

EFFECT OF THERMAL ACCLIMATION ON LOCOMOTOR ENERGETICS AND LOCOMOTOR PERFORMANCE IN A LUNGLESS SALAMANDER, *DESMOGNATHUS OCHROPHAEUS*

By MARTIN E. FEDER

*Department of Anatomy and The Committee on Evolutionary Biology, The
University of Chicago, 1025 East 57th Street, Chicago, IL 60637, USA*

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SUMMARY

To determine the effects of thermal acclimation upon locomotor performance and the rate of oxygen consumption (\dot{M}_{O_2}) during activity, small (<3 g), lungless salamanders, *Desmognathus ochrophaeus* Cope, were acclimated to three temperatures (5, 13 and 21°C) and exercised at various controlled speeds within an exercise wheel while their \dot{M}_{O_2} was measured. \dot{M}_{O_2} increased with speed at low speeds (<14 cm min⁻¹). Although animals could sustain greater speeds, \dot{M}_{O_2} did not increase further. These small, exclusively skin-breathing salamanders could increase their \dot{M}_{O_2} 9–11 times during exercise and could sustain nearly half of the oxygen flux expected across a similar surface area of the mammalian lung. However, their maximum aerobic speed was remarkably slow (14 cm min⁻¹) and their net cost of transport remarkably large (15–17 ml O₂ g⁻¹ km⁻¹). Thermal acclimation affected \dot{M}_{O_2} during activity, the maximum sustainable speed and locomotor stamina in different ways. During exercise at 13°C, cold-acclimated animals had a significantly greater \dot{M}_{O_2} than warm-acclimated animals, but did not differ in stamina or the maximum sustainable speed. During exercise at 21°C, cold acclimation did not affect the \dot{M}_{O_2} significantly, but it decreased the stamina and increased the rate of lactate accumulation. Thus, these results suggest that thermal acclimation of the \dot{M}_{O_2} is not tightly coupled to thermal acclimation of locomotor performance in salamanders.

INTRODUCTION

Thermal acclimation may markedly alter the rate of oxygen consumption (\dot{M}_{O_2}) in amphibians, with some salamanders undergoing a 10–35 % decrease in the resting or standard \dot{M}_{O_2} during acclimation to a moderately warm temperature (Feder, Gibbs, Griffith & Tsuji, 1984; Feder, 1985). Although such decreases in \dot{M}_{O_2} are often considered adaptive because they reduce the overall energy requirement, a decrease in \dot{M}_{O_2} may also reduce the capacity for physical activity, which may offset the advantage of a reduced energy requirement. The purpose of the present study was to characterize any changes in \dot{M}_{O_2} during exercise that are associated with acclimatory changes in resting \dot{M}_{O_2} , and to determine how acclimatory changes in \dot{M}_{O_2} during activity affect locomotor performance. Interspecific comparisons at a single

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temperature suggest that amphibians with a relatively high resting \dot{M}_{O_2} have a relatively high \dot{M}_{O_2} during activity as well, and *vice versa* (Taigen, 1983). If the \dot{M}_{O_2} during rest and during activity are likewise coupled in thermal acclimation of metabolism in amphibians, a reduced resting \dot{M}_{O_2} due to thermal acclimation should be accompanied by a reduced \dot{M}_{O_2} during activity. Such variation is likely to affect locomotor performance. According to Bennett's (1982) model, which relates the maximum aerobic speed (which approximates to the maximum sustainable speed) to the maximum \dot{M}_{O_2} during activity, acclimatory reductions in the \dot{M}_{O_2} during activity ought to: (1) reduce the maximum aerobic speed; (2) decrease the speed at which significant anaerobiosis is sustained (Taigen & Beuchat, 1984); and (3) decrease stamina at non-sustainable speeds. Similar logic underlies claims that inverse acclimation of resting \dot{M}_{O_2} at warm temperatures (i.e. increases in resting \dot{M}_{O_2}) should potentiate physical activity in amphibians active during the summer (Weathers, 1976).

A test of these expectations requires measurements of \dot{M}_{O_2} during graded exercise at known levels of activity, such as are available for fishes swimming in flow chambers or reptiles and mammals walking on treadmills. To obtain such measurements, previous investigators have used respirometers in which amphibians were prodded, shocked, or overturned to yield locomotor activity that is presumably maximal but is not amenable to quantification (Hillman, Shoemaker, Putnam & Withers, 1979). The present study, by contrast, used an exercise wheel in which small (<3 g), lungless salamanders would walk for as much as 2 h at controlled and relatively constant speeds.

MATERIALS AND METHODS

Animal care and acclimation regimes

Allegheny Mountain salamanders, *Desmognathus ochrophaeus*, were collected near Highlands, North Carolina, USA. This species experiences temperatures between 1 and 20°C in the field (Feder, Lynch, Shaffer & Wake, 1982) and prefers 17–20°C in laboratory gradients (Spotila, 1972). Salamanders were maintained in the laboratory at 13–14°C on an LD 14:10 photoperiod centred at 13.00 h local time. Animals were fed fruit flies regularly. Body masses of animals ranged between 0.89 and 3.24 g and averaged 2 g.

Preliminary experiments established that body mass was inversely related to \dot{M}_{O_2} both in resting salamanders and in salamanders moving at various speeds. To minimize the effect of body mass, animals were sorted into groups with similar distributions of body masses before experimentation. Groups were then maintained at one of three acclimation temperatures (ATs): 5–6°C (hereafter 5° AT), 13–14°C (13° AT), and 21–22°C (21° AT). Animals were acclimated 2–3 weeks before use. Animals were not fed during the acclimation period; in addition, 5° AT animals were not fed during the week preceding acclimation to allow clearing of the gut (Feder *et al.* 1984). The photoperiod was unchanged during acclimation.

