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Setlalekgomo MR
Department of Basic Sciences,
Botswana College of Agriculture,
Gaborone, Botswana

PED Winter
Department of Zoology, Nelson
Mandela Metropolitan
University, Port Elizabeth, RSA

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

Setlalekgomo MR and PED Winter

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 *Chersina 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].

Correspondence
Setlalekgomo MR
Department of Basic Sciences,
Botswana College of Agriculture,
Gaborone, Botswana

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 $26 \pm 1^\circ\text{C}$ 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 VO_2 , using open flow through respirometry. The configuration of the set-up for the VO_2 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 VO_2 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 VO_2 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 VO_2 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 VO_2 measurements were recorded at a constant temperature of $26 \pm 1^\circ\text{C}$ 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 VO_2 measurements using a MAJOR TECH 3-in-1 humidity, temperature and time meter. The humidity ranged between 55 and 60%.

The VO_2 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 VO_2 readings, while any movement increased the VO_2 of the individual [24, 25].

The specific resting oxygen consumption (sRVO_2) ($\text{ml O}_2 \text{ kg}^{-1} \text{ hr}^{-1}$) of each tortoise was calculated from the data collected using the following equation:

$$\text{sRVO}_2 (\text{ml O}_2 \text{ kg}^{-1} \text{ hr}^{-1}) = \{[(D/100)*FR]/Mb\} * 60 * 1000$$

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 statistica 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 sRVO_2 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 sRVO_2 of *Chersina angulata* ($F_{(2,530)} = 8.55$, $P < 0.001$). The Tukey post hoc test showed that the sRVO_2 at 14L:10D was significantly larger than the sRVO_2 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 sRVO_2 values of tortoises at different light regimes were calculated and plotted (see Figs. 1–3). Under 14L:10D (Fig.1), the highest values of sRVO_2 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.

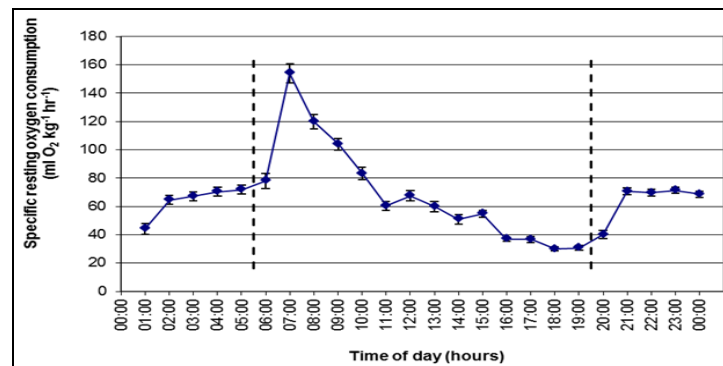


Fig 1: Mean hourly specific resting oxygen consumption of *Chersina angulata* determined under a 14 h light and 10 h dark regime at $26 \pm 1^\circ\text{C}$ over 24 hours. The broken vertical lines show when the lights were put on (left) and off (right) during acclimation and during the experiments.

The vertical bars are standard errors. N = 8.

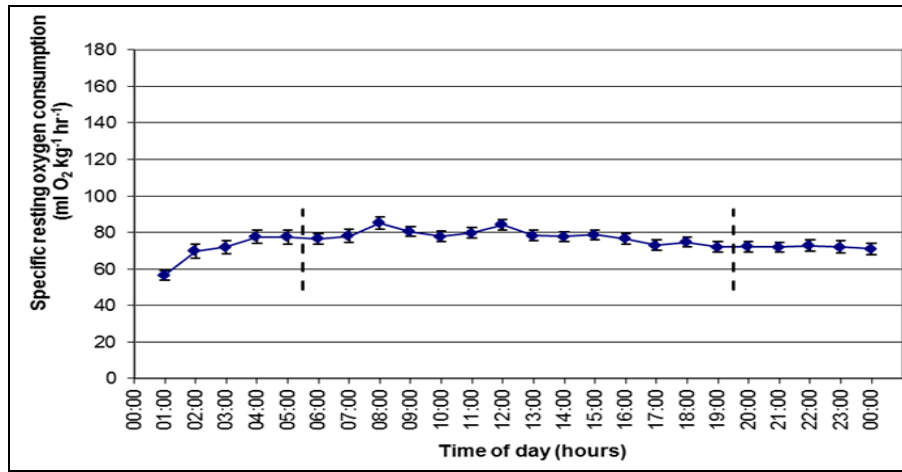


Fig 2: Mean hourly specific resting oxygen consumption of *Chersina angulata* determined under constant darkness at 26 ± 1 °C over 24 hours. The broken vertical lines show when the lights were put on (left) and off (right) during acclimation. The vertical bars are standard errors. N = 8.

