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Annual variation in the oxygen utilisation dynamics of the garden lizard, *Calotes versicolor*

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Abstract. Energy requirement of *Calotes versicolor*, as judged by the rate of oxygen consumption by whole body, liver and muscle, was recorded to be minimum during hibernation (quiescent phase) and maximum during breeding phase. Kidney metabolic rate was comparatively high during hibernation and post-hibernation. Statistically significant circannual rhythms were detected in the rate of oxygen consumption of whole body, liver, muscle and kidney but not in that of brain. However, significant but transient increase in the rate of brain oxygen uptake was recorded during February (arousal), May (initiation of breeding) and November (entry into hibernation). It seems that the annual changes in metabolic rate are reflections of energy requirements of the lizard and its tissues during different phases of the annual activity cycle. Further, climatic factors seem to affect the oxidative metabolism of *Calotes* acting through the endocrine glands.

Keywords. Reptiles; energy metabolism; respiration; oxygen consumption; hibernation; reproduction.

1. Introduction

There are various reports on physiological and biochemical reflections of circannual rhythmicity in homeotherms (Johansson and Senturia 1972; Senturia and Johansson 1974), however, information available on poikilotherms is scanty (Bennett and Dawson 1976; McDonald 1976). While information on behavioural thermoregulation and on the physiology of activity in lizards is well documented (Bennett 1973a,b, 1980; Bennett and Dawson 1972; Huey and Slatkin 1976; Avery 1976), little attention has been paid towards the energetics for reproduction and hibernation (McDonald 1976; Bennett and Dawson 1976). Unlike in temperate zone reptiles, little is known about the physiological correlates of metabolically critical annual events like hibernation, molting and reproduction in tropical reptilian species (McDonald 1976). Earlier reports were concerned mainly either with the whole body oxygen consumption or tissue respiration of lizards during and/or after hibernation. However, no attempt has been made to investigate the sequential changes simultaneously in both the whole body oxygen consumption and tissue respiration during different phases of the annual activity cycle, and especially in relation to the ever-changing both internal and external factors which are known to influence the over-all metabolism of reptiles (Bennett and Dawson 1976; McDonald 1976; Thapliyal 1980a,b; Gupta 1982). Most of the reports are confined to the energy metabolism of temperate species only (Bennett and Dawson 1976; Thapliyal and Gupta 1984). Moreover, reports on seasonal variations in the rate of tissue respiration is limited to two species only, i.e., *Thamnophis sirtalis* (Hoskins and Aleksiuk 1973) and *Natrix piscator* (Thapliyal and Sharan 1980). These studies were limited to few months only, and no attempt was made to study circannual variations in whole body oxygen consumption. Therefore, an attempt was made to study the annual changes in whole body oxygen consumption and the rate of tissue respiration especially in relation to reproduction and hibernation in a tropical lizard, *Calotes versicolor*.

2. Materials and methods

C. versicolor is a small-sized, non-poisonous, carnivore lizard found throughout the year in Varanasi (latitude, 25°18′ N; longitude, 83°01′ E). In nature as well as in laboratory conditions *C. versicolor* hibernates during winter months and breeds during monsoon. The annual cycle of activity of the lizard at Varanasi can be divided into 4 distinct phases: hibernation (November–January), post-hibernation (February–May), breeding (June–August), and pre-hibernation (September–October).

Adult male *C. versicolor* (average body weight 30 ± 2 g; snout to vent length 10 ± 2 cm) were collected from area during the first week of each month and were caged in wire-netted wooden cages fully exposed to environment: natural day length, temperature and other climatic factors. Animals were acclimatized for one week. During this period they were provided with live maggots (meal worms) and water *ad libitum*. At the end of 7 days, whole body oxygen consumption of 4 lizards was measured at 30°C in each month using a constant pressure volumetric respirometer under complete darkness (McDonald 1976).