Measurement of \dot{M}_{O_2} and locomotor performance

Each respirometer was essentially a hollow rectangular beam of Plexiglas (inner dimensions: 1.3 cm height \times 1.5–1.8 cm width \times 40 cm) bent in a circle to form a wheel. A port allowed access to the inside of the beam. Salamanders could walk inside the beam; i.e. within the wheel's rim. The top and bottom of the inside of the beam (salamanders would walk on both) were coated with plastic screening or roughened to facilitate traction. Several curved pieces of lead were cast to fit loosely inside the beam; these had the tips of 30-gauge syringe needles mounted in their ends. In use, the wheel respirometer was cleaned thoroughly, the interior of the beam was moistened, a salamander and the pieces of lead were placed in the track, and the port in the wheel's rim was bolted into place to form an air-tight seal. The wheel was then mounted vertically on a fixed axle connected by a pulley to an electric motor. A Minarik SL15 Motor Speed Controller (Minarik Electric Co., Los Angeles, CA) controlled motor speed and thus rotation of the wheel. As the wheel rotated, the lead pieces slid to the bottom of the wheel. Salamanders would walk forward in the track to avoid the sliding lead pieces. Accordingly, their speed would match that of wheel rotation.

Salamanders were placed in the wheel respirometer 2–3 h before measurement. The wheel was then immersed in a water bath at a predetermined temperature. Humidified air at that temperature entered and exited through stopcocks in the wheel's rim. To begin measurements of \dot{M}_{O_2} , a 20–30 ml gas sample was withdrawn from the excurrent gas stream and the stopcocks were sealed. After a measured time interval, a final 20–30 ml gas sample was withdrawn from the respirometer. The respirometer was then filled with water to determine the gas volume (typically between 58 and 71 ml), and the animal was weighed and measured. The fractional oxygen concentrations of the gas samples were determined with an Ametek Applied Electrochemistry S-3A Oxygen Analyzer (Pittsburgh, PA). Gas samples were injected into the analyser through a column of Drierite and Ascarite (to remove water vapour and carbon dioxide, respectively) with a Razel syringe pump (Stamford, CN). The oxygen consumption was calculated with the following equation (Vleck, 1978) and converted to $\mu\text{mol O}_2 \text{ h}^{-1}$:

$$\dot{M}_{O_2} = V(FI_{O_2} - FE_{O_2}) / (1 - FE_{O_2}) t,$$

where V is the volume of dry, CO_2 -free air in the respirometer at STP, t is the time between the initial and final samples, and FI_{O_2} and FE_{O_2} are the fractional concentrations of O_2 in the initial and final sample, respectively. When \dot{M}_{O_2} was measured several times in sequence, the respirometer was fully flushed with room air between determinations.

Relationships between locomotor speed, acclimation temperature and \dot{M}_{O_2} at 13°C

Animals from the three acclimation regimes were examined individually at 13°C. After 2–3 h for equilibration, \dot{M}_{O_2} of the inactive animal was determined for three successive 20-min intervals. Thereafter, the animal was exercised for 15 min at each of a series of increasing speeds separated by a 5-min rest. \dot{M}_{O_2} was determined during

each 15-min interval. The speeds were 6, 12, 22, 31, 39 and 47 cm min⁻¹. When animals were unable to sustain 15 min activity at a given speed, the run was terminated. Animals were considered exhausted when they could no longer maintain forward movement and instead were pushed continually by the lead pieces within the wheel.

Effect of acclimation temperature on locomotor stamina and \dot{M}_{O_2} at 21°C

Animals from the three acclimation regimes were examined individually at 21°C. After 2–3 h for equilibration, an initial gas sample was withdrawn. The animal was then exercised at a single speed, 31 cm min⁻¹, until it became exhausted. \dot{M}_{O_2} was determined during this period. Next, the animal was allowed to recover for 30 min. \dot{M}_{O_2} was determined for the last 20 min of this interval. Finally, the animal was again exercised at 31 cm min⁻¹ until exhaustion, during which \dot{M}_{O_2} was determined.

Effect of acclimation temperature on lactate accumulation during locomotion at 21°C

Animals from the three acclimation regimes were exercised at 21°C in wheels fashioned from plastic Petri dishes; the track within such wheels resembled that in the respirometer described above. Acclimated animals were weighed the day before measurement, placed in an individual wheel, and then returned to their respective acclimation temperatures overnight. Wheels containing salamanders were then placed at 21°C for 2–3 h for equilibration. Each wheel was individually affixed to the motor, and the animal within exercised at 31 cm min⁻¹ for either 0, 5, 10, 15, 20 or 30 min. Each animal was then immediately frozen in liquid nitrogen; the frozen carcass was homogenized in ice-cold 0.6 mol l⁻¹ perchloric acid. The lactate content of the homogenate was determined enzymatically according to Bennett & Licht (1972) with reagents from Sigma (St Louis, MO).

Standard \dot{M}_{O_2}

The standard (i.e. minimum) \dot{M}_{O_2} of acclimated animals was determined with a Gilson respirometer. Methods and complete data have been reported elsewhere (Feder *et al.* 1984; Feder, 1985). These previous data were adjusted to the body sizes of animals in the present study by assuming that \dot{M}_{O_2} scales as body mass raised to the 0.8 power (Feder, 1976).

