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# Scaling the duration of activity relative to body mass results in similar locomotor performance and metabolic costs in lizards

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## SUMMARY

**This study examines the physiological response to locomotion in lizards following bouts of activity scaled to body mass. We evaluate this method as a way to compare locomotor energetics among animals of varying body mass. Because most of the costs of brief activity in reptiles are repaid during recovery we focus on the magnitude and duration of the excess post-exercise oxygen consumption (EPOC). Lizards ranging from 3 g to 2400 g were run on a treadmill for durations determined by scaling the run time of each animal to the 1/4 power of body mass and allowing each animal to run at its maximum speed for that duration. This protocol resulted in each species traveling the same number of body lengths and incurring similar factorial increases in $\dot{V}_{O_2}$. Following activity, EPOC volume (ml $O_2$) and the cost of activity per body length traveled (ml $O_2$ per body length) scaled linearly with body mass. This study shows that the mass-specific costs of activity over an equivalent number of body lengths are similar across a broad range of body mass and does not show the typical patterns of allometric scaling seen when cost of locomotion are expressed on a per meter basis. Under field conditions larger animals are likely to travel greater absolute distances in a given bout of activity than smaller animals but may travel a similar number of body lengths. This study suggests that if locomotor costs are measured on a relative scale (ml $O_2$ per body length traveled), which reflects these differences in daily movement distances, that locomotor efficiency is similar across a wide range of body mass.**

Key words: energetics, lactate, locomotion, scaling.

## INTRODUCTION

Numerous biomechanical, biochemical and behavioral aspects of locomotion are significantly related to body mass. Therefore it is important to take body mass into account when asking questions about the energetic costs and locomotor patterns of animals, particularly when trying to compare animals of widely varying size. An important consideration in such studies is whether to compare animals in absolute or relative terms (Jones and Lindstedt, 1993; Van Damme and Van Dooren, 1999; Iriarte-Diaz, 2002). Comparing animals on the same absolute scale by holding speed, duration of activity or total distance constant all pose problems when asking questions relevant to the ecology and daily field energetics of each species examined. Home ranges, daily movement distances and maximum running distance have all been shown to increase with an animal's body mass in a variety of species (Garland, 1983a; Garland, 1993; Lindstedt et al., 1986; Perry and Garland, 2002) indicating that larger animals move greater distances to accomplish the same daily activities as smaller animals. Because of this it does not always make sense to compare the locomotor costs of traveling a fixed distance between animals of different size. Similarly, running speed (Garland, 1983b), endurance (Garland, 1994) and the speed at which the maximal rate of oxygen consumption ($\dot{V}_{O_2,max}$) is reached (Taylor et al., 1981) have been shown to scale positively with body mass. Because of these allometric relationships, holding the duration of activity or speed constant would result in animals traveling at different multiples of their maximum endurance time or maximum speed and at different multiples of their $\dot{V}_{O_2,max}$.

An alternative is to adjust activity patterns such that each animal is compared on the same relative scale. To accomplish this, locomotor performance is often compared at a common gait (Heglund and Taylor, 1988; Drucker, 1996), relative to the number of body lengths traveled (Bainbridge, 1958; Videler and Wardle, 1991), or at the same percentage of each animal's maximum aerobic speed (Weibel et al., 2004). These methods allow for comparisons that can be thought of as behaviorally or physiologically equivalent across a range of body masses. The importance of examining activity scaled to animal size is supported by Van Damme and Van Dooren (Van Damme and Van Dooren, 1999) who showed that relative speed (in body lengths per second) may be more important to predator avoidance than absolute speed. This idea also applies to studies of daily energetics and overall daily movement. Garland (Garland, 1984) showed that for *Ctenosaura similis* endurance scales in a similar fashion to maximum running distance (0.270 and 0.265 power of body mass, respectively) suggesting that, relative to how far lizards move, endurance is similar at different body masses.

Measuring locomotor energetics relative to home range, daily movement distance, or some other parameter that is relevant to each species' natural behavior would be an effective way to compare locomotor costs and gain insight into the actual energetic demands faced in the field. However, the difficulties involved in doing detailed metabolic measurements with even a moderate number of species makes collecting this kind of additional field behavior data prohibitive. In this study we test the use of an easily measured parameter, body mass, as a way to scale the duration of activity in order to compare locomotor energetics in lizards. By scaling run times to the 1/4 power of body mass this study attempted to establish a comparable activity pattern across an 800-fold range in mass. Based on the scaling of stride frequency to body mass and length (Heglund and Taylor, 1988; Marsh and Bennett, 1985; Irschick and Jayne, 1999) we predicted that scaling run times in this manner

would result in animals traveling a similar number of body lengths. Measurements of the cost of activity ($C_{act}$), which is the total metabolic cost incurred by activity during both the activity and recovery periods (Baker and Gleeson, 1998), and associated locomotor parameters were used to evaluate the effectiveness of scaling locomotion in this fashion. Although other aspects of resting and activity metabolism have been examined in relation to body mass, we are unaware of any data on how $C_{act}$ might vary with body mass. This study presents data on this relationship in lizards.

