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The Metabolic Adjustments Of The Angulate Tortoise (*Chersina Angulata*) To Seasonal Changes In Temperature And Photoperiod

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ABSTRACT

Seasonal effects were determined on the metabolic rate of adult *Chersina angulata*. Summer experiments were conducted in summer and winter experiments conducted in winter. The tortoises were acclimated to standard summer and winter conditions in an environmentally controlled room prior to experiments. Oxygen consumption (VO₂) was measured as an index of metabolic rate at experimental temperatures of 14°C, 18°C and 22°C. In addition, VO₂ of winter-acclimated tortoises was measured at 10°C. The metabolic rate for both winter-acclimated and summer-acclimated tortoises increased significantly with increasing experimental temperature within the temperature range tested. Season had no statistically significant effect on the metabolic rate of *C. angulata*. The metabolic rate–temperature curves of the summer-acclimated and the winter-acclimated tortoises cross each other.

**Key words:** Acclimation temperature; *Chersina angulata*; Metabolism; Photoperiod; Season.

Introduction

Varying environmental temperatures and seasonal changes are generally known to affect the physiology and the behaviour of ectotherms. Physiological effects have been shown for example by Cartland and Grimmond (1994), Hailey and Loveridge (1997), Ott et al. (2000) and Sepulveda et al. (2008), while behavioural effects have been shown for example by Huey and Pianka (1977), Gregory (1982), Zimmerman et al. (1994) and Rautenstrauch et al. (1998). Seasonal changes in food availability and rainfall are found to influence reptile metabolism (Bennett and Dawson, 1976; Felger et al., 1976; Kennett and Christian, 1994; Christian et al., 1995). Some reptiles reduce their metabolic rates during periods of food shortage (Nagy and Medica, 1986; Waldeschmidt et al., 1987), in order to conserve energy (Hailey and Loveridge, 1997) or to store lipids before going into hibernation (Heusner and Jameson, 1981).

Reptiles respond differently to seasonal changes in temperature, both physiologically and behaviourally. Some reptiles are active in summer and have metabolic depression in winter (Andrade et al., 2004; Milsom et al., 2008) while other reptiles elevate their metabolic rates in winter (Roberts, 1968). Variations in ambient temperature directly influence the biochemical, as well as the physiological processes in chelonians (Lutz et al., 1989; Penick et al., 1996; Seebacher et al., 2003; Southwood et al., 2003; Hochsheid et al., 2004). Some tortoises reduce their metabolic rates and over-winter in hibernacula (Gregory, 1982; Zimmerman et al., 1994; Rautenstrauch et al., 1998).

The effects of seasonal changes on chelonian metabolism have been extensively studied on turtles (for example Southwood et al., 2003; Hochsheid et al., 2004; Southwood et al., 2006). The influence of seasonal variations on the metabolic rates of tortoises, especially southern African tortoises, has not been extensively studied. Kuchling (1981) reported seasonal variations in *Testudo hermanni* while Kirsch and Vivien-Roels (1984) studied the responses of metabolic rate to time of day and temperature changes in the same species. Hailey and Loveridge (1997) reported an increase in the metabolic rates of *Kinixys spekii*, with increasing temperature. Nagy and Medica (1986) reported on the seasonal changes in the field metabolic rates of the desert tortoises in southern Nevada.

The reptiles of southern Africa are subjected to diverse climate and seasonal variations with fluctuations in ambient temperature in their natural habitat (Boycott and Bourquin, 2000; Kruger, 2004; McMaster and Downs, 2008). It is of importance to know how these reptiles react physiologically to these variations. This study focuses on the effect of season on the metabolic rate of the angulate tortoise (*Chesina angulata*).

*Chersina angulata* is widely distributed in Southern Africa and is adapted to a variety of climatic zones and habitats. Seasonal variations have been reported in the vegetation of some of these habitats in the Western Cape.

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The variations in the activity patterns of *C. angulata* over different seasons in the Eastern Cape (Els, 1989) and Western Cape (Ramsay et al., 2002; Keswick et al., 2006) have been reported. The occurrence of *C. angulata* in diverse environments, as well as their ability to be active throughout the year in mild winters (Els, 1989; Bourquin and Boycott, 2000), suggest that the tortoises may have the ability to thermoregulate physiologically to some extent within a preferred temperature range. This poses a question as to whether *C. angulata* have a distinct pattern of seasonal metabolic compensation. There are no published studies on the seasonal metabolism of this species.