Statistics

In experiments at 13°C, in which the \dot{M}_{O_2} of individual salamanders was measured repeatedly during or after exercise at several speeds, the data were analysed using analysis of covariance with repeated measures (Dixon, 1977). Body mass was the covariate, speed during exercise (including recovery) was the trial factor, and acclimation temperature was the grouping factor. In experiments 3 and 4, each measurement was analysed individually with analysis of covariance (Dixon, 1977); body mass was the covariate. When body mass significantly affected \dot{M}_{O_2} , the analysis

programmes 'adjusted' \dot{M}_{O_2} for this effect (Dixon, 1977); the adjusted mean \dot{M}_{O_2} is presented in the figures where appropriate. The 'honestly significant difference' was computed from the analyses of covariance (Sokal & Rohlf, 1969) and is presented in the figures; means differing by more than this amount differ significantly ($P < 0.05$). Nonparametric statistics (Kolmogorov-Smirnov test, Spearman's rank correlation coefficient) are according to Siegel (1956).

RESULTS

General patterns of locomotion and \dot{M}_{O_2} at 13°C

When placed in the wheel respirometer, salamanders typically explored the chamber but soon became quiescent. Despite the lack of obvious movement, the \dot{M}_{O_2} of salamanders resting in the wheel respirometer averaged twice that of salamanders measured in the Gilson respirometer chambers. For example, in preliminary measurements at 13°C, the \dot{M}_{O_2} of eight inactive salamanders in the two respirometer systems was 1.93 ± 0.18 (mean \pm standard error) and $0.89 \pm 0.02 \mu\text{mol g}^{-1} \text{h}^{-1}$, respectively. The Gilson chambers were small glass vials containing moist paper towelling, and thus more closely resembled natural retreats of salamanders than did the wheel respirometer. Accordingly, the \dot{M}_{O_2} of animals resting in the wheel respirometer is henceforth termed the 'routine \dot{M}_{O_2} ' and the lower \dot{M}_{O_2} determined with the Gilson respirometer is termed the 'standard \dot{M}_{O_2} '.

When rotation of the wheel was begun, salamanders moved forward in response to contact with the lead pieces, and generally matched their forward speed to the rate of wheel rotation. Most often, salamanders walked upward at approximately a 45° gradient. \dot{M}_{O_2} increased with the average forward speed at speeds between 0 and approximately 14 cm min^{-1} (Fig. 1). At greater average speeds, \dot{M}_{O_2} was independent of speed and averaged 4–6 times the routine \dot{M}_{O_2} . This increment corresponds to 9–12 times the standard \dot{M}_{O_2} . Because \dot{M}_{O_2} did not increase at speeds greater than approximately 14 cm min^{-1} , this speed is the 'maximum aerobic speed' (Bennett, 1982). However, all but a single animal were able to sustain activity at greater speeds for at least 30 min.

Effect of acclimation temperature on \dot{M}_{O_2} and maximum sustainable speed at 13°C

Acclimation to a warm temperature (21°C) clearly decreased \dot{M}_{O_2} during locomotion at 13°C (Fig. 1). The \dot{M}_{O_2} of animals acclimated to 21°C averaged 74 % of that of animals acclimated to 5 and 13°C (combined) at speeds between 6 and 39 cm min^{-1} . Moreover, the routine \dot{M}_{O_2} of the 21° AT animals was just 61 % of the routine \dot{M}_{O_2} of the 5° AT and 13° AT animals. Complete data were available for 15 animals at 0, 6, 12, 22 and 31 cm min^{-1} (many animals became exhausted at greater speeds). These data were subjected to an analysis of variance with repeated measures, which confirmed that the effect of acclimation temperature on \dot{M}_{O_2} was statistically significant ($P = 0.0027$). Thus, the total aerobic cost of locomotion was lower in warm-acclimated salamanders than in cool-acclimated salamanders.