We also used this approach to test the hypothesis that the metabolic strategy for clearing post-activity lactate should depend on body mass in lizards. Following exhaustive exercise, lactate is elevated to a similar concentration across lizards of different mass. However, reptiles appear to remove lactate at a slower rate as they get larger (Moberly, 1968; Bennett and Licht, 1972; Coulson, 1980; Gleeson, 1980; Gleeson, 1982; Gleeson and Bennett, 1982; Hailey et al., 1987; Gleeson and Dalessio, 1989). In addition, activities of key glycolytic and oxidative enzymes in muscles have been shown to vary with body mass, based on studies in both mammals and fish (Emmett and Hochachka, 1981; Somero and Childress, 1990; Norton et al., 2000; Davies and Moyes, 2007), further suggesting that rates of lactate metabolism might be sensitive to body mass. Mass-specific resting rates of oxidative metabolism decrease as body mass increases suggesting that less oxidative fuel would be needed in larger animals per gram of body mass. Based on the patterns in enzyme activities and oxidative metabolism we predicted that the fate of lactate would be mass sensitive, with a smaller proportion of the post-exercise lactate load being oxidized as body mass increased and a greater proportion being converted to glycogen.

## MATERIALS AND METHODS

### Animal care

Animals were obtained from a variety of sources. *Uta stansburiana* Baird and Girard 1852 and *Sceloporus occidentalis* Baird and Girard 1852 were collected from field study sites in Oregon by Dr Peter Zani, Lafayette College. *Dipsosaurus dorsalis* Baird and Girard 1852 were collected near Cathedral City, CA, USA on permit from the California Department of Fish and Game. *Ctenosaura similis* Gray 1831 and *Iguana iguana* Linnaeus 1758 were purchased from commercial dealers. All animals were kept under a 12 h:12 h light:dark cycle, fed three times per week on a diet appropriate for each species and had constant access to water. All procedures described here were approved by the Institutional Animal Care and Use Committee of the University of Colorado.

### Metabolic gas analysis

During all procedures involving gas analysis, exhaled breath was analyzed for $CO_2$ and $O_2$ content using an Anarad AR-411 $CO_2$ analyzer and an Applied Electrochemistry S3A $O_2$ analyzer. All gases were passed through Drierite to remove water vapor before analysis. Gas analysis was run in 20 min intervals with the first 2 min of each interval used for calibration. Respiratory gases from smaller animals (*U. stansburiana*, *S. occidentalis*) were sampled using an open-flow chamber. Larger animals (*D. dorsalis*, *C. similis*, *I. iguana*) were fitted with masks covering the mouth and nares. Air flow through the mask or chamber was at a rate that ensured all exhaled gases were drawn through the analyzers (flow rates: *U. stansburiana*, 0.15 l min$^{-1}$; *S. occidentalis*, 0.2 l min$^{-1}$; *D. dorsalis*, 0.4 l min$^{-1}$; *C. similis*, 2.5 l min$^{-1}$; *I. iguana*, 4.0 l min$^{-1}$). To minimize washout and mixing effects the chambers used for the smaller species were fitted to the animal's body size to reduce movement and ensure that the head remained inside a close fitting cone attached to the outgoing air tube. Larger animals were also placed inside appropriately sized containers to similarly restrict movement during recovery. For the larger lizards, masks were removed before running and replaced immediately following activity.

### Trial 1: recovery from scaled activity

All animals were fasted for 3 days prior to trials. On the day before the trial, animals were weighed and their snout–vent length was measured then placed in individual cages overnight. On the morning of the trial they were moved to a temperature-controlled cabinet until they reached their preferred body temperature (35°C for all animals except *D. dorsalis*, which was at 40°C) (Norris, 1953; McGinnis, 1966; Waldschmidt and Tracy, 1983; Garland, 1984; van Marken Lichtenbelt et al., 1997). Cloacal body temperature was checked using a Yellow Springs Instruments thermistor. Animals were kept at their preferred temperature for the entire trial. After reaching a stable body temperature animals were fitted with a mask or placed in an open-flow chamber and then placed back in the temperature-controlled cabinet for 3 h to determine their resting metabolic rate (RMR). The lowest 15 min average $\dot{V}_{O_2}$ (ml $O_2$ h$^{-1}$) over this span was used to determine RMR.

Following measurements of RMR animals were placed on the treadmill surface and immediately induced to run at their maximum speed for a duration determined by the following equation:

$$\text{Run time} = 4.94 \times \text{mass}^{0.25}, \quad (1)$$

where run time is in seconds and mass in grams.

