Evidence for a circadian rhythm in the oxygen consumption of resting angulate tortoise 
(Chersina angulata)

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Abstract

Some ectotherms show daily fluctuations in their metabolic rate (MR) within their experienced temperature range. The rhythms may disappear or persist in constant conditions in the laboratory. The resting oxygen consumption (RVO2) of resting Chersina angulata was measured using open flow through respirometry over 24 hours to detect the presence of daily fluctuations in MR. The tortoises were acclimated in an environmentally controlled room to an ambient temperature of 26 ± 1 °C and a 14L:10D light regime prior to the resting VO2 measurements. The VO2 was measured when tortoises were resting at a constant temperature of 26 ± 1 °C and at three different light regimes, 14L:10D, DD and LL. Daily rhythms in the MR were detected at all three light regimes. However, the rhythm was more pronounced at 14L:10D than at DD and LL. Higher values of resting MR were registered in the early hours of the day, followed by a decrease as the day progressed, while the lowest values were recorded in the late hours of the day. Under constant conditions, the rhythmic pattern persisted, but with smaller amplitudes. Consequently, the rhythm is considered to be circadian and endogenous.

Keywords: Circadian rhythm, ectotherm, metabolism, specific resting oxygen consumption, thermoregulation

1. Introduction

Animals in their natural habitats experience changes in environmental conditions like photoperiods and ambient temperatures. Some physiological parameters, like oxygen consumption (VO2), have been shown to exhibit daily rhythmicity in many organisms [1, 2]. Some of these circadian rhythms tend to persist under constant conditions in the laboratory and are said to be endogenous. Endogenous circadian rhythms prepare the organism to adjust physiologically to any anticipated environmental change [3].

Circadian rhythms in MR vary among species and photoperiods [4]. In MR studies conducted on chelonians e.g. those of [5, 6, 7], a few studies have investigated the circadian rhythms of VO2 [8, 9]. [8] Reported a day night rhythm in the oxygen consumption of Testudo hermanni °C while [9] reported no circadian rhythm in the oxygen consumption of Caretta caretta.

Even though there are few studies in the literature of circadian rhythms in the MR of chelonians, there is a voluminous amount of information on the subject in other ectotherms. Daily fluctuations in VO2 have been reported for the tuatara [2], snakes [3, 10] and lizards [11, 12, 13]. The results of the study of circadian rhythms of the metabolic rates of C. angulata would be useful in the study of the energetics of the tortoise and will contribute to the literature on the circadian rhythms in the metabolic rate of reptiles. The rhythms possibly contribute to variations in standard metabolic rate studies in reptiles [10, 14].

This study investigated possible daily variations in the MR of C. angulata. Knowing the daily pattern in the VO2 of C. angulata may have ecological relevance in that it would help estimating their daily energy budgets.

2. Materials and methods

Chersina angulata is a medium-sized tortoise with a plastron length of 15-25 cm and a mass of up to 2.1 kg [15]. It is endemic to southern Africa and it is distributed along the Cape coastal regions of South Africa and the southern part of Namibia [16]. Adult C. angulata (four males and four females) having more or less similar body conditions, mass ranging from 467.80 to 787.95 g used in this study were obtained from the Sardinia Conservancy in the western part of Port Elizabeth (33° 58’ S, 25° 42’ E), South Africa. The site has a temperate climate and dune thicket vegetation [17].
At NMMU, each tortoise was given an identity number written on the carapace with a non-toxic permanent marker. The tortoises were weighed to two decimal places using an electronic scale (ADAM AFP–3100L, d = 10mg). They were acclimated to 26 ± 1 °C and 14L:10D light regime in an environmentally controlled room for at least two weeks prior to experiments. The acclimation conditions simulated summer conditions in the Port Elizabeth area, because the experiments were conducted in austral summer, between January and March 2008. The 26 °C is within the summer temperature range (17 °C–27 °C) in the area [18] and the preferred ambient temperature range (14 °C–28.7 °C) for the activity of C. angulata [16, 19, 20]. The 26 °C was also used so as to isolate the effect of photoperiod. Light was provided by fluorescent lamps phased on and off automatically at 05h30 and 19h30 respectively. The phasing of the lights was done over a period of 30 minutes to simulate sunrise and sunset respectively. The tortoises were maintained on fresh chopped vegetables [15] and clean water ad-libitum.