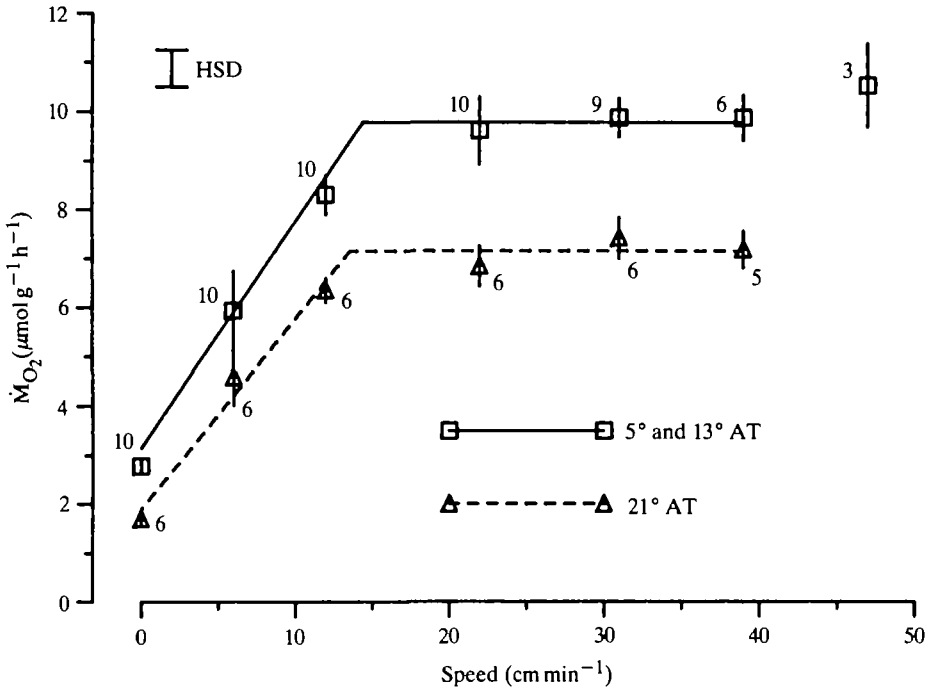


Fig. 1. Effect of acclimation temperature (AT) on the \dot{M}_{O_2} of *Desmognathus* walking at various speeds. Numbers above or below symbols represent the number of non-fatiguing animals whose \dot{M}_{O_2} was measured at each speed, the symbols represent the means of these \dot{M}_{O_2} values, and the vertical lines signify the means ± 1 standard error. The \dot{M}_{O_2} of animals active at 47 cm min⁻¹ was excluded from the statistical analysis and computation of the honestly significant difference (HSD). The ascending lines were calculated from linear regressions of \dot{M}_{O_2} at 0, 6 and 12 cm min⁻¹ versus speed. The horizontal lines were calculated as the means of the \dot{M}_{O_2} values at 22, 31 and 39 cm min⁻¹. Experimental temperature was 13°C.

This acclimatory difference in \dot{M}_{O_2} during activity, however, had no obvious consequences for the maximum speed that salamanders could sustain at 13°C. The maximum aerobic speeds of the two groups were similar (Fig. 1). Of the cold-acclimated animals ($N = 10$), 90 % could sustain 15 min of activity at 31 cm min⁻¹, 60 % could sustain 39 cm min⁻¹, 30 % could sustain 47 cm min⁻¹ and 10 % could sustain 56 cm min⁻¹. Of the warm-acclimated animals ($N = 6$), 100 % could sustain 31 cm min⁻¹, 84 % could sustain 39 cm min⁻¹ and none could sustain 47 cm min⁻¹. These two distributions do not differ significantly (Kolmogorov-Smirnov test, $P > 0.5$). Within the cold-acclimated group, by contrast, animals with the greatest \dot{M}_{O_2} also had the greatest sustainable speed (Spearman's $r = 0.623$; $P = 0.05$).

Effect of acclimation temperature on \dot{M}_{O_2} , stamina, and recovery from fatigue at 21°C

The unexpected finding of a major effect of thermal acclimation on \dot{M}_{O_2} during locomotion *without* a corresponding effect on the maximum sustainable speed prompted a second series of measurements, in which procedures differed in two

respects. First, the experimental temperature was increased to 21°C in the hope of eliciting greater differences in \dot{M}_{O_2} among acclimation treatments (see Feder, 1978). Second, stamina and recovery from fatigue were used as measures of locomotor performance in the expectation that they might reflect acclimatory differences in \dot{M}_{O_2} better than had the maximum sustainable speed.

Contrary to expectations, acclimation temperature had no significant effect ($P=0.52$) on \dot{M}_{O_2} during exercise to exhaustion at the test speed, 31 cm min⁻¹ (Table 1). Moreover, acclimation failed to affect \dot{M}_{O_2} during a 30-min recovery period ($P=0.41$) and during a second period of exercise to exhaustion ($P=0.25$).

Although \dot{M}_{O_2} during locomotion at 21°C was unaffected by thermal acclimation, stamina differed markedly among the acclimation treatments during both the first exercise period ($P=0.011$) and the second exercise period ($P=0.002$) (Table 1). In both exercise periods, 5° AT animals had a significantly lower stamina than either the 13° AT animals or the 21° AT animals, which did not differ from one another significantly. Stamina of animals in the latter two groups was as much as 133 min (>41 m total distance) and averaged more than 1 h. Stamina during the second exercise period, which followed 30 min of rest, was 51–55% of the initial stamina; this percentage was not affected by thermal acclimation.

Lactate accumulation during locomotion at 21°C

One possible explanation for the rapid fatigue of 5° AT animals, whose \dot{M}_{O_2} was not different from that of the other groups, was that the 5° AT animals accumulated lactate at a greater rate than did the other groups. To test this explanation, whole-body lactate concentration was determined in acclimated animals at various times during 30 min of exercise at 31 cm min⁻¹. The experimental temperature was 21°C. Salamanders acclimated to 13°C increased lactate concentrations seven-fold during the first 10 min of activity, but accumulated little or no additional lactate during

Table 1. *Effect of acclimation temperature on \dot{M}_{O_2} , locomotor stamina and recovery from exhaustion during exercise at 21°C*

	Acclimation temperature (°C)			<i>P</i>
	5	13	21	
Sample size	10	8	8	
\dot{M}_{O_2} ($\mu\text{mol g}^{-1} \text{h}^{-1}$) during				
1st exercise to exhaustion	14.8 ± 1.3	14.3 ± 0.7	12.9 ± 1.0	0.320
30 min recovery	8.9 ± 1.5	10.8 ± 1.2	8.9 ± 0.9	0.410
2nd exercise to exhaustion	16.1 ± 0.7	17.0 ± 1.5	14.1 ± 1.2	0.250
Stamina (min) during				
A 1st exercise	35.3 ± 5.7	59.6 ± 9.6	72.3 ± 9.8	0.011
B 2nd exercise	16.3 ± 1.9	31.0 ± 4.0	37.3 ± 5.5	0.002
Percentage recovery from exhaustion during 30 min [(B/A) × 100 %]	51.4 ± 5.5	55.4 ± 5.0	52.0 ± 4.4	0.840