Animals were fasted for 24 hr before measuring the rate of respiration. Further, to minimise the effect of circadian changes on the rate of oxygen consumption (Pati *et al* 1982), the rate of respiration was always measured 2 hr before sunset. A 30 day interval was maintained between subsequent measurements. Just after the measurement of whole body oxygen consumption, lizards were decapitated, tissues (liver, skeletal muscle, kidney and brain) removed, rinsed in ice-cold reptilian Ringer's solution (Gupta 1982) and stored in a freezer at −15°C. The rate of tissue respiration was measured in a Warburg apparatus (Warburg Apparat V166; B Braun Melsungen, Federal Republic of Germany) following the method of Umbreit *et al* (1964). α-Ketoglutaric acid (30 mM) was used as substrate (for details see Gupta and Thapliyal 1983). Results were expressed in ml O_2/g/hr and μl O_2/mg wet tissue/hr for the rate of oxygen consumption by whole body and tissue respiration, respectively.

The mean environmental temperature was measured at the cage level (figure 1). Data were analysed statistically with the help of Student's 't' test (Snedecor 1961). For evaluating the circannual rhythms in 12 months data, least square fitting of cosine function was utilized (Halberg *et al* 1972).

3. Results

An annual pattern of variation in the rate of oxygen consumption by whole body of the lizard and its tissues was recorded (figure 1). While the rate of oxygen consumption of whole body, liver and muscle was minimum during hibernation, it increased gradually during post-hibernation and reached a maximum during the breeding phase. The metabolic rate of kidney and brain were comparatively more during hibernation and were greater during the post-hibernation period (table 1). Further, a significant

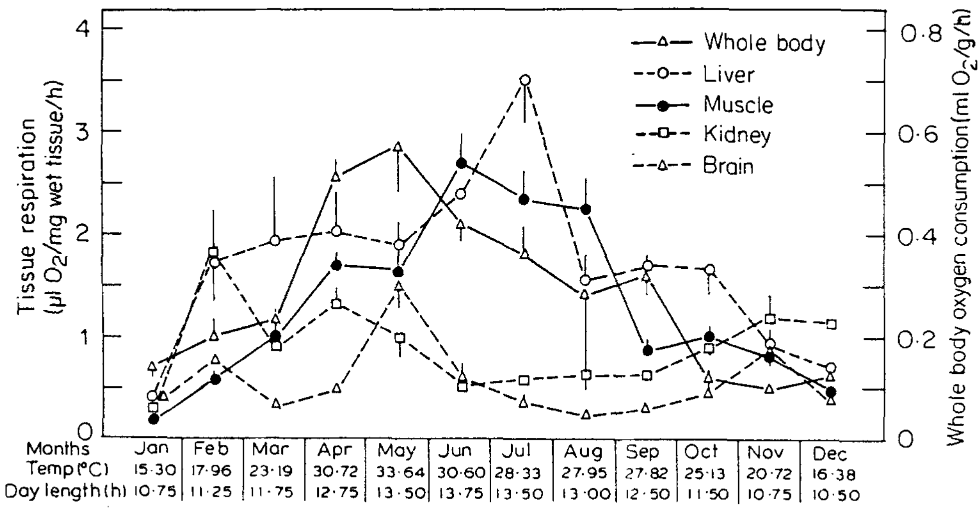

Figure 1. Annual variations in the rate of oxygen consumption by whole body and isolated tissues (liver, muscle, kidney and brain) of *C. versicolor*. Vertical bars represent 1 Standard error ($n=4$).

Table 1. Average metabolic rates of whole body and different tissues during different phases of the annual activity cycle of *C. versicolor*.