The MR of each tortoise was measured indirectly as the VO₂ using open flow through respirometry. The configuration of the set-up for the VO₂ measurements was a metabolic chamber, oxygen analyzer, flow meter and pump. A 5.7 litre rectangular metabolic chamber made of a translucent material, allowing visual observations of the tortoise during the measurements, was used. The air from the metabolic chamber was pulled into an Applied Electrochemistry N37 oxygen sensor and analyzed by an Applied Electrochemistry S-3A/II oxygen analyzer. The ambient air was from the environmentally controlled room. The airflow through the system was controlled by an Applied Electrochemistry Model R-2 air pump and a flow meter. The oxygen analyzer was calibrated with ambient air and set at 20.95% oxygen prior to the VO₂ measurements. Behavioural observations and visual oxygen percentage readings were recorded every five minutes. In the dark, any sound of movement was noted instead of making behavioural observations. The tortoise was considered resting when lying down quietly, and active when walking in the chamber. The experimental tortoise was starved for at least 48 hours prior to the VO₂ measurements. The tortoise was weighed to the nearest 0.01g, and placed in a metabolic chamber, unrestrained. The measurements commenced at 22h00 and readings were recorded from midnight to midnight the following day. The VO₂ readings of the first two hours (22h00 to 00h00) were not used in the MR calculations in order to avoid variations due to handling [21, 22, 23].

The VO₂ measurements were recorded at a constant temperature of 26 ± 1 °C and repeated for three different light regimes namely; light and dark (14L:10D), constant light (LL) and constant darkness (DD). The sequence of exposure was 14L:10D, LL and Lastly DD and each tortoise was measured once under each light regime. After each trial, the experimental tortoise was weighed again and taken back to the acclimation room where it was kept for at least two weeks prior to any measurements in the next light regime. The humidity was not controlled during acclimation, but was measured in an environmentally controlled room during the VO2 measurements using a MAJOR TECH 3-in-1 humidity, temperature and time meter. The humidity ranged between 55 and 60%.

The VO₂ data were discarded when the tortoise was active, sounds of movements were heard in the dark, or where the tortoises defaecated or urinated in the chamber. Faeces depleted oxygen in the chamber, causing an error in the VO₂ readings, while any movement increased the VO₂ of the individual [24, 25].

The specific resting oxygen consumption (sRVO₂) (ml O₂ kg⁻¹ hr⁻¹) of each tortoise was calculated from the data collected using the following equation:

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sRVO₂ (\text{ml O}_2 \text{ kg}^{-1} \text{ hr}^{-1}) = \left\{ \frac{D}{100} \times FR \right\} / Mb \times 60 \times 1000
\]

Where D is the difference between the preset ambient oxygen percentage (20.95%) and the recorded excurrent datum; FR is flow rate (ml min⁻¹) and Mb is the body mass (g).

Statistical analyses were performed using stata computer software. Statistical tests were considered significant where P<0.05 [26].

3. Results

Male and female C. angulata used in the trials had similar body masses (14L:10D, P = 0.18; at DD, P = 0.08 and at LL, P = 0.42). There was no significant effect of gender (P = 0.36) on the sRVO₂ of C. angulata. There was also no significant effect of body mass on metabolic rate of tortoises used in this study (P>0.10).

Analysis of variance (ANOVA) showed that different light regime exposure had a significant effect on the average sRVO₂ of Chersina angulata (F(2,530) = 8.55, P<0.001). The Tukey post hoc test showed that the sRVO₂ at 14L:10D was significantly larger than the sRVO₂ at both constant conditions (P<0.001), but not significantly different between constant conditions; P = 0.33).

To examine the daily patterns of the MR of C. angulata, mean hourly sRVO₂ values of tortoises at different light regimes were calculated and plotted (see Figs. 1–3). Under 14L:10D (Fig.1), the highest values of sRVO₂ of C. angulata were reached in the early hours of the light phase between 06h00 and 10h00, while the lowest values were recorded in the late afternoons, between 17h00 and 19h00.