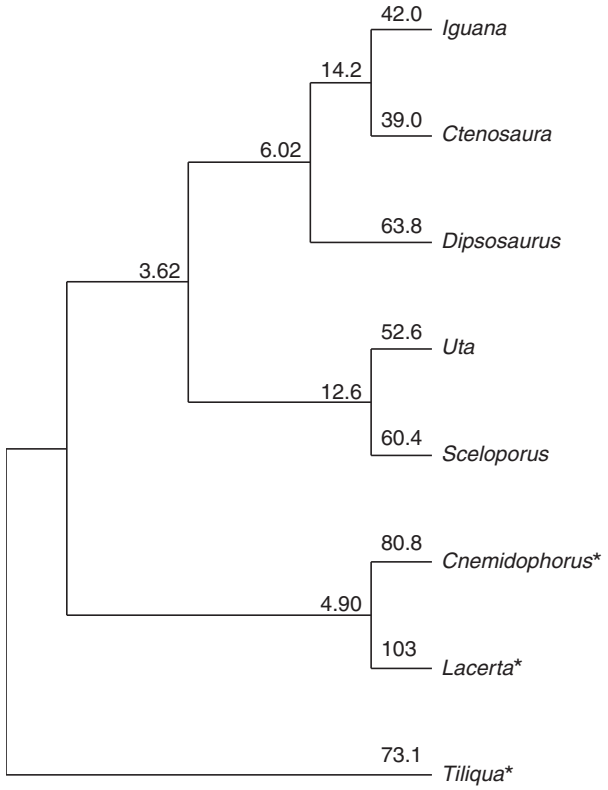


Fig. 1. Phlyogenetic relationships among lizard species. Numbers above branches indicate branch lengths in units of total character difference using the cytochrome *b* gene sequence. See Materials and methods for details on phylogeny construction. An asterisk indicates species that were included as outgroups and not measured.

Although there is still significant debate over exactly how metabolic parameters scale with body mass (White and Seymour, 2003; Savage et al., 2004), mass-specific resting metabolic rates in reptiles do appear to scale to the 1/4 power of body mass (White et al., 2006). This scaling value was also chosen based on the frequency with which it has been found to be associated with the allometric scaling of animal locomotion (Lindstedt, and Calder, 1981; Huey and Hertz, 1982; Garland, 1983a; Garland, 1984; Heglund and Taylor, 1988). The constant (4.94) was set so that the middle species in the body mass range, *D. dorsalis*, would run for 15 s, the longest interval that *D. dorsalis* has been observed to run in the field (Hancock et al., 2001). The run time of this species was chosen to determine the scaling constant as it was the only one for which data on voluntary locomotory duration were available. Animals were induced to run by tapping the base of the tail. Only trials where an animal ran consistently for the entire time period were used. The width of the treadmill surface was adjusted for each species so that each animal was forced to run in a straight line with minimal side-to-side motion. Treadmill speed was continuously adjusted to match running speed, and distance was determined by integrating treadmill speed over the run.

At the end of the run, animals were masked or put back in respiratory chambers, placed inside the temperature-controlled chamber and allowed to recover undisturbed for 2 h. Following the run it took approximately 5 s to mask an animal or place it into its respiratory chamber. $\dot{V}_{O_2,peak}$ (ml $O_2$ $h^{-1}$) was calculated as the highest 10 s average oxygen consumption rate immediately following activity. During recovery the excess post-exercise oxygen consumption (EPOC) (Gaesser and Brooks, 1984), which is the total volume of oxygen consumed in excess of the animal's RMR, was measured and used to determine the cost of activity ($C_{act}$) (Baker and Gleeson, 1998). $C_{act}$ is defined here as the total EPOC during recovery per unit distance traveled. We used oxygen consumption during just the recovery period to represent $C_{act}$ because lizards breathe irregularly while running (Wang et al., 1997) and oxygen consumption during 15 s sprints in *D. dorsalis* accounts for only 2% of the metabolic costs incurred by activity (Donovan and Gleeson, 2006).

The duration of the EPOC period was determined by how long it took each animal's $\dot{V}_{O_2}$ to fall to 1.5× their RMR (Baker and Gleeson, 1999; Hancock and Gleeson, 2002). Each animal was run twice and the results were averaged within each species. Trials for individual animals were separated by at least 5 days.

### Trial 2: profile of post-exercise lactate metabolism

A second round of trials was conducted to determine the metabolic fate of lactate during recovery. The animal handling and exercise protocols were the same as above except for the following changes. After reaching a resting state, but prior to running, each animal was injected intra-peritoneally with 0.0125 μCi $g^{-1}$ of U-$^{14}$C-lactate (ICN Biomedicals, Irvine, CA, USA). To account for the increase in circulation time as body mass increases (Holt et al., 1968; Prothero, 1980) each animal was allowed to rest following the injection for a time scaled to its body mass:

$$\text{Equilibration time} = 6.47 \times \text{body mass}^{0.25}, \quad (2)$$

where equilibration time is in minutes and body mass in grams.

The constant in this equation was chosen to set the equilibration time for *D. dorsalis* to 20 min, which has been shown to be sufficient time for injected lactate to reach a steady state of oxidation (Donovan and Gleeson, 2006). At the end of the equilibration time each animal was run in the same fashion as described above and then allowed to recover for a duration equal to twice the EPOC period determined from the first trial.