We hypothesized no significant effect of season on the metabolic rate of adult *C. angulata*. If there was an effect of season on the metabolic rate of *C. angulata*, we predicted winter-acclimated tortoises to have a slightly reduced metabolic rate in order to conserve energy.

**Materials And Methods**

**Study Animal:**

*Chersina angulata* is a medium-sized tortoise of carapace length of up to 272 mm and mass of up to 2.1 kg (Boycott and Bourquin, 2000). Seventeen adult tortoises of mass range 358 g to 867.81 g and plastron length 130 mm to 213 mm were used in this study. Nine males and eight females were used for summer measurements, while eight of the males and seven of the females were used for winter measurements.

**Site:**

*Chersina angulata* is endemic to southern Africa (Ramsay et al., 2002). It is distributed along the Cape coastal regions of South Africa and the southern part of Namibia (Hofmeyr, 2009). The tortoises used in this study were obtained from Sardinia Conservancy and neighbouring privately owned land in the western approach of Port Elizabeth (33° 58' S, 25° 42' E). The tortoises were free ranging in both sites. The sites have temperate climate and dune thicket vegetation (Greig and Burdett, 1976). The annual rainfall is 600–700 mm and it falls throughout the year (Port Elizabeth Weather Bureau).

**Permits:**

Permits to capture *Chersina angulata* transport and keep them in captivity; were obtained from the Department of Economic Development and Environmental Affairs, Port Elizabeth. The protocols used in this study were non-invasive and were approved by the Nelson Mandela Metropolitan University (NMMU) Research Animal Ethics Committee.

**Acclimation:**

The tortoises were transferred from an outdoor enclosure where they were kept initially, into an environmentally controlled room (ECR) for acclimation to experimental conditions for a period of at least one week. They were maintained on fresh chopped vegetables (cabbage, carrots, tomatoes and lettuce) and water. The summer acclimation conditions in the ECR were a temperature of 22°C ± 1°C and a 14L:10D light regime. These are mean summer temperature and photoperiod for Port Elizabeth respectively (Port Elizabeth Weather Bureau). Light was provided by the fluorescent lamps on the ceiling which were phased on automatically at 05h30, and off at 19h30. Full light or full darkness was phased in over 30 minutes to simulate sunrise and sunset respectively. For winter acclimation, the set-up was as for summer acclimation, but the temperature was reduced to 14°C and the light regime changed to 11L:13D. Light was provided as in summer acclimation but phased in automatically at 06h30 and out at 17h30. These are the winter mean temperature and light regimes of Port Elizabeth respectively (Port Elizabeth Weather Bureau).

**Measurement of Resting Oxygen Consumption:**

The resting metabolic rates of both summer-acclimated and winter-acclimated *Chersina angulata* were determined indirectly, as rates of resting oxygen consumption (RVO2) at varying experimental temperatures. The summer RVO2 measurements were taken in summer (from October to February) and the winter measurements were taken in winter (from April to August). The RVO2 of each tortoise was measured using open flow through respirometry. The RVO2 measurements were taken at experimental temperature intervals 4°C from 10°C–22°C for winter, while the summer measurements were taken from 14°C–22°C.
Procedure for Measuring Resting Oxygen Consumption:

After acclimation of tortoises to experimental conditions, one tortoise at a time was starved for at least forty-eight hours prior to VO\textsubscript{2} measurements. Water was provided ad libitum but withdrawn during the VO\textsubscript{2} measurements. The tortoise was weighed using an electronic scale (ADAM AFP–3100L, d = 10mg), then placed into a metabolic chamber, unrestrained, and the lid placed into position after the tortoise had settled down. All the VO\textsubscript{2} measurements were recorded over a period of six hours, from 08h30 to 14h30, to avoid any possible circadian variation in the VO\textsubscript{2} (Kirsch and Vivien-Roels, 1984). Oxygen percentage readings and behavioural observations were recorded every five minutes. Readings were recorded from an oxygen analyser pre-calibrated at 20.95% oxygen prior to the placement of the tortoise in the metabolic chamber. For each tortoise, the VO\textsubscript{2} was measured once at each season and experimental temperature. Each tortoise was given at least one week of rest before the next VO\textsubscript{2} measurements.

The tortoises were taken back to a large outdoor enclosure at intervals to have access to sunlight which is vital for their carapace development (Bourquin and Boycott, 2000). The tortoises were brought back into the ECR on time for acclimation to experimental conditions prior to VO\textsubscript{2} measurement in the next experimental temperature.