![Fig 1: Mean hourly specific resting oxygen consumption of Chersina angulata determined under a 14 h light and 10 h dark regime at 26 ± 1 °C over 24 hours. The broken vertical lines show when the lights were put on (left) and off (right) during acclimation and during the experiments. The vertical bars are standard errors. N = 8.](image)
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Fig 2: Mean hourly specific resting oxygen consumption of Chersina angulata determined under constant darkness at 26 ± 1 °C over 24 hours. The broken vertical lines show when the lights were put on (left) and off (right) during acclimation. The vertical bars are standard errors. N = 8.

Fig 3: Mean hourly specific resting oxygen consumption of Chersina angulata determined under constant light regime at 26 ± 1 °C over 24 hours. The broken vertical lines show when the lights were put on (left) and off (right) during acclimation. The vertical bars are standard errors. N = 7.

4. Discussion
The introduction of light seemed to increase the sRVO$_2$ that enabled the tortoises to be active in the morning. Increased activity of tortoises was observed in the morning but was not documented since the study focused on the resting metabolic rates. In their natural habitat, [27] reported Chersina angulata to start being active in the morning after warming up. The decrease in the sRVO$_2$ as the day progressed, together with the late evening decrease, may be anticipatory to the nightfall.

The decrease in the sRVO$_2$ occurred in the afternoon which agree with the findings by [27] who found Chersina angulata to be inactive in the afternoons, which may be a way of conserving energy by the tortoises [8, 9, 12, 28]. The phasing out of light resulted in the increasing of the sRVO$_2$ after the decrease observed towards the end of the light phase, to almost the same value prior to the introduction of light in the morning. It is not clear why this happened. It is possible that the phasing out of light caused a disturbance that increased alertness of the tortoises, leading to increased metabolic rate. This seems to suggest that the fall of darkness cued the increase in the MR. There appeared to be a relationship between the switching of the lights and the sRVO$_2$. The phasing on of the lights in the morning and the phasing off of the lights in the evening were found to increase the sRVO$_2$ of C. angulata. This may suggest that even though the phasing on and off of the light was done over a period of thirty minutes to simulate sun rise and sun set respectively, that had an impact on sRVO$_2$ of C. angulata.

The daily pattern observed in this study corroborates the findings of [8] who reported a diurnal rhythm in the VO$_2$ in male Testudo hermanni at constant temperatures with peak values during the daylight hours. The values of sRVO$_2$ of C. angulata are slightly higher than those of Testudo hermanni which may be attributed to interspecific differences among the tortoises or to the effects of thermal acclimation. The acclimation light regime of Testudo hermanni was not mentioned, but their maximum experimental temperature was 28 °C, which is close to the 26 °C used in this study. The diurnal rhythm other than the amplitude would not be expected to change at different temperatures. [29] Reported seasonal variations in the VO$_2$ and the blood glucose concentration under low temperature conditions in the male Testudo hermanni of Yugoslavia.

The existence of a daily variation, at constant temperatures, in the sRVO$_2$ of Chersina angulata in this study and in Testudo hermanni [8] suggests the possibility of similar rhythms in other tortoises. The variations in the VO$_2$ rhythms, as well as their absence in some chelonian species studied so far for example [9] show the importance of taking VO$_2$ rhythms into account when measuring metabolic rate values.

To detect the possible existence of an endogenous nature of the VO$_2$ rhythm in Chersina angulata, VO$_2$ measurements
were recorded under constant darkness and under constant light. We found consistent \( \text{sRVO}_2 \) rhythms but different amplitudes, which were more pronounced during the 14L:10D regime than under constant conditions. This may imply that the circadian rhythm is endogenous. The results from this study, and other studies conducted on ectothermic reptiles, showing the presence of an endogenous circadian rhythm in the metabolism (for example the study of circadian cycles on metabolism in the lizard \textit{Sceloporus occidentalis} by \cite{11}, suggests that the circadian rhythm may have a significant physiological function in some ectotherms like in thermoregulation. The presence of endogenous circadian rhythm in \textit{C. angulata} may suggest that \textit{C. angulata} has the capability to thermoregulate physiologically to some extent. The effect of varying ambient temperature on the \( \text{sRVO}_2 \) of \textit{C. angulata} should be studied to find out if the tortoises can thermoregulate physiologically or if their body temperature is completely dependent on the ambient temperature, that is increasing with increasing ambient temperature. It is imperative to take into account the time of day in measurements of MR in tortoises and other ectotherms.

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