During the recovery period exhaled $CO_2$ was collected using 15 ml $CO_2$ traps consisting of ethanolamine and methycellusolve mixed in a 1:3 ratio (Brooks et al., 1973). Traps were changed every 10 min and analyzed for $^{14}CO_2$ content as described by Donovan and Gleeson (Donovan and Gleeson, 2006). At the end of the recovery period muscle samples were taken. For samples of *D. dorsalis*, *S. occidentalis* and *U. stansburiana*, animals were decapitated into liquid nitrogen and hind legs were clamped between aluminum blocks cooled in liquid nitrogen, cut off and submerged in liquid nitrogen. The gastrocnemius was then removed from the frozen legs. For *I. iguana* and *C. similis*, because of the limited availability of these larger species, biopsies from the gastrocnemius were taken to allow for a second sample from the other leg, if needed. After cleaning the area of skin over the gastrocnemius with iodine, Lidocaine (20 mg $ml^{-1}$) was injected to anaesthetize the area. A 1 cm incision was made and a strip of muscle (approximately 200 mg) was cut away and frozen in liquid nitrogen. The site of the incision was sutured and sealed with a liquid wound sealant (Liquid Bandage, Johnson and Johnson). For one *I. iguana* a second trial was needed because the animal refused to run during the first trial. The second trial was conducted 1 month following the first to allow for complete healing from the initial biopsy and for exhaled $^{14}CO_2$

Table 1. Allometric parameters for activity and recovery metabolism

| | Slope *vs* mass | Intercept | *P* | *F* | 95% CI for slope |
|---|---|---|---|---|---|
| Trial 1 | | | | | |
| log RMR (ml $O_2$ $h^{-1}$) | 0.71 | –0.16 | 0.006 | 227.75 | 0.56, 0.86 |
| log distance (m) | 0.35 | 0.68 | 0.001 | 284.11 | 0.28, 0.42 |
| Distance (BL) | 8.14 | 151.74 | 0.39 | 0.98 | –18.04, 34.32 |
| log recovery time (min) | 0.33 | 0.91 | 0.002 | 123.83 | 0.24, 0.43 |
| log EPOC (ml $O_2$) | 1.09 | –0.83 | 0.001 | 171.33 | 0.83, 1.36 |
| log $C_{act}$ (ml $O_2$ $m^{-1}$) | 0.74 | –1.50 | 0.002 | 96.87 | 0.50, 0.98 |
| log $C_{act}$ (ml $O_2$ $BL^{-1}$) | 1.07 | –3.0 | 0.001 | 180.70 | 0.82, 1.32 |
| log $\dot{V}_{O_2,peak}$ (ml $O_2$ $h^{-1}$) | 0.74 | 0.72 | 0.0001 | 1927.83 | 0.69, 0.80 |
| $\dot{V}_{O_2,peak}$ $RMR^{-1}$ (ml $O_2$ $h^{-1}$) | 0.56 | 7.84 | 0.44 | 0.78 | –1.45, 2.56 |
| Trial 2 | | | | | |
| log lactate oxidized during recovery (% of total injected) | 0.10 | –1.10 | 0.15 | 3.69 | –0.07, 0.28 |
| log lactate converted to glycogen during recovery (% $g^{-1}$ muscle tissue) | –0.77 | –1.33 | 0.03 | 15.47 | –1.39, –0.15 |

RMR, resting metabolic rate; BL, body length; EPOC, excess post-exercise oxygen consumption; CI, confidence interval; $C_{act}$, cost of activity; $\dot{V}_{O_2,peak}$, peak rate of oxygen consumption.

Table 2. Animal size, activity traits and metabolic results

| | *U. stansburiana* | *S. occidentalis* | *D. dorsalis* | *C. similis* | *I. iguana* |
|---|---|---|---|---|---|
| Trial 1 | | | | | |
| *N* | 3 | 3 | 3 | 3 | 3 |
| Body mass (g) | 3.76 (1.18) | 19.77 (1.99) | 80.99 (10.53) | 690.16 (186.52) | 2149.83 (480.15) |
| Body length (mm) | 50.60 (4.26) | 83.00(3.00) | 131.22 (8.46) | 279.5 (28.64) | 396.67 (28.87) |
| Run time (s) | 7.2 (0.8) | 10.4 (0.6) | 15.0 (0.7) | 25.0 (2.0) | 33.3 (1.7) |
| Distance (m) | 7.27 (0.89) | 13.86 (2.62) | 23.61 (3.57) | 51.97 (8.41) | 64.97 (9.06) |
| Distance (BL) | 143.62 (12.16) | 166.45 (26.46) | 179.98 (24.82) | 185.35 (15.30) | 164.31 (26.14) |
| RMR ($ml\,O_2\,h^{-1}$) | 1.89 (0.14) | 4.88 (0.79) | 12.25 (1.43) | 72.11 (10.51) | 189.79 (53.63) |
| EPOC recovery time (min) | 11.67 (3.02) | 22.79 (2.82) | 29.49 (4.29) | 80.13 (7.74) | 96.95 (2.55) |
| EPOC ($ml\,O_2$) | 0.68 (0.32) | 2.48 (0.45) | 12.48 (3.08) | 230.76 (50.58) | 698.64 (153.35) |
| $C_{act}$ ($ml\,O_2\,m^{-1}$) | 0.10 (0.03) | 0.20 (0.02) | 0.57 (0.11) | 4.34 (0.48) | 10.85 (2.44) |
| $C_{act}$ ($ml\,O_2$ body $length^{-1}$) | 0.005 (0.002) | 0.016 (0.002) | 0.077 (0.02) | 1.21 (0.23) | 4.35 (1.29) |
| $\dot{V}_{O_2,peak}$ ($ml\,O_2\,h^{-1}$) | 12.84 (4.94) | 44.57 (12.13) | 122.85 (35.58) | 640.02 (165.50) | 1645.31 (135.70) |
| $\dot{V}_{O_2,peak}$ $RMR^{-1}$ ($ml\,O_2\,h^{-1}$) | 6.92 (3.13) | 9.38 (3.21) | 10.43 (3.66) | 8.87 (1.21) | 9.11 (2.38) |
| Trial 2 | | | | | |
| *N* | 2 | 2 | 3 | 2 | 2 |
| Body mass (g) | 3.00 (0.85) | 22.62 (0.54) | 91.20 (14.04) | 850.40 (160.22) | 2650.50 (180.31) |
| Lactate oxidized during recovery (% of total injected) | 9.78% (3.79) | 8.07% (2.19) | 15.88% (8.33) | 13.62% (1.72) | 20.77% (11.65) |
| Lactate converted to glycogen during recovery (% $g^{-1}$ muscle tissue) | 0.81% (0.64) | 0.77% (0.13) | 0.42% (0.10) | 0.022% (0.01) | 0.0058% (0.003) |