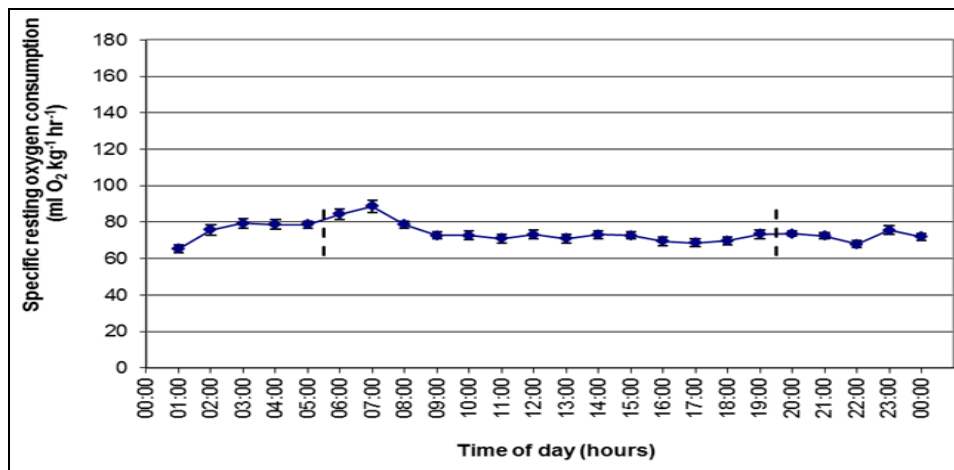


Fig 3: Mean hourly specific resting oxygen consumption of *Chersina angulata* determined under constant light regime at 26 ± 1 °C over 24 hours. The broken vertical lines show when the lights were put on (left) and off (right) during acclimation. The vertical bars are standard errors. N = 7.

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 sRVO₂ 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 *Sceloporus occidentalis* by [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 *C. angulata* may suggest that *C. angulata* has the capability to thermoregulate physiologically to some extent. The effect of varying ambient temperature on the sRVO₂ of *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.