Means are given ± standard error. *P* is the significance of the difference among means in a row.

the subsequent 20 min. Salamanders acclimated to 5°C, by contrast, continued to accumulate lactate for the entire 30-min period (Fig. 2). Salamanders acclimated to 21°C, which had the greatest average stamina, had relatively low lactate

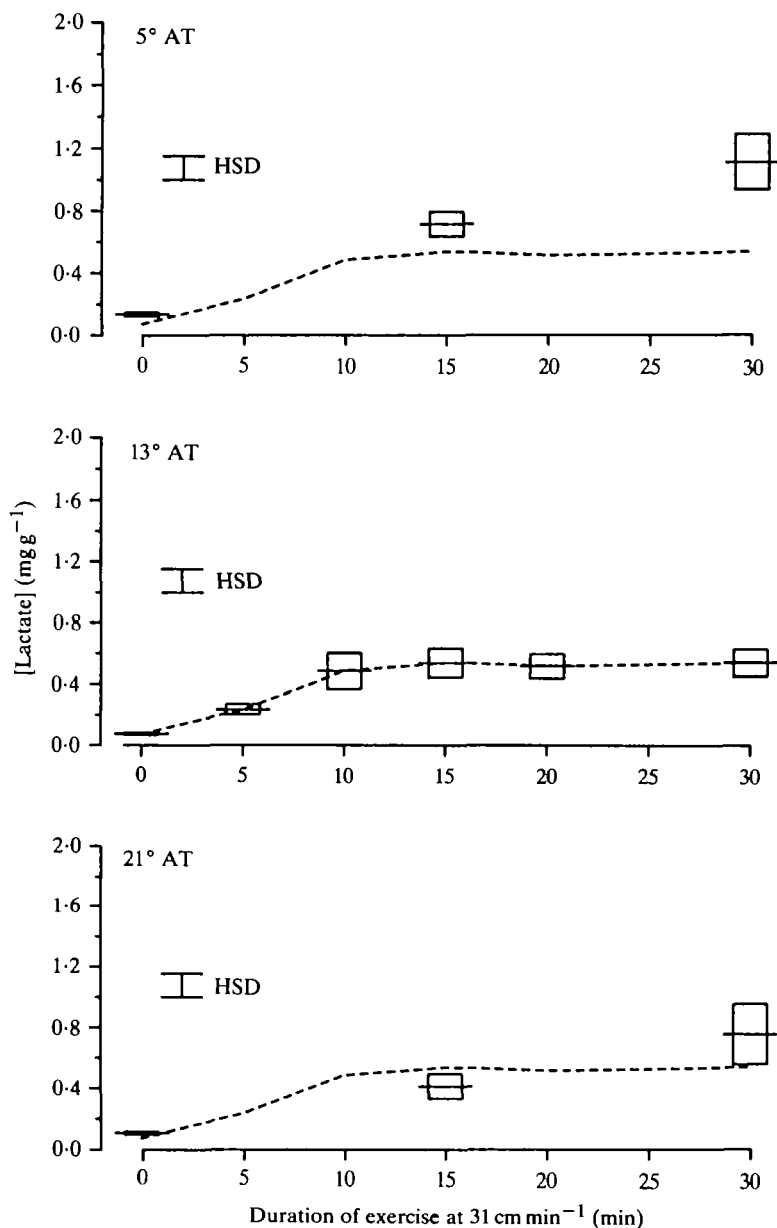


Fig. 2. Lactate concentrations at rest and after different durations of exercise in salamanders acclimated to three different temperatures (AT). Animals were exercised at 21°C. The horizontal line represents the mean of 5-6 animals; the vertical bar signifies the mean ± 1 standard error. A broken line connects the means for 13° AT animals; this line is repeated in the top and bottom graphs to facilitate comparison of the data. A single honestly significant difference (HSD) was determined for all data.

concentrations after 15 min of exercise but unexpectedly high lactate levels after 30 min of exercise. The latter value may have stemmed from exceptionally vigorous activity in two animals in the sample; levels in the three other animals were low.

DISCUSSION

Energetics of locomotion in salamanders

This study includes the first measurements of \dot{M}_{O_2} during controlled locomotion in a small (<3 g) terrestrial ectothermic vertebrate. The \dot{M}_{O_2} of *Desmognathus* in the exercise wheel increased with speed at low speeds and was independent of speed at high speeds, and thus resembled the pattern of \dot{M}_{O_2} in larger lizards exercising on treadmills (Bennett, 1982) as well as many other terrestrial animals. Also, the mass-specific \dot{M}_{O_2} was inversely related to the body size of salamanders both at rest and during activity. In other aspects, however, *Desmognathus* is unusual. The maximum aerobic speed, approximately 14 cm min^{-1} or 0.008 km h^{-1} , was spectacularly low. This speed is three orders of magnitude less than the maximum aerobic speed predicted for mammals by Garland's (1983) equation 5 and thus two orders of magnitude less than the maximum aerobic speed expected for lizards (Garland, 1982). This last comparison is confounded by the gross extrapolation that is required, the use of an exercise wheel rather than a treadmill in the present study, and the low body temperatures at which salamanders are active. Nonetheless, *Desmognathus*, which is by no means sedentary among salamanders, clearly is exceptionally slow.