Numbers in parentheses are standard deviations.
RMR, resting metabolic rate; BL, body length; EPOC, excess post-exercise oxygen consumption; $C_{act}$, cost of activity; $\dot{V}_{O_2,peak}$, peak rate of oxygen consumption.

to return to background levels (Donovan and Gleeson, 2006). The second sample was taken from the other leg, not from the same site as the first sample. All muscle samples were kept at –70°C until analyzed for [$^{14}$C]glycogen content to determine conversion of lactate into glycogen, as described previously (Donovan and Gleeson, 2006).

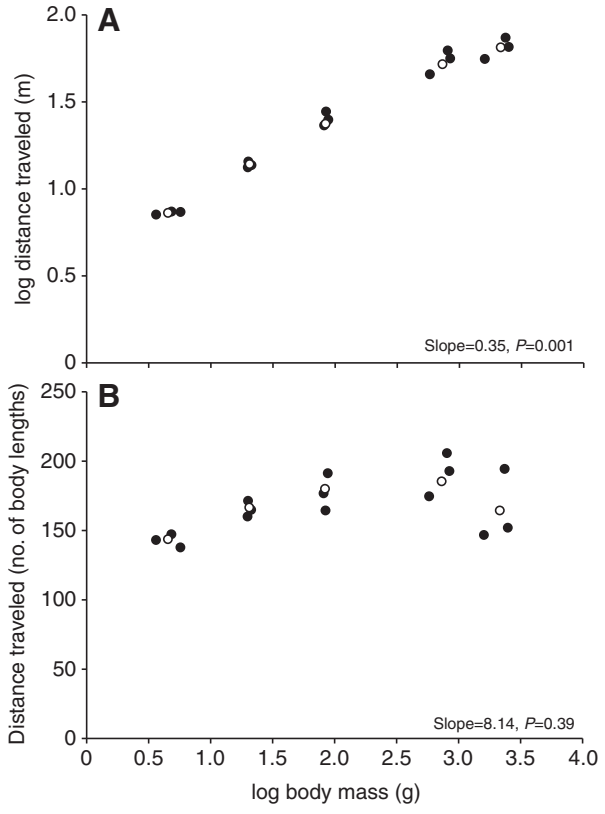


Fig. 2. Distance traveled in (A) meters and (B) body lengths (BL) as a function of body mass. Open circles are species means, filled circles are individual values.

### Statistical analysis

Metabolic traits frequently show an increase in variance with increases in body mass. Therefore, all measured variables were checked for uniform variances by regressing the within-species variances for a given variable on the mean value for each species. Traits that showed a significant increase in variance with increasing means were log transformed and checked again to verify uniform variance.

The mean trait values for each species, transformed when appropriate, were used to generate phylogenetically independent contrasts (PIC) which correct for the non-independence due to the phylogenetic relationships between species (Felsenstein, 1985; Harvey and Pagel, 1991). The phylogeny for this analysis was produced from sequences for the highly conserved mitochondrial cytochrome b gene (Graybeal, 1993; Jonhs and Avise, 1998; Bradley and Baker, 2001) obtained from GenBank (Petren and Case, 1997; Radtkey et al., 1997; Paulo et al., 2001; Whiting et al., 2003; Hodges and Zamudio, 2004; Kumazawa and Nishida, 1995) and aligned using ClustalX v.1.81 software (Thompson et al., 1997). PAUP v.4.0b10 was used to construct a rooted phylogeny, using a minimum evolution model, and to determine branch lengths in units of total character differences between each species (Fig. 1). In addition to the five species mentioned above, sequences for *Cnemidophorus tigris*, *Tiliqua gigas* and *Lacerta agilis* were also included as outgroups to root the tree. Physiological measurements were not made for the three outgroup species. Mesquite v.1.06 (Maddison and Maddison, 2004) and the PDTREE package v.1.07 (Midford et al., 2005) were used to calculate the PIC values for each node in Fig. 1 (Garland et al., 1999; Garland and Ives, 2000).