Data Handling:

The data recorded in the first two hours for all the RVO\textsubscript{2} measurements in this study were not used in the metabolic rate calculations (Litzgus and Hopkins, 2003; McCue and Lillywhite, 2002; Coulson and Hernandez, 1980). This was done to avoid variations in the resting metabolic rates due to the handling and commencement effects on the measurements. The data where the experimental tortoise was found active, defeacated or urinated were discarded. Activity of chelonians increases their metabolic rates while the presence of faeces resulted in inaccurate metabolic rates due to oxygen depletion in the metabolic chamber (Hughes et al., 1971; Prange and Ackerman, 1974; Lutz et al., 1989; Hailey and Loveridge, 1997).

Data Analysis:

Repeated measures analysis of variance (RMANOVA) was used to compare the body masses of tortoises between different experimental temperatures and seasons. RMANOVA was used to test for the effects of season (summer and winter), gender (male and female) and experimental temperature (14 °C–22°C) on the mean sRVO\textsubscript{2} of tortoises. Tukey post hoc test was employed where necessary. The Q\textsubscript{10} values were calculated (Schmidt-Nielsen, 1997; Lighton, 2008) at temperature intervals for each season. Statistical significance was assumed at P < 0.05 (Zar, 1998). All mean values are presented as mean ± SE. Throughout the text n refers to the number of observations and N refers to the number of animals. Due to tortoise mortalities, data were obtained from a different number of tortoises per experimental temperature and season. Twelve to fifteen tortoises were used at each experimental temperature for each season.

Results:

There was no significant difference in the body masses between males and females at all experimental temperatures (P > 0.05), and no significant differences in body mass of tortoises between experimental temperatures (P = 0.74) and between seasons (P = 0.09). RMANOVA showed no significant effects of gender (F(1,82) = 0.72, P = 0.40), no significant effects of season (F(1,82) = 0.04, P = 0.84), but a significant effect of temperature (F(2,81) = 56.62, P < 0.001) on the sRVO\textsubscript{2} of tortoises. The Tukey post-hoc test revealed that the mean values for the sRVO\textsubscript{2} of tortoises at all the tested temperatures were significantly different from each other (F(2,81) = 67.69, P < 0.001).

The data for male and female tortoises at each season were then combined. The mean sRVO\textsubscript{2} values of summer–acclimated and winter–acclimated tortoises were computed for each experimental temperature (Table 1). The relationships of the computed means and the experimental temperatures at each season are shown in Fig. 1. The mean sRVO\textsubscript{2} increased with increasing temperatures in both summer–acclimated and winter–acclimated tortoises (Fig. 1, Table 1). The winter–acclimated tortoises had almost equal mean sRVO\textsubscript{2} at 14°C, a slightly elevated mean sRVO\textsubscript{2} at 18°C and a slightly lower mean sRVO\textsubscript{2} at 22°C in comparison with the summer–acclimated tortoises (Fig. 1, Table 1). The metabolic rate–temperature curves for winter–acclimated and summer–acclimated tortoises cross each other (Fig. 1). The mean sRVO\textsubscript{2} of the winter–acclimated tortoises was lowest at 10°C, only 7.58% of that at 22°C.

The Q\textsubscript{10} values were calculated from the means of the sRVO\textsubscript{2} of the winter–acclimated and the summer–acclimated tortoises over the experimental temperature range used (Table 2). The highest Q\textsubscript{10} value for the winter–acclimated tortoises was at 10°C–14°C, while the lowest was at 18°C–22°C. The summer–acclimated
tortoises had a higher Q₁₀ value of 8.82 at 18°C–22°C in comparison with the winter–acclimated tortoises. The Q₁₀ values of the winter–acclimated tortoises increased with decreasing temperature, while the Q₁₀ values of the summer–acclimated tortoises decreased with decreasing temperature. The overall Q₁₀ value was higher for summer–acclimated tortoises between 14 and 22 °C (Table 2).