Variable (Unit)	Phase of the annual activity cycle: Hibernation ($n=12$)	Post-hibernation ($n=16$)	Breeding ($n=12$)	Pre-hibernation ($n=8$)
Whole body oxygen consumption (ml O_2/g/h)	0·12±0·01*	0·38±0·05^{b}	0·35±0·02^{b}	0·22±0·04^{a,c}
Tissue O_2 consumption (O_2/mg wet tissue/h)				
a) Liver	0·64±0·09	1·90±0·20^{b}	2·42±0·31^{b}	1·67±0·11^{b,c}
b) Muscle	0·48±0·09	1·25±0·14^{b,d}	2·47±0·16^{b}	0·94±0·07^{b,d}
c) Kidney	0·92±0·13	1·29±0·14^{d}	0·58±0·05^{a}	0·77±0·06^{c}
d) Brain	0·55±0·08	0·76±0·12^{c}	0·39±0·06	0·38±0·05

*Mean ± SE
^{a,b}Differ from the value of hibernation: $P<0·05$ and $P<0·001$, respectively.
^{c,d}Differ from the value of breeding: $P<0·05$ and $P<0·001$, respectively.
Based on the data plotted in figure 1.

transient increase in the brain O_2 uptake was found during the months of February, May and November (figure 1).

When the data were analysed for circannual rhythm test with the help of least square fitting of cosine function, significant circannual rhythms were detected in the rate of oxygen consumption by whole body, liver, muscle and kidney (table 2, figure 2). However, no significant circannual rhythm was recorded for brain oxygen consumption. While acrophases for metabolic rate of whole body, liver and muscle were obtained during breeding phase, that of kidney was recorded during February—the period of arousal from the winter sleep (table 2, figure 2).

Table 2. Rhythmometric summary based on least-square fitting of 365·25-d cosine function (365·25 = 360°) to data plotted in figure 1.

Variable (Unit)	Key to elipses	No. obs.[a]	PR[b]	P[c]	M[d] ± SE[e]	A[f] ± SE	ϕ^g ± SE (95% CL[h]) Degree	ϕ^g ± SE (95% CL[h]) Month/date
Whole body oxygen consumption (ml O_2/g/h)	A	48	67	0·001	0·281 ± 0·014	0·197 ± 0·020	−205 ± 06 (−193; −217)	July 14 (July 2; July 26)
Tissue O_2 uptake (μl O_2/mg wet tissue/h)								
Liver	B	48	41	0·001	1·679 ± 0·111	0·873 ± 0·158	−186 ± 10 (−166; −206)	June 25 (June 5; July 15)
Muscle	C	48	73	0·001	1·298 ± 0·065	1·023 ± 0·092	−182 ± 05 (−172; −192)	June 21 (June 11; July 1)
Kidney	D	48	21	0·005	0·933 ± 0·065	0·313 ± 0·092	−47 ± 17 (−14; −80)	Feb. 7 (Jan. 5; March 11)
Brain	E	48	9	0·129	0·552 ± 0·054	0·159 ± 0·077	−110 ± 28 (−0; −0)	April 10 (00; 00)

[a]Number of observations; [b]Percent rhythm (percentage of variability accounted for by cosine curve); [c]Probability of hypothesis that amplitude = 0; [d]Mesor (arithmatic mean of observations made over 12 months); [e]Standard error; [f]Amplitude (one half difference between highest and lowest values); [g]Acrophase (lag between the timing of peak metabolic function and arbitrary reference time (Ref., December 22); [h]Confidence limit.

See figure 2 for elipses.

