature over the limited thermal range of these experiments. Nevertheless, our results were consistent with previous findings for other ectotherms that the elevation (*y*-intercept), but not the slope (C_{\min}), of the \dot{V}_{O_2} *versus* speed relationship is temperature-dependent (Taylor, 1977; John-Alder and Bennett, 1981; Herreid *et al.* 1981).

Somewhat surprising was the finding that C_{\min} scaled isometrically with body mass when C_{\min} was expressed in units of oxygen consumption per distance travelled (i.e. $\text{ml O}_2 \text{ km}^{-1}$). Consequently, mass-specific C_{\min} ($\text{ml O}_2 \text{ g}^{-1} \text{ km}^{-1}$) was independent of body mass. In contrast, broad-scale comparisons among diverse taxa indicate that mass-specific C_{\min} scales allometrically and decreases with body mass (e.g. Taylor *et al.* 1970; Full, 1991; Gatten *et al.* 1992). However, few analyses of the intraspecific allometry of the cost of transport have been undertaken. Our intraspecific results are similar to interspecific findings for amphibians (Fig. 2). Mass is unrelated to mass-specific C_{\min} among the seven anuran species ($r^2=0.045$, $P=0.65$, data for other anurans from Walton and Anderson, 1988; Walton, 1993) or among all of the 13 amphibian species examined to date ($r^2=0.021$, $P=0.63$, data for salamanders from Gatten *et al.* 1992). The data for amphibians are, however, as yet too few to make generalizations concerning the scaling of C_{\min} in this group.

Maximum oxygen consumption

The maximum rate of oxygen consumption of *B. b. halophilus* measured in closed, rotating respirometers ($0.97 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$) was similar to values reported previously for this species at similar temperatures (Carey 1979a,b; Hillman and Withers, 1979). In fact, the value reported here is well within the 95 % confidence intervals of predicted values based on the allometric equation of Gatten *et al.* (1992) relating body mass to $\dot{V}_{\text{O}_2\text{max}}$ of anurans measured in rotating respirometers at 25 °C: $\dot{V}_{\text{O}_2\text{max}} = 1.06 \text{ mass}^{1.04}$, $r^2 = 0.97$, $N = 16$.

The maximum rates obtained on the treadmill during non-sustainable locomotion were, however, lower than the closed-system estimates and are below the 95 % confidence limits of the general anuran equation of Gatten *et al.* (1992). Furthermore, maximal rates of oxygen consumption measured during sustained locomotion on the treadmill were lower than the closed-system estimates of $\dot{V}_{\text{O}_2\text{max}}$. Our observations suggest that the discrepancy between closed-system and treadmill maxima reflects both differences due to experimental procedures and differences in locomotor behavior in the two contexts. Animals in rotating respirometers are turned upside down, jostled and exhibit clear signs of distress. Most toads, for example, inflate themselves at the start of these ‘tumbling’ experiments, just as they do as a defensive response in nature. In contrast, toads rarely showed defensive behavior, at least at lower speeds, while walking on the treadmill. Further, treadmill locomotion may activate a smaller fraction of the total aerobic muscle mass than the rotating respirometer technique.

The results of this study differ from those of Walton and Anderson (1988), who found that closed-system ‘tumbling’ and treadmill respirometry elicited similar maximal oxygen consumption rates for *B. w. fowleri*. Perhaps locomotion in rotating respirometers more closely approximates vigorous hopping by *B. w. fowleri* than pedestrian locomotion by *B. b. halophilus*. Even in hopping species, however, rotating respirometers and treadmills may elicit different metabolic responses. Taigen and Beuchat (1984) found that tumbling *B. americanus* showed significant accumulation of lactate after just a few minutes of submaximal exercise, whereas Anderson *et al.* (1991) found no such accumulation in *B. w. fowleri*, even after 30 min of treadmill locomotion at speeds that elicited $\dot{V}_{\text{O}_2\text{max}}$.