The standardized independent contrasts for each variable were regressed on the standardized contrasts values for mass. Visual assessment of all plots following transformation indicated that a linear regression was appropriate to determine if a significant allometric trend was present in the data and all variables were

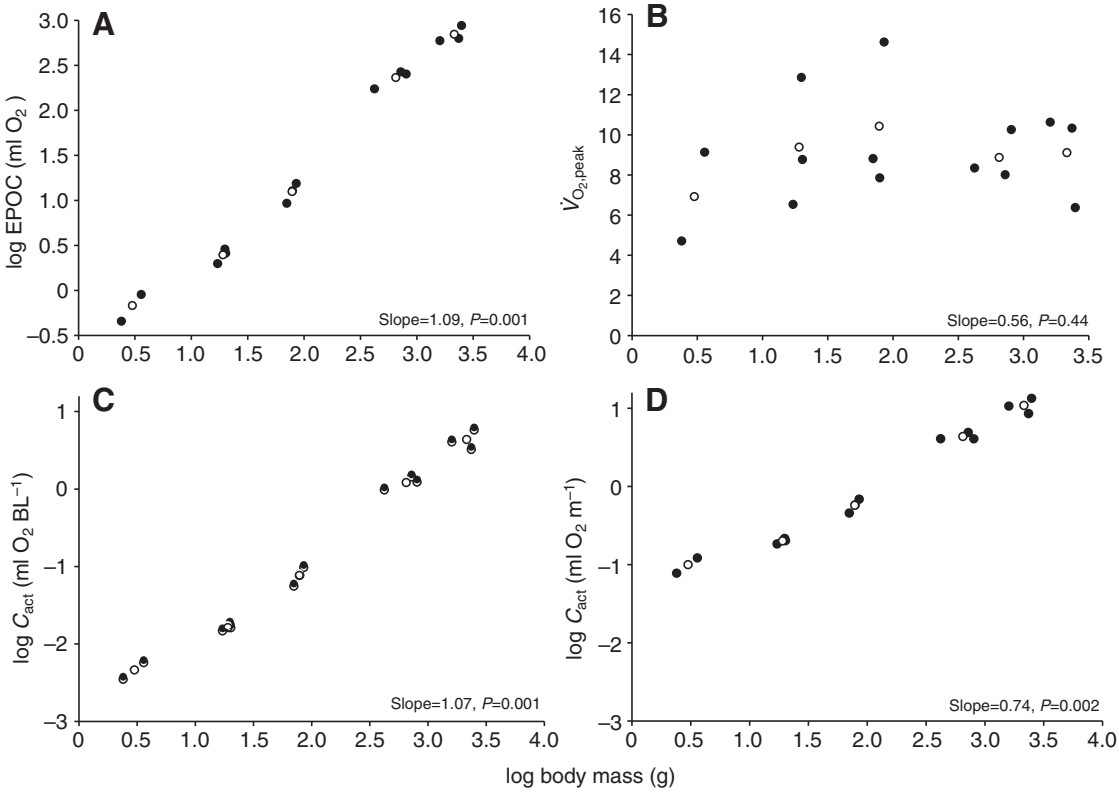


Fig. 3. Oxygen consumption during recovery and the cost of activity ($C_{act}$) as a function of body mass. (A) Excess post-exercise oxygen consumption (EPOC); (B) factorial increase in metabolic rate expressed as the ratio of $\dot{V}_{O_2,peak}$ following activity and the resting metabolic rate (RMR); (C) $C_{act}$ relative to the number of body lengths (BL) traveled; (D) $C_{act}$ relative to the number of meters traveled. Open circles are species means, filled circles are individual values.

analyzed using least squares regression. All of the statistical results (Table 1) are based on these phylogenetically independent contrasts (PIC). Figures show the variables in their original units (log transformed when appropriate), not those of the PIC values. Figures also show the trait values of individuals within each species to illustrate the within-species variance.

## RESULTS

The phylogenetic relationships between the species used in this study have been established by previous studies (Petren and Case, 1997; Wiens and Hollingsworth, 2000; Townsend et al., 2004) and the phylogeny generated for this study agrees with the evolutionary relationships shown by these previous studies. However, we chose to construct our own phylogeny in order to generate a consistent set of branch length values for use in our analysis. The appropriateness of the branch lengths of the phylogeny used in this analysis were checked by regressing the absolute values of the standardized PIC values on their standard deviations (Garland et al., 1992). Results for this diagnostic showed no significant relationships for any of the physiological variables (all $P$-values >0.37), suggesting that the branch length assumptions are adequate.