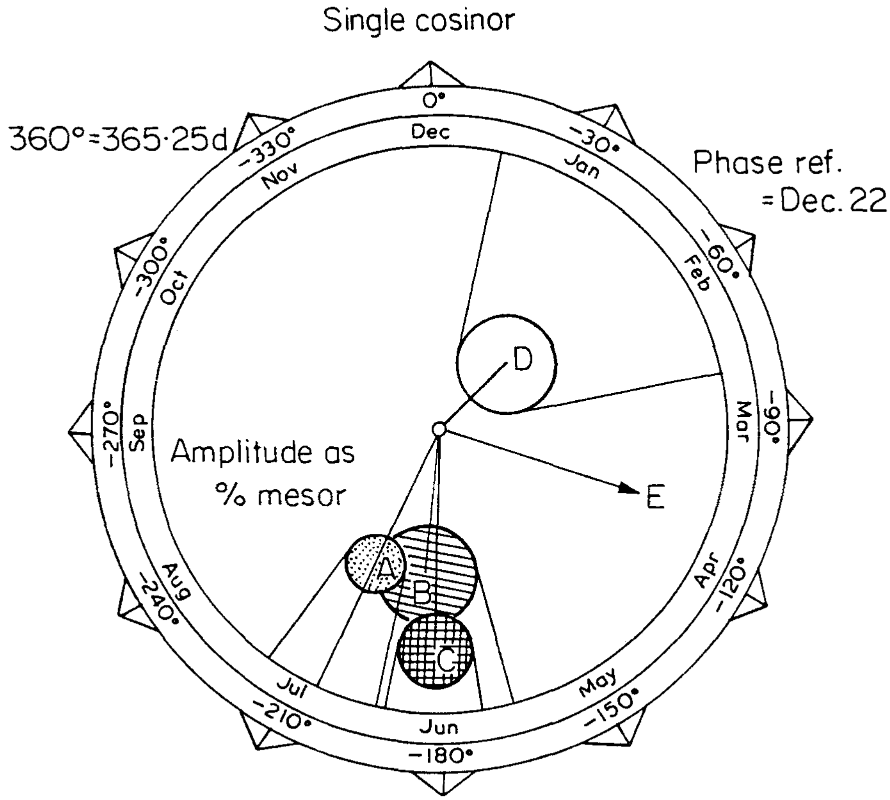

Figure 2. Microscopic parameter estimations on circannual rhythms of oxygen consumption rate by whole body and different tissues of *C. versicolor*. The direction of the vector and the area covered by two lines which touch the inner circle at the periphery indicate the span of greater metabolic activity. The length of the same vector indicates the extent of predictable periodic changes, i.e., the rhythm amplitude (expressed as % mesor). The circle does not overlap the pole indicates detection of rhythm by cosinor method. Calculation is based on least square fitting of 365·25 d cosine curve. (A), Whole body; (**B**), liver; (C), muscle; (**D**), kidney; (E), brain.

4. Discussion

The present study is a comprehensive study of the annual variation in the rate of oxygen consumption of a tropical reptile and of its vital tissues (liver, muscle, kidney and brain) during the year.

Seasonal variations in the rate of whole body oxygen consumption indicate that the metabolic rate of *C. versicolor* changes with the energy demand during different phases of the annual activity cycle. During hibernation, when most of the metabolic pathways are either non-operative or running at a low level, and endocrine glands (except chromaffin tissues) are inactive or minimally active (Singh 1968; Thapliyal and Chandola 1973; Kashinathan and Basu 1973; Haldar 1977; Thapliyal 1980b), the lizard becomes lethargic and inactive, and needs minimum energy just sufficient for the maintenance of life. During this phase the metabolic rate of the lizard is not influenced by high ambient temperature even when raised artificially (Gupta and Thapliyal 1985; Thapliyal 1980b). Further, monthly changes in whole body O_2

consumption and tissue respiration exhibit no direct relationship with the monthly variation in the ambient temperature (figure 1). Gradual increase in the metabolic rate during post-hibernation seems to indicate an increased capacity to meet the enhanced energy requirements for the increased activity associated with feeding/foraging, offence/defence etc. During the breeding phase the metabolic rate of the lizard remained fairly high probably due to a further increase in energy demand for the maintenance of vigorous activity related to reproductive functions. Higher metabolic rate during pre-hibernation than that during hibernation may be due to high energy demand for post-nuptial molting (figure 1). It is thus obvious that the metabolic rate of the lizard depends upon the need of energy production during different phases of the annual activity cycle.