According to Bennett's (1982) characterization of the maximum aerobic speed, activity at greater speeds is supported increasingly by anaerobic metabolism, whose end-products gradually accumulate and result in fatigue; thus, activity at greater speeds should not be sustainable. Some aspects of locomotion in *Desmognathus* are obviously not in accord with this scheme. For example, *Desmognathus* can sustain speeds greater than the maximum aerobic speed for considerable periods. At 13°C , most animals could move at a series of greater speeds for at least 45 min. At 21°C , some animals walked at 31 cm min^{-1} for nearly 2 h. In a different apparatus at 20°C (Feder & Londos, 1984), the same species could maintain 150 cm min^{-1} for 10 min before fatiguing. Although all animals accumulated lactate during the first 10 min of activity at 31 cm min^{-1} , most of the warm-acclimated animals accumulated little or no additional lactate when active at this speed for up to 20 min more (Fig. 2). Moreover, because \dot{M}_{O_2} did not increase at speeds greater than the maximum aerobic speed, animals could be active at increasing speeds without a concomitant increase in \dot{M}_{O_2} (and, apparently, lactate production). Increases in the speed of sustainable locomotion without parallel increases in \dot{M}_{O_2} , although uncommon, have been reported and generally involve specializations such as elastic storage (Dawson & Taylor, 1973) or ram ventilation (Freadman, 1981). One possible explanation for this pattern in *Desmognathus* is that salamanders change to a more efficient gait at higher speeds (Edwards, 1976). Another (but unlikely) explanation is that the time course of lactate production at 21°C is greatly different from the time course of

lactate production at 13°C, at which the maximum aerobic speed was determined. Both of these explanations are currently under investigation.

The slopes of the ascending lines relating \dot{M}_{O_2} to speed in Fig. 1 express the net cost of transport (Taylor, Schmidt-Nielsen & Raab, 1970) in *Desmognathus*. These are 17.2 ml O₂ g⁻¹ km⁻¹ for the cold-acclimated animals and 14.5 ml O₂ g⁻¹ km⁻¹ in the warm-acclimated animals. Comparable values have been obtained for another species of similar size, *Bolitoglossa subpalmata*, in the wheel respirometer (M. E. Feder, unpublished data). The net costs of transport for *Desmognathus* and *Bolitoglossa* thus differ dramatically from the net cost of transport predicted for 2-g animals by the summary equation 8 of Taylor, Heglund & Maloiy (1982), 3.8 ml O₂ g⁻¹ h⁻¹. Although the equation of Taylor *et al.* (1982) is based exclusively on data for mammals, it satisfactorily describes the cost of transport in a variety of invertebrates (0.01–100 g) and lizards (Full, 1984; Schmidt-Nielsen, 1984). By contrast, the net cost of transport in a third, larger species (*Plethodon jordani*, 4 g) walking on a treadmill is only 2.2 ml O₂ g⁻¹ km⁻¹ (Full, 1985), which is consistent with the equation of Taylor *et al.* (1982). Accordingly, it is not clear whether the remarkably high net cost of transport in *Desmognathus* and *Bolitoglossa* reflect their peculiar mode of sporadic movement within the respirometer (see above), their small size, the use of an exercise wheel instead of a treadmill, or an unusual case of costly locomotion. Additional studies are under way to clarify this point.

A final comparison concerns the respiratory gas exchange of *Desmognathus*, which lacks lungs and gills and thus breathes exclusively through its skin and buccopharynx. Cutaneous gas exchange has often been considered inadequate or limited because of the large diffusive resistance of skin and inadequate regulatory mechanisms (Feder & Burggren, 1985). Despite their reliance upon cutaneous respiration, the animals showed a considerable ability to increase \dot{M}_{O_2} during activity. The factorial aerobic scope (maximum \dot{M}_{O_2} /standard \dot{M}_{O_2}) was 9.66 in experiment 1 and 11.7 for the cold-acclimated animals in experiment 2. The maximum \dot{M}_{O_2} of the 21° AT animals in experiment 3 was 17 $\mu\text{mol g}^{-1} \text{h}^{-1}$. This corresponds to an O₂ flux of 2.5 $\mu\text{mol cm}^{-2} \text{h}^{-1}$ across the 13.6 cm² of skin surface (Whitford & Hutchison, 1967) of a 2-g salamander; fluxes are even greater at warmer body temperatures (Feder, 1977; Hillman *et al.* 1979). An average 10 kg mammal, with a pulmonary surface area of 2.96 × 10⁵ cm² (Gehr *et al.* 1981) and a maximum oxygen consumption of 1.88 × 10⁶ $\mu\text{mol h}^{-1}$ (Taylor *et al.* 1981), has an O₂ flux of 6.3 $\mu\text{mol cm}^{-2} \text{h}^{-1}$. Thus, despite a vastly 'improved' cardiovascular system, presence of a ventilatory system, and a thinner diffusion barrier (Feder & Burggren, 1985), each cm² of lung surface area in an average mammal sustains little more than twice the O₂ flux of a similar area of salamander skin.

Thermal acclimation and its consequences

A major purpose of this study was to determine whether acclimatory changes in resting \dot{M}_{O_2} were reflected in the \dot{M}_{O_2} during activity, and in turn whether acclimatory changes in the \dot{M}_{O_2} during activity were reflected in locomotor performance (e.g. stamina, maximum sustainable speed). Although acclimatory

changes in resting \dot{M}_{O_2} , \dot{M}_{O_2} during activity and locomotor performance all occurred in *Desmognathus*, these changes individually bore little resemblance to one another. Thus, while acclimation to warm temperatures improved locomotor stamina at warm temperatures in *Desmognathus*, this improvement was not mediated by acclimatory changes in aerobic metabolism. These findings parallel the conclusions of Feder (1978), Carey (1979) and Miller & Zoghby (1983), who likewise reported a lack of concordance among acclimatory patterns in resting \dot{M}_{O_2} , \dot{M}_{O_2} during activity and stamina.

