Original article

Metabolism of the spade-headed Amphisbaenian worm lizard, *Diplometopon zarudnyi* (Nikolsky, 1907), in Saudi Arabia (Reptilia: Trogonophidae)

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A B S T R A C T

The oxygen consumption rate $\dot{V}O2$ and lactate production of the Amphisbaenian worm lizard *Diplometopon zarudnyi* were measured at temperatures ranging from 15 °C to 35 °C at 5 °C intervals. The $\dot{V}O2$ was significantly different between resting and active states at any specified temperature, while the average value at the resting state generally rose with increased temperature from 15 °C (0.05 ml O_2/g/h) to 25 °C (0.111 ml O_2/g/h). The aerobic respiration scopes at resting and active states were also significantly different. The highest Q10 values (3.24 and 1.69) were obtained at 15 °C–20 °C and 30 °C–35 °C during resting and active states, respectively, with these values being significantly different. Lactate concentrations were significantly higher during active states than when resting, and the anaerobic scope was found to increase with increased temperature. There was a proportional increase in ATP molecules (μmoles/g/2 min) during aerobic or anaerobic respiration, as well as in total metabolic scope, with increasing temperature, and the anaerobic scope showed significantly higher values than the aerobic scope, confirming the importance of anaerobic behavior for this species.

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1. Introduction

In most reptilian species, an increase in the surrounding temperature increases the body temperature, which in turn increases the oxygen consumption (Al-Farraj, 1993; Al-Shammeri, 2007). As with endotherms, who internally regulate their body temperature using the heat by-product of their metabolic processes, ectotherms must sustain exercise by generating adenosine-5-triphosphate (Randall et al., 2002). In reptiles, especially lizards, at temperatures less than the preferred body temperature (PBT), oxygen consumption rates when they are active exceed those when they are resting, with oxygen consumption reaching the highest level at the PBT (Bennett and Dawson, 1976; Al-Sadoon, 1986b; Al-Farraj, 1993; Al-Sadoon et al., 2014). The total metabolic scope (aerobic and anaerobic) is therefore highest at PBT (Al-Sadoon, 1986a,b). Previous studies have shown that the PBT ranges from 16.6 °C in *Goniurosaurus kuroiwae* (Werner et al., 2005) to 37.9 °C in *Sauromalus ater* (Brattstrom, 1965).

Intense activity in ectotherms is primarily supported anaerobically. This is due to the limits of their aerobic scope. In vertebrates, however, the literature on anaerobic scope is not rich due to the practical difficulties in measuring the rate of anaerobic energy production (Seymour, 2013). While the aerobic energy of exercising animals can be determined easily by measuring $\dot{V}O2$, anaerobic energy must be measured by rates of lactate production in muscle as determined by whole body homogenization and biopsy or by blood lactate levels before and after exercise.

The Amphisbaenian, *Diplometopon zarudnyi,* is the only species of the family Trogonophidae present in Central Arabia (Al-Sadoon, 1988). *D. zarudnyi* is a monospecific worm lizard and occurs in arid habitats and sand deposits but is known to emerge onto the surface to breed (Maisano et al., 2006). Previous work on *D. zarudnyi* includes investigations into changes in the metabolic rate-temperature curves (the semi-logarithmic plot of oxygen consumption rates and temperatures) for many different ambient temperatures in comparison with two other lizards *Acanthodacty-*

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lus boskianus and *Scincus mitranus* (Al-Sadoon, 1986a). Currently, however, little is known about the metabolic rate of this species. Most previous studies of reptilian activity metabolism have focused on surface- dwelling, quadrupedal species; little emphasis has been placed on limbless and/ or fossorial forms (Kamel and Gatten Jr, 1983). The purpose of this study was to determine the metabolic rates at the resting and active states during aerobic and anaerobic respiration for *D. zarudnyi* across a wide temperature range (from 15 to 35 °C). This study also aimed to determine the contribution percentage of both aerobic and anaerobic respiration to the total metabolic scope used by the animal when active so as to examine the adaptations employed by this species during its active mode. This is intersecting since most of the time *D. zarudnyi* lives below the soil surface. Our study could provide insights into metabolic adaptations for the fossorial life-style of this worm lizard.

2. Materials and methods

2.1. Animal collection and maintenance

All specimens of *D. zarudnyi* (n = 70) were collected from different locations within the Riyadh region of Saudi Arabia (24°38″N 46°43″E) during monthly field trips. Lizards were located by tracking the traces they left on the soil surface. The captured worm lizards were transferred to the Zoology Department of the College of Science, King Saud University. They were housed in glass boxes with a substrate of fine sandy soil, food (mealworm larvae and minced beef) and water were supplied *ad libitum* under a light/dark cycle of 12 h each at an air temperature (25 ± 0.5 °C). Lizards were starved for two days prior to the start of the experiment. The animals were treated in accordance with the standards set out in the guidelines for the care and use of experimental animals by the King Saud University, Riyadh; Kingdom of Saudi Arabia.