The annual patterns of oxygen consumption of the tissues were not similar. While changes in the oxidative capacity of liver seem to be correlated with the availability of food and feeding behaviour, the oxidative capacity of muscle is correlated with the level of activity of the lizard during different months of the year (figure 1). The higher rate of kidney oxygen consumption in mammals is reportedly associated with energy production required for conservation of water and electrolytes, and excretion of harmful metabolites (Kill 1971). Thus, the high metabolic rate of kidney during hibernation seems to indicate high rate of energy production. The increased kidney O_2 uptake during hibernation, when the lizard experiences prolonged cold stress, may be of a great physiological significance. Since the presence of excretory products (i.e., urea, uric acid, and other nitrogenous waste products) in blood even in minute quantity may endanger survival of the lizard during hibernation. Therefore, kidney probably requires more energy to meet the excretory demand. High oxidative capacity of brain during winter months seems to indicate increased activity of the brain. In *C. versicolor* as well as in other reptiles, brain oxygen consumption has been shown to be increased under artificially lowered room temperatures (Das and Patnaik 1979; Sharan *et al* 1981). It thus seems that the increase in the central oxidative capacity in response to low temperatures of winter protects the animal against freezing during hibernation phase. Transient increase in the metabolic capacity of brain during February (month of arousal), May (when *Calotes* enters in breeding phase) and November (time to enter hibernation) may be associated with the increased synthesis of chemical transmitters and/or transmission of neural impulses which are critical for signalling critical time for initiation and termination of reproduction and hibernation (Thapliyal and Sharan 1980). The high metabolic capacity may also be required to meet the increased energy demand of the brain tissue to maintain ion gradients for high electrical activity during these critical periods.

Statistically significant (table 2) circannual rhythms in the metabolic rate of whole body, liver and muscle with acrophases during breeding phase indicate that, to meet greater energy demand during breeding phase, annual cycles in metabolic rate and reproductive functions are synchronized by some common factor(s). Acrophase of Kidney O_2 uptake during February may be associated with sudden acceleration of catabolic processes at the time of arousal. The absence of a significant circannual rhythm in brain tissue respiration seems to suggest a constant level of brain activity throughout the year, and also its independence from the annually changing factors, both internal and external. Obviously, the stabilized and self-regulated metabolic capacity of brain probably helps the lizard in co-ordinating vital metabolic processes for successful survival under the high summer and low winter temperatures.

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References

Avery R A 1976 Thermoregulation, metabolism and social behaviour in Lacertidae; in *Morphology and physiology of reptiles*; (eds) A d' A Bellairs and C Barry Cox; (New York: Academic Press) pp 245–259

Bennett A F 1973a Ventilation in two species of lizards during rest and activity; *Comp. Biochem. Physiol.* **A46** 653–671

Bennett A F 1973b Blood physiology and oxygen transport during activity in two lizards *Varanus gouldii* and *Sauromalus hispidus*; *Comp. Biochem. Physiol.* **A46** 673–690

Bennett A F 1980 The metabolic foundations of vertebrate behaviour; *BioScience* **30** 452–456

Bennett A F and Dawson W R 1972 Aerobic and unaerobic metabolism during activity in the lizard *Dipsosaurus dorsalis*; *J. Comp. Physiol.* **61** 289–299

Bennett A F and Dawson W R 1976 Metabolism; in *Biology of the reptilia*, (eds) C Gans and W R Dawson (London, New York, San Fransisco: Academic Press) Vol 5, pp 127–224

Das K and Patnaik B K 1979 Effect of age, sex and cold exposure on pyruvate oxidation by midbrain of the garden lizard, *Calotes versicolor*; *Indian J. Exp. Biol.* **17** 1371–1372

Gupta B B P 1982 *Endocrine control of oxidative metabolism in Indian gardan lizard, Calotes versicolor*, Doctoral Dissertation, Banaras Hindu University, Varanasi

Gupta B B P and Thapliyal J P 1983 Adrenal hormones and the oxidative metabolism in the garden lizard (*Calotes versicolor*); *J. Endocrinol.* **99** 211–216

Gupta B B P and Thapliyal J P 1985 Role of thyroid and testicular hormones in the oxidative metabolism of the Indian garden lizard, *Calotes versicolor*; *Gen. Comp. Endocrinol.* **58** 20–27