Several major discrepancies between aerobic metabolism and performance appear in the data. Acclimation to 21°C reduced the routine \dot{M}_{O_2} and maximum \dot{M}_{O_2} of salamanders walking at 13°C; the standard \dot{M}_{O_2} is reduced as well in similar circumstances (Fitzpatrick, Bristol & Stokes, 1971; Feder *et al.* 1984). These changes presumably should have reduced the maximum sustainable speed of these animals. However, 21° AT animals and those acclimated to cooler temperatures did not differ in maximum sustainable speed. In contrast to this pattern of acclimation of \dot{M}_{O_2} without acclimation of the maximum sustainable speed, salamanders measured at 21°C showed acclimation of locomotor stamina without acclimation of \dot{M}_{O_2} . At this temperature, warm acclimation improved stamina but did not affect \dot{M}_{O_2} during activity. Significant acclimation of the standard \dot{M}_{O_2} occurs at this temperature (Fitzpatrick *et al.* 1971; Feder *et al.* 1984; Feder, 1985), which was not reflected in the \dot{M}_{O_2} during activity.

One pronounced effect of thermal acclimation in the present study was a reduction in the rate of lactate accumulation during sustained activity at 21°C. The increased reliance upon anaerobiosis in the 5°C-acclimated animals is consistent with the reduced stamina in this group, both of which are seemingly independent of \dot{M}_{O_2} . Indeed, several recent studies suggest that changes in neuromuscular phenomena and anaerobic metabolism rather than in aerobic metabolism underlie acclimatory changes in locomotor performance. For example, changes in myofibrillar ATPase, muscle volume and the speed at which white muscle was recruited were all associated with acclimatory differences in sustainable activity in carp (Rome, Loughna & Goldspink, 1985); carp not acclimated to the cool experimental temperature accumulated lactate more readily than acclimated animals. Also, frogs acclimated to cool temperatures fail to jump well at warm temperatures, apparently because of failures in neural control of jumping (Renaud & Stevens, 1983, 1984; Rome, 1983; Hirano & Rome, 1984; Rome *et al.* 1985). Prosser and his colleagues (e.g. Prosser & Nelson, 1981) have long maintained that improvements in neuromuscular control (rather than in aerobic metabolism) underlie the improved locomotor performance that may result from thermal acclimation.

The present study does not compellingly demonstrate that acclimatory changes in aerobic metabolism in any way potentiate changes in locomotor performance in *Desmognathus*. Lack of acclimation of locomotor performance has also been reported for both anurans and urodeles that undergo thermal acclimation of metabolism (Putnam & Bennett, 1981; P. Else & A. Bennett, personal communication). A parallel study of salamanders suggests that acclimation of aerobic metabolism

provides at best a trivial saving in long-term energy expenditure (Feder, 1985). Thus, at least in salamanders, the 'adaptive significance' of thermal acclimation remains to be demonstrated.

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REFERENCES

- BENNETT, A. F. (1982). The energetics of reptilian activity. In *Biology of the Reptilia*, Vol. 13 (ed. C. Gans & F. H. Pough), pp. 155–199. London: Academic Press.
- BENNETT, A. F. & LICHT, P. (1972). Anaerobic metabolism during activity in lizards. *J. comp. Physiol.* **81**, 277–288.
- CAREY, C. (1979). Effect of constant and fluctuating temperatures on resting and active oxygen consumption of toads *Bufo boreas*. *Oecologia* **39**, 201–212.
- DAWSON, T. J. & TAYLOR, C. R. (1973). Energetic cost of locomotion in kangaroos. *Nature, Lond.* **246**, 313–314.
- DIXON, W. J. (1977). *BMDP-77 Biomedical Computer Programs P-Series*. Berkeley: University of California Press.
- EDWARDS, J. L. (1976). A comparative study of locomotion in terrestrial salamanders. Ph.D. dissertation, University of California, Berkeley.
- FEDER, M. E. (1976). Lunglessness, body size, and metabolic rate in salamanders. *Physiol. Zool.* **49**, 398–406.
- FEDER, M. E. (1977). Oxygen consumption and activity in salamanders: effect of body size and lunglessness. *J. exp. Zool.* **202**, 403–414.
- FEDER, M. E. (1978). Environmental variability and thermal acclimation in neotropical and temperate-zone salamanders. *Physiol. Zool.* **51**, 7–16.
- FEDER, M. E. (1985). Acclimation to constant and variable temperatures in plethodontid salamanders. I. Rates of oxygen consumption. *Comp. Biochem. Physiol.* **81A**, 673–682.
- FEDER, M. E. & BURGGREN, W. W. (1985). Cutaneous gas exchange in vertebrates: design, patterns, control, and implications. *Biol. Rev.* **60**, 1–45.
- FEDER, M. E., GIBBS, A. G., GRIFFITH, G. A. & TSUJI, J. (1984). Thermal acclimation of metabolism in salamanders: fact or artefact? *J. therm. Biol.* **9**, 255–260.
- FEDER, M. E. & LONDOS, P. L. (1984). Hydric constraints upon foraging in a terrestrial salamander, *Desmognathus ochrophaeus* (Amphibia: Plethodontidae). *Oecologia* **64**, 413–418.
- FEDER, M. E., LYNCH, J. F., SHAFFER, H. B. & WAKE, D. B. (1982). Field temperatures of neotropical and temperate zone salamanders. *Smithson. herpet. Inform. Serv. Publ.* **52**, 1–23.
- FITZPATRICK, L. C., BRISTOL, J. R. & STOKES, R. M. (1971). Thermal acclimation and metabolism in the Allegheny Mountain salamander, *Desmognathus ochrophaeus*. *Comp. Biochem. Physiol.* **40A**, 681–688.
- FREADMAN, M. A. (1981). Swimming energetics of striped bass (*Morone saxatilis*) and bluefish (*Pomatomus saltatrix*): gill ventilation and swimming metabolism. *J. exp. Biol.* **83**, 217–230.
- FULL, R. J. (1984). Energetics of invertebrate terrestrial locomotion: a comparison of metabolic responses in exercising decapod crustaceans. Ph.D. dissertation, State University of New York, Buffalo.
- FULL, R. J. (1985). Exercising without lungs: energetics and endurance in a lungless salamander, *Plethodon jordani*. *Physiologist* **28**, 342.