Table 1: Mean specific resting oxygen consumption (sRVO₂) and mean mass of winter–acclimated and summer–acclimated adult *Chersina angulata* at different experimental temperatures.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Winter–acclimated Chersina angulata</th>
<th>Mean sRVO₂ (ml O₂ kg⁻¹ hr⁻¹ ± SE)</th>
<th>Mean mass (g±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>13</td>
<td>5.52 ± 0.51</td>
<td>657.18 ± 40.58</td>
</tr>
<tr>
<td>14</td>
<td>15</td>
<td>20.42 ± 2.61</td>
<td>680.15 ± 40.00</td>
</tr>
<tr>
<td>18</td>
<td>15</td>
<td>52.00 ±4.02</td>
<td>681.96 ± 40.09</td>
</tr>
<tr>
<td>22</td>
<td>15</td>
<td>72.82 ± 5.04</td>
<td>677.21 ± 37.82</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Summer–acclimated Chersina angulata</th>
<th>Mean sRVO₂ (ml O₂ kg⁻¹ hr⁻¹ ± SE)</th>
<th>Mean mass (g±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>12</td>
<td>20.70 ± 2.54</td>
<td>689.17 ± 43.60</td>
</tr>
<tr>
<td>18</td>
<td>13</td>
<td>37.12 ± 3.93</td>
<td>580.11 ± 37.72</td>
</tr>
<tr>
<td>22</td>
<td>14</td>
<td>88.70 ± 8.43</td>
<td>598.51 ± 35.56</td>
</tr>
</tbody>
</table>

Table 2: Q₁₀ values for the mean specific resting oxygen consumption (sRVO₂) in winter–acclimated and summer-acclimated *Chersina angulata* at different experimental temperatures (10°C–22°C).

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Winter–acclimated tortoises</th>
<th>Q₁₀</th>
<th>Summer–acclimated tortoises</th>
<th>Q₁₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 - 14</td>
<td>26.32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 - 18</td>
<td>10.35</td>
<td>4.31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 - 22</td>
<td>2.32</td>
<td>8.82</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 – 22 (Overall)</td>
<td>4.90</td>
<td>6.17</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1: Comparison of seasonal effects on the mean specific resting oxygen consumption for summer–acclimated and winter–acclimated *Chersina angulata* at different experimental temperatures (10°C–22°C). Each point represents the mean of the different individuals at an experimental temperature. The vertical lines above and below each mean are standard errors. Twelve to fifteen tortoises were used at each experimental temperature for each season.

Discussion:

*Chersina angulata* used in this study showed no distinct or little seasonal variation in metabolic rate. However, some behavioural differences were noted. The metabolic rate of both winter–acclimated and summer–acclimated tortoises increased with experimental temperature (Fig. 1 and Table 1). The metabolic rate at 22°C was almost four times that at 14°C, for both summer–acclimated and winter–acclimated tortoises. The lowest metabolic rate was recorded on the winter–acclimated tortoises at 10°C. The values of the metabolic rate of tortoises in this study are slightly higher than those reported in *Kinixys spekii*, (Hailey and Loveridge, 1997), *Testudo hermanni* (Kirsch and Vivien-Roels, 1984) and *Malacochersus tornieri* (Wood et al., 1978). Differences may be attributed to the variations in body mass, time of day at which VO₂ measurements were made and the temperature and photoperiod of acclimation history of the tortoises. The differences may also be attributed to the interspecific differences among tortoises.
The winter–acclimated tortoises in this study had a steeper decrease in $sRVO_2$ ($Q_{10} = 10.35$) than the summer–acclimated tortoises ($Q_{10} = 4.31$) at lower temperatures ($14^\circ C$–$18^\circ C$). This seasonal difference in the $Q_{10}$ value at low temperatures indicates a metabolic depression in winter–acclimated tortoises (Hailey and Loveridge, 1997). The depression of the metabolic rate of the winter–acclimated *Chersina angulata* between $10^\circ C$ and $14^\circ C$ ($Q_{10} = 26.32$) may occur during inactivity in order to conserve energy on cool winter nights. The depression may be ascribed to reduced energy demand of cells leading to a reduction in heat production (Rocha and Branco, 1998). The hypo metabolic state may be beneficial in extending the survival of the tortoises while relying on stored fuel supply during winter (Rocha and Branco, 1998). *C. angulata* are reported to have reduced activity during cold weather, with the lowest ambient temperature at which they were found active in their natural habitat being $14.1^\circ C$ (Els, 1989). However, their preferred activity temperature range was reported to be $17^\circ C$–$27^\circ C$ (Els, 1989; Ramsay et al., 2002). Almost all the tortoises used in this study were found inactive for nearly the entire oxygen consumption measurement period at $10^\circ C$ and $14^\circ C$.