Halberg F, Johnson E A, Nelson W and Sothern R 1972 Autorhythmometry procedures for self measurements and their analysis; *Physiol. Teacher* **1** 1–11

Haldar C 1977 *Pineal in reptiles*, Doctoral Dissertation, Banaras Hindu University, Varanasi

Hoskins M A H and Aleksiuk M 1973 Effects of temperature, photoperiod and season on *in vitro* metabolic rate of tissues from *Thamnophis sirtalis parietalis*, a cold climate reptile; *Comp. Biochem. Physiol.* **A45** 737–756

Huey R B and Slatkin M 1976 Cost and benefits of lizard thermoregulation; *Q. Rev. Biol.* **31** 363–384

Johansson B W and Senturia J B 1972 Seasonal variations in the physiology and biochemistry of the European hedgehog, *Erinaceus europaeus* including comparison with non-hibernators, guinea pig and man; *Acta Physiol. Scand. Suppl.* **380** 1–159

Kasinathan S and Basu S L 1973 Seasonal variation in testes, accessory ducts and adrenal glands of *Calotes versicolor* (Daudin); *Acta Morphol. Acad. Sci. Hung.* **21** 271–279

Kill F 1971 Blood flow and oxygen utilization by the kidney; in *Kidney hormones*; (ed) J W Fisher (London, New York: Academic Press) pp 1–30

McDonald H S 1976 Methods for physiological study of reptiles; in *Biology of the reptilia*; (eds) C Gans and W R Dawson (London, New York, San Fransisco: Academic Press) Vol 5, pp 19–126

Pati A K, Gupta B B P and Thapliyal J P 1982 Circadian rhythm in oxidative metabolism of the Indian garden lizard, *Calotes versicolor*; *Proc. Natl. Acad. Sci. India* **B51** 250–256

Senturia J B and Johansson B W 1974 Physiological and biochemical reflections of circannual rhythmicity in the European hedgehog and man: in *Circannual clocks*; (eds) Erick T Pengelley; (New York, San Fransisco, London: Academic Press) pp 253–296

Sharan S, Thapliyal J P and Pati A K 1981 Influence of cooling of the hypothalamus on circadian rhythm of oxygen uptake of the brain and blood morphology of chequered water snake, *Natrix piscator*; *Int. J. Chronobiol.* **17** 122

Singh G S 1968 *Sexual cycle and secondary sex characters of Indian garden lizard, Calotes versicolor*, Doctoral Dissertation, Banaras Hindu University, Varanasi

Snedecor C W 1961 *Statistical methods* (Bombay: Pacific Private Ltd.)

Thapliyal J P 1980a Thyroid in reptiles and birds; in *Hormones, adaptation and evolution* (eds) S Ishii, T Hirano and M Wada (Tokyo/Springer-Verlag: Japan Sci. Soc. Press) pp 240–250

Thapliyal J P 1980b Thyroid in reptiles; *Proc. Natl. Acad. Sci. India* (*Golden Jubilee Commemoration Volume*) pp 530–540

Thapliyal J P and Chandola A 1973 Seasonal variation in thyroid hormonogenesis in the Indian garden lizard, *Calotes versicolor*; *J. Endocrinol.* **56** 451–462

Thapliyal J P and Gupta B B P 1984 Hormonal Regulation of the Oxidative Metabolism in Reptiles; in *Recent Trends in Life Sciences* (eds) A Gopal Krishna, S B Singh and A K Saxena (Kanpur: Manu Publication) pp 260–275

Thapliyal J P and Sharan S 1980 Seasonal variation in the oxygen consumption of tissues in the chequered water snake, *Natrix piscator*: with special reference to hibernation; *Indian J. Exp. Biol.* **18** 236–239

Umbreit J E, Burris R H and Stauffer J E 1964 *Manometric Techniques* (Mineapolis, Minnesota: Burgess)