- GARLAND, T. (1982). Scaling maximal running speed and maximal aerobic speed to body mass in lizards and mammals. *Physiologist* **25**, 338.
- GARLAND, T. (1983). The relation between maximal running speed and body mass in terrestrial mammals. *J. Zool., Lond.* **99**, 157–170.
- GEHR, P., MWANGI, D. K., AMMAN, A., MALOIY, G. M. O., TAYLOR, C. R. & WEIBEL, E. R. (1981). Design of the mammalian respiratory system. V. Scaling morphometric pulmonary diffusing capacity to body mass: wild and domestic mammals. *Respir. Physiol.* **44**, 61–86.
- HILLMAN, S. S., SHOEMAKER, V. H., PUTNAM, R. & WITHERS, P. C. (1979). Reassessment of aerobic metabolism in amphibians during activity. *J. comp. Physiol.* **129**, 309–313.
- HIRANO, M. & ROME, L. C. (1984). Jumping performance of frogs (*Rana pipiens*) as a function of muscle temperature. *J. exp. Biol.* **108**, 429–439.
- MILLER, K. M. & ZOGHBY, G. M. (1983). Thermal acclimation of locomotor performance in anuran amphibians. *Am. Zool.* **23**, 916.
- PROSSER, C. L. & NELSON, D. O. (1981). The role of nervous systems in temperature adaptation of poikilotherms. *A. Rev. Physiol.* **43**, 281–300.
- PUTNAM, R. W. & BENNETT, A. F. (1981). Thermal dependence of behavioural performance of anuran amphibians. *Anim. Behav.* **29**, 502–509.
- RENAUD, J. M. & STEVENS, E. D. (1983). The extent of long-term temperature compensation for jumping distance in the frog, *Rana pipiens*, and the toad, *Bufo americanus*. *Can. J. Zool.* **61**, 1284–1287.
- RENAUD, J. M. & STEVENS, E. D. (1984). The extent of short-term and long-term compensation to temperature shown by frog and toad sartorius muscle. *J. exp. Biol.* **108**, 57–76.
- ROME, L. C. (1983). The effects of long-term exposure to different temperatures on the mechanical performance of frog muscle. *Physiol. Zool.* **56**, 33–40.
- ROME, L. C., LOUGHNA, P. T. & GOLDSPIK, G. (1985). Thermal acclimation: improved sustained swimming performance in carp at low temperatures. *Science* **228**, 194–196.
- SCHMIDT-NIELSEN, K. (1984). *Scaling: Why is Animal Size so Important?* Cambridge: Cambridge University Press.
- SEIGEL, S. (1956). *Nonparametric Statistics for the Behavioral Sciences*. New York: McGraw-Hill.
- SOKAL, R. R. & ROHLF, F. J. (1969). *Biometry*. San Francisco: W. H. Freeman.
- SPOTILA, J. R. (1972). Role of temperature and water in the ecology of lungless salamanders. *Ecol. Monogr.* **42**, 95–125.
- TAIGEN, T. L. (1983). Activity metabolism of anuran amphibians: implications for the origin of endothermy. *Am. Nat.* **121**, 94–109.
- TAIGEN, T. L. & BEUCHAT, C. A. (1984). Anaerobic threshold of anuran amphibians. *Physiol. Zool.* **57**, 641–647.
- TAYLOR, C. R., HEGLUND, N. C. & MALOIY, G. M. O. (1982). Energetics and mechanics of terrestrial locomotion. I. Metabolic energy consumption as a function of speed and body size in birds and mammals. *J. exp. Biol.* **97**, 1–21.
- TAYLOR, C. R., MALOIY, G. M. O., WEIBEL, E. R., LANGMAN, V. A., KAMAU, J. M. Z., SEEHERMAN, H. J. & HEGLUND, N. C. (1981). Design of the mammalian respiratory system. III. Scaling maximum aerobic capacity to body mass: wild and domestic mammals. *Respir. Physiol.* **44**, 25–38.
- TAYLOR, C. R., SCHMIDT-NIELSEN, K. & RAAB, J. L. (1970). Scaling of energetic cost of running to body size in mammals. *Am. J. Physiol.* **219**, 1104–1107.
- VLECK, D. (1978). The energetics of activity and growth. Ph.D. dissertation, University of California, Los Angeles.
- WEATHERS, W. W. (1976). Influence of temperature acclimation on oxygen consumption, haemodynamics and oxygen transport in bullfrogs. *Aust. J. Zool.* **24**, 321–330.
- WHITFORD, W. G. & HUTCHISON, V. H. (1967). Body size and metabolic rate in salamanders. *Physiol. Zool.* **40**, 127–133.