Table 2 summarizes the size, activity patterns and metabolic variables measured for each species. Although the absolute distance in meters traveled by each species did increase with body mass (Fig. 2A; $P$=0.001) the relative distance in total body lengths did not vary with body mass (Fig. 2B; $P$=0.39). The scaling of run times to the 1/4 power of body mass resulted in each species running an average of 168±11 body lengths. RMR ($ml\,O_2\,h^{-1}$) scaled significantly with body and showed an allometric slope of 0.71. Both the total EPOC (Fig. 3A; $P$=0.001) and the $C_{act}$ per body length traveled (Fig. 3C; $P$=0.001) scaled linearly with body mass. $\dot{V}_{O_2,peak}$ ($P$=0.0001) and $C_{act}$ per meter traveled (Fig. 3D; $P$=0.002) were both significantly dependent on body mass and showed a negative allometric trend with slope significantly less than 1. The ratio of $\dot{V}_{O_2,peak}$ to RMR was calculated to determine the factorial increase in $\dot{V}_{O_2}$ following activity (Fig. 3B). This ratio had a mean of 8.94±0.56 and did not vary with body mass ($P$=0.44).

Data for the removal of lactate are presented as the percentage of the total injected $^{14}C$ from lactate appearing in either the exhaled $CO_2$ or the muscle glycogen pools during the recovery period (Table 2). Data are expressed per whole animal and per gram of muscle mass, respectively. Whole-body oxidation of lactate (Fig. 4A) during the recovery period was not significantly related to body mass ($P$=0.15). Glycogen synthesis per gram of muscle (Fig. 4B) showed a significant negative correlation with body mass ($P$=0.03).

## DISCUSSION

### Locomotor performance and intensity following scaled activity

The scale on which activity is measured is a major concern when trying to compare energetic costs between animals of different size. In terms of daily activity a 5 g *U. stansburiana* moving 10 m covers a much greater portion of its total home range than a 2.5 kg *I. iguana* moving the same distance (Perry and Garland, 2002). In addition, the total number of limb cycles and muscle contractions would vary widely. Using estimates for the scaling of stride length with body size (Heglund and Taylor, 1988; Marsh and Bennett, 1985; Irschick and Jayne, 1999) a *U. stansburiana* would need 69 strides to cover

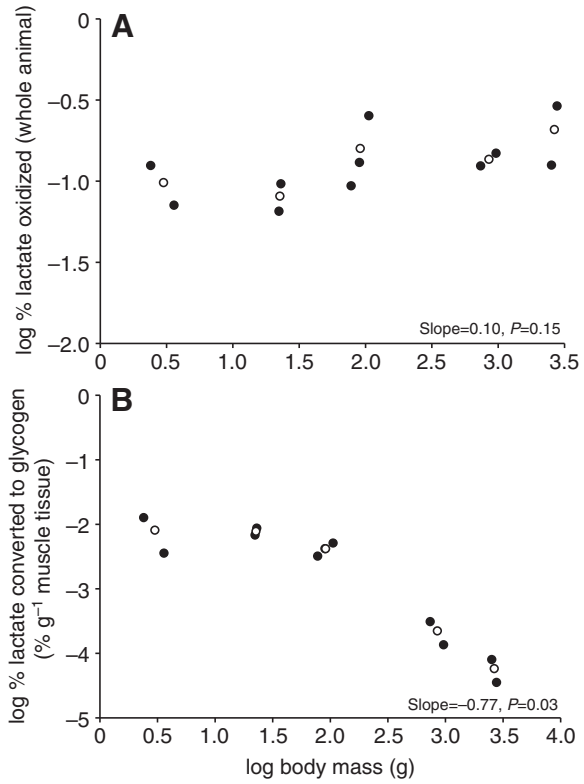


Fig. 4. Metabolic fate of lactate. (A) Oxidation of lactate expressed as a percentage of the total amount of $^{14}C$ that was injected that appeared in exhaled $CO_2$ during the recovery period; (B) conversion of lactate to glycogen expressed as a percentage of the total amount of $^{14}C$ that was injected that appeared in glycogen during the recovery period per gram of gastrocnemius muscle. Open circles are species means, filled circles are individual values.

10 meters whereas *I. iguana* would need only seven strides. In this study each animal ran for an approximately equivalent number of body lengths (Fig. 2B). In addition, from the estimates of the scaling of stride length to body mass cited above, each animal used roughly the same number of strides during activity. This supports the use of our method of scaling run times to body mass as a way to compare relatively equivalent behaviors between animals covering a wide mass range. This method allows for comparisons of distances that probably correlate more closely to the actual distances they might cover in their daily movements than would runs of the same absolute distance.