The tortoises were observed to have lower food consumption during winter acclimation ($14^\circ C$, $11L:13D$) in comparison with summer acclimation ($22^\circ C$, $14L:10D$) (pers. obs.). This may be their way of energy conservation, since food digestion needs energy and is dependent on temperature. The low food consumption during cold acclimation has also been reported in other chelonians, for example the cold–acclimated Kemp’s ridley turtles and the green turtles (Moon et al., 1997) and the loggerhead turtle, *Caretta caretta* (Hochscheid et al., 2004). The cessation of feeding has also been noted on green turtles at water temperatures of $15^\circ C$ and lower temperatures (Felger et al., 1976).

The results of this study showed no significant effect of season on the metabolic rate of *C. angulata*. The metabolic rate–temperature curves of the winter–acclimated and the summer–acclimated tortoises (Fig. 1) cross each other, showing no distinct pattern in the seasonal metabolic compensation in *C. angulata*. The seasonal metabolic pattern exhibited by *C. angulata*, is close to the rotational and translational type described by Pohl (1976), whereby the metabolic rate–temperature curves of the summer–acclimated and winter–acclimated animals cross each other.

Reptiles that hibernate are known to reduce their metabolic rates in the cold, while those reptiles that are active in the winter increase their metabolic rates in response to cold. Unlike the results obtained from this study, studies from some tortoises and turtles have shown a reduction in metabolic rates during winter, for example, the study by Kuchling (1981) indicated a metabolic preparation in autumn prior to hibernation in the cold by the tortoise *Testudo hermanni*. Hailey and Loveridge (1997) reported a significant metabolic depression during dormancy in the tortoise *Kinixys spekii*. The study by Hochscheid et al. (2004) showed a decrease in the $VO_2$ of the turtle *Caretta caretta* between summer and winter.

Seasonal variations in the field metabolic rate (FMR) of the desert tortoise have been reported (Nagy and Medica, 1986; Peterson, 1996; Henen, 1997; Henen et al., 1998). The tortoises had elevated rates in spring and summer, and reduced rates in winter. Desert tortoises are said to increase their body energy content in summer; which is then conserved for use in the winter.

The lack of a distinct pattern in the seasonal metabolic compensation in *Chersina angulata* is not strange in chelonians. No significant difference was exhibited in the metabolic rates of cold and warm–acclimated box turtles (*Terrapene Carolina*) at $5^\circ C$ (Gatten, 1987). Southwood et al. (2003) reported no significant difference in the $VO_2$ of summer–acclimated and winter–acclimated juvenile green turtles, but a $24$–$27\%$ decrease in the $VO_2$ during exposure to winter. The study by Kennett and Christian (1994) showed that the metabolic rate of the long-neck turtles (*Chelodina rugosa*) during estivation dropped by $28\%$, but was not significantly different from that during pre-estivation. The study by Southwood et al. (2006) showed a $43\%$ drop in the mean FMR of juvenile green turtles (*Chelonia mydas*) during winter. However, there was no statistically significant difference ($P = 0.17$) in the mean FMR between winter and summer.

In some lizards, inverse and partial compensation in seasonal metabolic rates have been reported. Some lizards have reduced metabolic rates in winter (for example, Mayhew, 1965; Gregory, 1982; Gatten, 1985; Zari, 1999). It may be assumed that this is in order to conserve energy during times of inactivity and to possibly make it available for use during the active season. On the other hand, some lizards elevate their metabolic rates during winter to maintain their levels of activity at lower temperatures (for example, studies by Gregory, 1982; Gatten, 1985; and Zari, 1996).

The lack of a distinct pattern of seasonal metabolic compensation in *Chersina angulata* as compared to other tortoises studied may suggest that some species of tortoises are active throughout the year, despite the heterogeneous thermal environments in which they live. In turtles that do not show any seasonal metabolic compensation, this may be explained by their natural habitat. In the sea the turtles experience a narrowed temperature range due to the large specific heat capacity of water, unless they travel long distances or dive deep into the water (Hochscheid et al., 2004).
Conclusions:

*Chersina angulata* used in this study showed an increase in metabolic rate with temperature for both summer–acclimated and winter–acclimated tortoises within the temperatures tested. There was no significant difference between the mean specific resting oxygen consumption of the tortoises at same experimental temperatures and different seasons. The pattern appeared to be correlated with their behaviour in their natural habitat within the temperature ranges tested. This may also indicate that *C. angulata* and some reptiles are not purely ectothermic, they have some endothermic traits. However, the high Q₁₀ values calculated at the lower temperatures, especially for winter–acclimated tortoises between 10°C and 14°C may be an indication that below 10°C the tortoises may hibernate.

Acknowledgments

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References