2.2. Determination of resting and active oxygen consumption rates, $\dot{V}O2$

Sandard metabolic rates of ten resting worm lizards ($\bar{x}$ weight, 6.34 ± 0.27 g) were determined using the double chamber volumetric system described by Al-Sadoon and Spellerberg (1985) at 15 °C, 20 °C, 25 °C, 30 °C and 35 °C. Oxygen consumption $\dot{V}O2$ was estimated according to the Gas laws at standard temperature and pressure (STP). The thermal quotient (Q10) was calculated using the formula mentioned below.

$$Q10 = (R_2/R_1)^{10/(T2-T1)}$$

where R = Rate, T = Temperature (°C).

Before measurements of standard metabolic rates in the active state, further ten worm lizards ($\bar{x}$ weight, 4.25 ± 0.31 g) were allowed to rest in a Plexiglas container (15.0 × 7.8 × 3.5 cm) ventilated with room air for at least 2 h at 25 °C prior to adjustment of respirometer to different temperature treatments. A flexible port was fitted at top of the chamber which allowed manual stimulation of the animal to activity. Just before the oxygen measurements, the animal chamber was flushed with dry, CO_2-free air. The chamber was then sealed, a preactivity air sample taken, and its fractional oxygen concentration determined using the S-3A analyzer. The chamber was again flushed and sealed. The animal was stimulated to activity by manual prodding, shaking, and tapped on the tail for 2 min. Air sample was immediately taken after activity and its oxygen concentration was determined. The difference in the fractional concentration of oxygen between pre- and post-activity samples was used to calculate the amount of O_2 consumed. Resting and active oxygen consumption values were expressed as

ml O_2/g body weight/h and corrected to standard temperature and pressure.

2.2. Estimation of lactic acid in resting and active states

The extent of anaerobiosis was determined by measuring the total lactic acid concentration in 25 worm lizards at rest and further 25 in an active state. Ten lizards (five resting and five active) were used to measure anaerobic metabolism for each temperature point investigated. Total lactate production at different temperatures (15 °C, 20 °C, 25 °C, 30 °C and 35 °C) was determined in whole body homogenates of resting and active worm lizards following the methodology of Gatten (1985). Each chamber was opened and resting lizards were quickly frozen in liquid nitrogen. Other lizards were induced by forced stimulation for two minutes within a chamber before being dumped into liquid nitrogen. Frozen lizards were weighed and homogenized in cold 1.0 N perchloric acid. The homogenate was centrifuged at 12,000 × g for 20 min and the resulting supernatant was filtered and frozen for further analysis. The lactate concentration of each animal was determined in duplicate using the enzymatic method of Bergmeyer (1974) and a spectrophotometer at 340 nm. The data was expressed as mg lactate/g body weight. The total metabolic scope was estimated according to Bennett and Licht (1972) to determine the contribution level of each type of respiration following conversion to equivalent ATP millimoles at STP.

2.3. Data analysis

Comparisons of the mean oxygen consumption between experimental groups were made using two-tailed t-tests. Analysis of variance for total body lactate was carried out to determine the contribution of temperature and activity level to the observed variation. Differences were considered to be statistically significant when p < .05. All means are presented ± Standard Error (SE).

3. Results

3.1. Oxygen consumption

$\dot{V}O2$ was significantly different (P < .05) between resting and active states at any assigned temperature (Fig. 1). The average value for $\dot{V}O2$ at a resting state generally rose as the temperature increased from 15 °C (0.05 ml O_2/g/h) to 25 °C (0.111 ml O_2/g/h). This increase was statistically significant (P < .05), but no significant increment (P > .05) occurred between 25 °C and 30 °C. When the ambient temperature changed from 30 °C to 35 °C, however, the overall mean $\dot{V}O2$ showed a significant increase (from 0.111 to 0.153 ml O_2/g/h). When the worm lizards were active, oxygen consumption was observed to rise with increased temperature from 15 °C to 35 °C (Fig. 1). The Q10 values of active lizards were significantly lower than those of resting ones, except at temperatures between 25 °C and 30 °C, where resting lizards showed a lower value (Table 1). It was found that the highest Q10 values (3.24) were obtained at 15–20 °C at a resting state, whereas the highest value in the active state (1.69) was observed at 30–35 °C, with these values being significantly different (P < .05). The aerobic scope for this process showed a significant increase in line with increased temperature, with the maximum value occurring at 30–35 °C (1.5 ml O_2/g/h).