Another consideration for studies of different body mass is how to evaluate comparisons of the intensity of their metabolic effort. During steady-state locomotion in mammals this comparison is frequently based on $\dot{V}_{O_2,max}$, and activities are often considered metabolically comparable if each animal performs at the same percentage of this value. Reptiles are generally only able to sustain low levels of steady-state activity and $\dot{V}_{O_2,max}$ is achieved at speeds much lower than those reached during typical running behavior (Bennett, 1978). However, $\dot{V}_{O_2}$ dramatically increases soon after the end of vigorous activity (Scholnick and Gleeson, 2000; Hancock et al., 2001; Donovan and Gleeson, 2006) and the current study uses $\dot{V}_{O_2,peak}$ following each run instead of $\dot{V}_{O_2,max}$ to compare the relative intensity of metabolic expenditure by each animal. The model of activity used here resulted in the same factorial-increase in $\dot{V}_{O_2}$ across the range of body mass measured (Fig. 3B) which indicates a similar amount of metabolic effort by each species.

### Cost of activity

In general, costs of locomotion increase with body mass but with a slope of less than 1 when measured on a per meter or per hour basis (Taylor et al., 1982; Garland, 1983b; Walton et al., 1990; Rome, 1992) showing that on a mass-specific scale, larger animals have lower costs of locomotion than smaller animals. However, for reasons discussed previously, comparing activity over a fixed time or distance is very different from comparisons among animals that have traveled a similar number of body lengths. Comparisons of costs of activity per meter or per hour may not be as relevant when trying to compare the daily energetics of the animals of varying body mass.

Our data show that when traveling the same number of body lengths locomotor costs scale isometrically and that larger lizards did not have a lower mass-specific cost of activity, per body length traveled, than smaller lizards (Fig. 3C). The fact that $C_{act}$ scales linearly with body mass under these conditions suggests that mass-specific locomotor costs, relative to the actual distances traveled by an animal during normal daily locomotion may be similar across a wide range of body mass. These findings are similar to previous work showing that when mammals are run at equivalent gaits, the cost of locomotion per stride is independent of body mass (Heglund and Taylor, 1988). Heglund and Taylor (Heglund and Taylor, 1988) showed that the increase in mass-specific metabolic rate seen as body mass decreases is due to the greater stride frequency in smaller animals.

### Post-activity lactate metabolism

Lizards generate large amounts of lactate during vigorous activity. In *D. dorsalis* the conversion of lactate into glycogen is the major metabolic cost during recovery while oxidation of lactate provides most of the ATP required to fuel recovery costs (Gleeson and Dalessio, 1989; Donovan and Gleeson, 2006; Hancock and Gleeson, 2008). We hypothesized that the mass-specific metabolic fate of lactate between glycogen synthesis and oxidation would shift with body mass. Use of lactate as an oxidative substrate to pay recovery costs was predicted to occur to a greater degree in smaller species because of the higher mass-specific metabolic rate, and therefore greater rate of cellular ATP use. Conversely, we hypothesized that the ATP-consuming process of converting lactate to glycogen would be preferred in larger species. Although this strategy would increase the short-term metabolic recovery costs for larger animals, the immediate oxidation of lactate could provide more ATP than would be needed by larger animals with lower mass specific metabolic rates. Converting the lactate back to glycogen would preserve much of the chemical energy in the lactate pool for later use.

Data presented here provide mixed support for this hypothesis. Across all species whole-animal lactate oxidation did not show a clear allometric trend and the data indicate that each species is oxidizing a similar percentage of its total lactate load during recovery. This suggests that mass-specific oxidation of lactate would scale with an exponent close to –1 and decrease to a greater degree with increasing body mass that does the conversion of lactate to glycogen (slope=–0.77). This trend supports our hypothesis of an increase in the relative mass-specific fate of lactate metabolism towards conversion to glycogen. However, the confidence intervals around these slopes and the variation in the percentage values (Table 1 and Table 2) make it difficult to draw precise conclusions on the allometric scaling of lactate metabolism and suggest that the metabolic fate of lactate may be only weakly linked to body mass.

Although the fate of lactate following activity may not be heavily determined by body mass, clearing lactate may still influence an animal's locomotor patterns. Following maximal activity, lactate levels rise to a similar concentration in reptiles regardless of body

mass and remain elevated for a prolonged period (Moberly, 1968; Bennett and Licht, 1972; Coulson, 1980; Gleeson, 1980; Gleeson, 1982; Gleeson and Bennett, 1982; Hailey et al., 1987; Gleeson and Dalessio, 1989). This results in a significant drop in plasma and tissue pH (Bennett, 1973; Gleeson and Bennett, 1982; Wagner et al., 1999). Even though the relative fate of lactate may be similar in large and small lizards, the time to clear the accumulated lactate is much greater in larger animals. As a result, larger lizards might be inclined to move less frequently, travel shorter relative distances (i.e. fewer body lengths) or run at slower relative speeds during daily activities to avoid this prolonged recovery and acidosis.

The activity model presented here provides support for using body mass as a scaling parameter when comparing activity and metabolism between animals of different body mass. These results have important implications for the ecological energetics of naturally occurring field behaviors. When lizards across a wide range of body mass are compared over relatively equivalent distances, such as the same number of body lengths traveled, the metabolic consequences do not show the negative allometric scaling patterns seen when examining locomotion over the same absolute distances. This suggest that if animals are compared over distances typical of natural daily activities that locomotor efficiency is similar across a wide range of body mass.

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