Acknowledgements

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References

- Rutter J, Reick M, McKnight SL. Metabolism and the control of circadian rhythms. Annual Review of Biochemistry. 2002; 71:307-331.
- Birchard GF, Nelson NJ, Daugherty CH. A circadian rhythm in oxygen consumption rate in juvenile tuatara (*Sphenodon punctatus*). New Zealand Journal of Zoology. 2006; 33:185-188.
- Cruz JGP, Grimes C, Ronchi DL, Cunha VP. The influence of circadian rhythms on the metabolism of the snake *Bothrops jararaca* (Serpentes, Viperidae). Maringa. 2008; 30:327-331.
- Andrews RM, Pough FH. Metabolism of squamate reptiles: allometric and ecological relationships. Physiological Zoology. 1985; 58:214-231.
- Brown TK, Nagy KA, Morafka DJ. Costs of growth in tortoises. Journal of Herpetology. 2005; 39:19-23.
- Scantlebury M, Minting P. Differences in resting metabolic rates of two southern African tortoises: *Psammobates oculiferus* and *Geochelone pardalis*. Journal of Herpetology. 2006; 55:161-165.
- Clark NJ, Gordos MA, Franklin CE. Thermal plasticity of diving behaviour, aquatic respiration, and locomotor performance in the Mary River turtle, *Elusor macrurus*. Physiological and Biochemical Zoology. 2008; 81:301-309.
- Kirsch R, Vivien-Roels B. Oxygen consumption in the tortoise, *Testudo hermanni* G., subjected to temperature changes in summer and autumn. Comparative Biochemistry and Physiology A. 1984; 79:513-517.
- Hochscheid S, Bentivegna F, Speakman JR. Long-term cold acclimation leads to high Q₁₀ effects on oxygen consumption of loggerhead sea turtles *Caretta caretta*. Physiological and Biochemical Zoology. 2004; 77:209-222.
- Roe JH, Hopkins WA, Snodgrass JW, Congdon JD. The influence of circadian rhythms on pre- and post-prandial metabolism in the snake *Lamprophis fuliginosus*. Comparative Biochemistry and Physiology A. 2004; 139:159-168.
- Roe JH, Hopkins WA, Talent LG. Effects of body mass, feeding and circadian cycles on metabolism in the lizard *Sceloporus occidentalis*. Journal of Herpetology. 2005; 39:595-603.
- Hare KM, Pledger S, Thompson MB, Miller JH, Daugherty CH. Daily patterns of metabolic rate among New Zealand lizards (Reptilia: Lacertilia: Diplodactylidae and Scincidae). Physiological and Biochemical Zoology. 2006; 79:745-753.
- Milsom WK, Andrade DV, Britto SP, Toledo LF, Wang T, Abe AS. Seasonal changes in daily metabolic patterns of tegu lizards (*Tupinambis merianae*) placed in the cold (17 °C) and dark. Physiological and Biochemical Zoology. 2008; 81:165-175.
- Zaidan F. Variation in cottonmouth (*Agkistrodon piscivorus leucostoma*) resting metabolic rates. Comparative Biochemistry and Physiology A. 2003; 134:511-523
- Boycott RC, Bourquin O. *The Southern African Tortoise Book: A guide to the southern African tortoises, terrapins and turtles*. Lowry Publishers, Johannesburg, South Africa, 2000.
- Ramsay SL, Hofmeyr MD, Joshua QI. Activity patterns of the angulate tortoise (*Chersina angulata*) on Dassen Island, South Africa. Journal of Herpetology. 2002; 36:161-169.
- Greig JC, Burdett PD. Patterns in the distribution of southern African terrestrial tortoises (Cryptodira: Testudinidae). African Zoology. 1976; 11:249-273.
- Els SF. Aspects of thermoregulation of *Chersina angulata*. Herpetological Association of Africa. 1989a; 36:74.
- Branch WR. Preliminary observations on the ecology of the angulate tortoise (*Chersina angulata*) in the Eastern Cape Province, South Africa. Amphibia-Reptilia 1984; 5:43-55.
- Els SF, Erasmus T, Winter PED. Heating and cooling rates and their effects upon heart rate in the angulate tortoise, *Chersina angulata*. South African Journal of Zoology. 1988; 23:230-234.
- Coulson RA, Hernandez T. Oxygen debt in reptiles: relationship between the time required for repayment and metabolic rate. Comparative Biochemistry and Physiology A. 1980; 65:453-457.
- McCue MD, Lillywhite HB. Oxygen consumption and the energetics of island dwelling Florida cottonmouth snakes. Physiological and Biochemical Zoology. 2002; 75:165-178.
- Litzgus JD, Hopkins WA. Effect of temperature on metabolic rate of the mud turtle (*Kinosternon subrubrum*). Journal of Thermal Biology. 2003; 28:595-600.
- Hailey A, Loveridge JP. Metabolic depression during dormancy in the African tortoise *Kinixys spekii*. Canadian Journal of Zoology. 1997; 75:1328-1335.

25. Hailey A. The specific dynamic action of the omnivorous tortoise *Kinixys spekii* in relation to diet, feeding pattern and gut passage. *Physiological Zoology*. 1998; 71:57-66.
26. Zar JH. Biostatistical analysis. Edn. 4, Prentice Hall, Pearson, 1998.
27. Els SF. Eco physiological studies on the tortoise, *Chersina angulata*. PhD thesis, University of Port Elizabeth, Port Elizabeth, South Africa, 1989b.
28. Bennett AF, Dawson WR. Metabolism. In: *Biology of the Reptilia*, (eds) Gans C and Pough FH. Academic Press, New York. 1976; 5:127-223.
29. Kuchling G. Seasonal variations of the oxygen consumption and the blood glucose concentration under low temperature conditions in the male tortoise, *Testudo hermanni Gmelin*. *Amphibia-Reptilia*. 1981; 2:235-241.