The hypothesis that high $\dot{V}_{\text{O}_2\text{max}}$ is associated with a walking gait in *Bufo* is not supported by our findings. The maximal rate of oxygen consumption of *B. b. halophilus* measured either in a rotating respirometer or on a treadmill is not higher than that of other *Bufo* species. It is in fact lower than expected for toads of similar mass. Fig. 3 presents the data for current and previous measurements of $\dot{V}_{\text{O}_2\text{max}}$ at 25 °C for *Bufo*, including four previous measurements of *B. b. halophilus*. Based on these data, the relationship between $\dot{V}_{\text{O}_2\text{max}}$ ($\text{ml O}_2 \text{ h}^{-1}$) and body mass (g) for *Bufo* is $\dot{V}_{\text{O}_2\text{max}} = 0.184 \text{ mass}^{0.905}$ ($N = 12$, $r^2 = 0.90$, $P < 0.01$). All measurements of *B. b. halophilus* fall below this line. Analysis of variance indicates that *Bufo b. halophilus* has a significantly lower mass-independent maximum rate of oxygen consumption (i.e. $\dot{V}_{\text{O}_2\text{max}}/\text{mass}^{0.905}$) than cogenetic species that rely less heavily on walking [$F(1,10) = 6.07$, $P = 0.033$]. These findings contrast with those of Taigen *et al.* (1982), who found that high $\dot{V}_{\text{O}_2\text{max}}$ was associated with walking in a comparison of 17 species belonging to seven families of Anura.

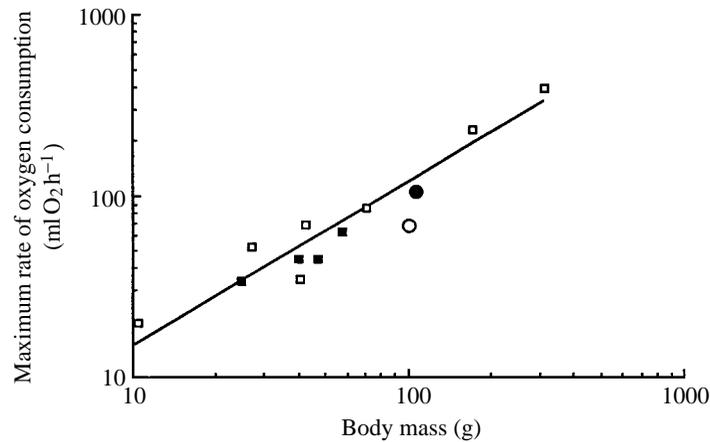


Fig. 3. Maximum rate of oxygen consumption as a function of body mass for the genus *Bufo*. The solid line represents the linear regression through these data (see text). Filled squares indicate means for *Bufo boreas halophilus* from previous studies; open squares indicate means for all other *Bufo* species. The large, filled circle represents the mean $\dot{V}_{O_{2max}}$ measured in closed, rotating respirometers, the open circle represents the mean $\dot{V}_{O_{2max}}$ achieved on the treadmill. Data from previous analyses were obtained from the compilation of Gatten *et al.* (1992).

Locomotor energetics and walking in Bufo

Anderson *et al.* (1991) demonstrated that a gait change from walking to hopping was energetically advantageous and allowed sustained locomotion at speeds greater than the apparent MAS of *B. w. fowleri*. We hypothesized that, among species such as *B. b. halophilus* that do not change gait, reliance on an expensive walking gait should be associated with a high $\dot{V}_{O_{2max}}$. Our results, however, indicate that walking is not an exceptionally costly gait in general, nor is dependence on walking predictive of high $\dot{V}_{O_{2max}}$ within the genus *Bufo*. On the contrary, the economy of locomotion in *B. b. halophilus* is similar to that predicted for terrestrial animals of similar size, and the $\dot{V}_{O_{2max}}$ of this species is actually lower than predicted for its body size. Consequently, our findings do not support the hypothesis of co-adaptation of gait and aerobic capacity in this group.

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