3.2. Lactate production

Resting lactate concentrations did not show a significant thermal dependence (Fig. 2: P > .05). Lactate production during the
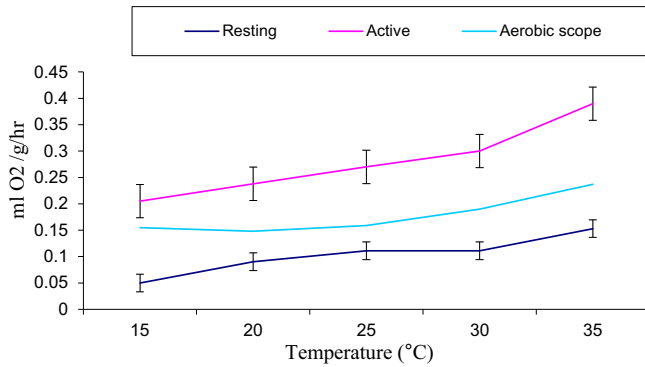
Fig. 1. Comparison of oxygen consumption during resting and active states and aerobic scope of *D. zarudnyi* at different temperatures.

Table 1

Thermal Quotient (Q10) for oxygen consumption and aerobic scope of *D. zarudyni* during resting and active states under different temperature ranges.

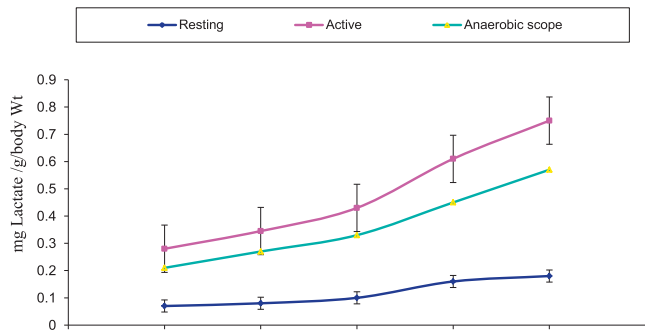| Temperature in interval (°C)	Resting Q10	Active Q10	Aerobic scope
15–20	3.24	1.53	0.912
20–25	1.52	1.29	1.15
25–30	1.00	1.23	1.43
30–35	1.90	1.69	1.50
Over all Q10 values (15–20 °C)	1.92	1.39	1.25

Fig. 2. Comparison of lactate concentration (mg/g body wt) during resting and active states and aerobic scope of *D. zarudnyi* at different temperatures.

Table 2

Thermal Quotient (Q10) for lactate concentration and anaerobic scope of *D. zarudnyi* during resting and active states under different temperature ranges.

Temperature in interval (°C)	Resting Q10	Active Q10	Anaerobic scope
15–20	1.31	1.52	1.59
20–25	1.56	1.55	1.49
25–30	2.56	2.01	1.86
30–35	1.26	1.51	2.78
Over all Q10 values (15–20 °C)	1.67	1.65	1.93

resting state ranged from 0.07 to 0.18 mg/g body wt. at temperatures of 15 °–35 °C. Lactate concentrations during active states showed values higher than those during a resting state (P < .05). At 15 °C, lactate production during the active state was significantly lower (0.28 mg/g body wt) than that at all higher temperatures (P < .05). Mean lactate values during the active state were highest (0.75 mg/g body wt) at 35 °C, indicating the greatest anaerobic debt at this temperature. The anaerobic scope was also found to increase as temperature increased, ranging from 0.21 to 0.57 mg/g (Fig. 2). Overall Q10 values in the process of lactate production were not significantly different between resting and active states (1.67 and 1.65, respectively; P > .05). The highest Q10 values were 2.56 and 2.01 for resting and active lizards, respectively, at 25–30 °C (Table 2).

3.3. Total metabolic scope during outburst forced activity

The ATP production measured for *D. zarudnyi* from the net amount of lactate produced and oxygen consumed during the first 2 minutes of forced stimulation is given in Table 3. The maximum value of the anaerobic and aerobic scope (9.52 and 3.46 µmoles/g/2 min, respectively) occurred at 35 °C. The contribution of the anaerobic respiratory scope to ATP production increased from 60.98% to 73.34% as the temperature increased from 15 °C to 35 °C.

4. Discussion

In line with previous studies of reptiles (Lowe and Vance, 1955; Kamel and Gatten Jr, 1983; Huang et al., 2006; Yang et al., 2008; Al-Sadoon et al., 2014), variations in temperature had a significant effect on the overall metabolism in *D. zarudnyi*, following the general rule of the temperature dependency of metabolism. It was found that oxygen consumption was temperature dependent, and the active $\dot{V}O2$ exceeded the resting $\dot{V}O2$ at all the different experimental temperatures. A significant difference was found between the $\dot{V}O2$ of *D. zarudnyi* when tested in resting and active states at different temperatures in agreement with the findings of Al-Farraj (1993) in respect to the sand fish lizard, *Scincus mitranus*, and Al-Sadoon (1986a) in respect to three desert lizard species (*Acanthodactylus boskianus*, *Scincus mitranus*, *D. zarudnyi*) who share the same ecological features. Al-Sadoon and Abdo (1988) reported oxygen consumptions with a low thermal dependence over the temperature range 15–25 °C in *Ptyodactylus hasselquistii* and *Bunopus tuberculatus* that may coincide with their preferred body temperature. This is understandable, since like *D. zarudnyi*, these two lizard species (*P. hasselquistii* and *B. tuberculatus*) are nocturnal and consequently their activities and rhythms are associated with low temperatures.

In *D. zarudnyi*, the aerobic scope increases linearly with temperature and was highest in the range of the animal's PBT, when it was resting. This is in agreement with the findings observed in some other fossorial lizards (Kamel and Gatten Jr, 1983). At temperatures exceeding the PBT, the aerobic scope of several lizard species has been shown to be temperature independent (Bennett and Dawson, 1972; Wilson, 1974; Al-Sadoon, 1986b). This also applies

Table 3

Aerobic and anaerobic contribution in total scope within the first two minutes of forced stimulation in *D. zarudyni*.

Temp (°C)	Aerobic scope ATP (µ moles/g/2 min)	%	Anaerobic scope ATP (µ moles/g/2 min)	%	Total metabolic scope ATP (µ moles/g/2 min)
15	2.24	39.02	3.50	60.98	5.74
20	2.14	32.18	4.51	67.82	6.65
25	2.31	29.57	5.50	70.42	7.81
30	2.80	27.13	7.52	72.80	10.32
35	3.46	26.60	9.52	73.34	12.98

to the worm lizard since the metabolic scope averaged 0.19 at 30 °C and 0.23 at 35 °C while the Q10 value between temperatures 30–35 °C was 1.5. These results could be compared with another fossorial lizard *S. mitranus* inhabiting the same habitat. The metabolic scope of *S. mitranus* was recorded as 0.37 at 30 °C and 0.42 at 35 °C (Al-Farraj, 1993) which is almost double than *D. zarudnyi*. The high aerobic scope of *S. mitranus* might be a reflection of the pressure of predation exerted on this lizard since it is a diurnal species. Even though *D. zarudnyi* is out and active at low temperatures, the degree of activity is quite low. In general, for *D. zarudnyi*, the Q10 values during the active state were less than the values when resting, and this is in line with the prior findings of Al-Sadoon (1986b) in respect to *C. ocellatus* and Al-Farraj (1993) in respect to *S. mitranus*. These skinks are active, alert and warry animals which flee rapidly to safety at any sign of movement in their habitat (Al-Sadoon, 1986a,b).

Earlier reports regarding lactic acid concentrations of small lizards resting in the inactive phase of their daily cycle have shown little inter or intraspecific variation, with resting lactate values ranging between 0.25 and 0.35 mg lactate g^{-1} body mass in most of the lizards (Bennett and Dawson, 1972; Bennett and Licht, 1972). Resting lactate levels for *D. zarudnyi* fall below this range, but this coincides with the findings of Kamel and Gatten Jr (1983) in the fossorial lizards, *Anneilla pulchara and Tronophis weigmanni* whereas that of *Ophisaurus ventralis* was within this range. The lactic acid concentration was shown to be significantly more in active *D. zarudnyi* when compared with rested lizards, which could be attributed to the increased muscular activity requiring more energy. At the end of a 2-min burst of activity, the lactate concentrations of *D. zarudnyi* ranged from 0.28 ± 0.001 to 0.75 ± 0.011 mg g^{-1} body mass. Other reptiles have total body lactate concentrations after 2 min of activity of 1.1–2.1 mg g^{-1} (Bennett and Dawson, 1972).

Since, *D. zarudnyi* is a nocturnal sand burrower, it primarily shows anaerobic behavior and, as predicted, the high dependency on anaerobic respiration in this worm lizard was evident. The fossorial species studied show a low standard metabolic rate which might be beneficial in a subterranean habitat; however, some other non-fossorial reptiles also exhibit low resting metabolic rates. Thus, a low rate of oxygen consumption at rest is not unique to burrowing forms. Anaerobiosis seems to be the ideal solution to the energetic problems posed by activity to small behaviorally-thermoregulating poikilotherms. The anaerobic and total metabolic scopes of *D. zarudnyi* are low to moderate. The fact that the metabolic scope of the fossorial lizard examined was equal to or higher than those of non-burrowing species leads us to conclude that fossorial and surface-dwelling reptiles cannot be distinguished on the basis of metabolic patterns.

Acknowledgement

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