

Seasonal changes in daily metabolic patterns of *Lacerta viridis*

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Summary. *Lacerta viridis* maintained under natural photoperiodic conditions show daily and seasonal changes in metabolic rates and body temperature (T_b) as well as seasonal differences in sensitivity to temperature change. At all times of the year lizards have a daily fluctuation in oxygen consumption, with higher metabolic rates during the light phase of the day when tested at a constant ambient temperature (T_a) of 30 °C. Rhythmicity of metabolic rate persists under constant darkness, but there is a decrease in the amplitude of the rhythm.

Oxygen consumption measured at various T_a s shows significant seasonal differences at T_a s above 20 °C. Expressed as the Arrhenius activation energy, metabolic sensitivity of *Lacerta viridis* shows temperature dependence in autumn, which changes to metabolic temperature independence in spring at T_a s above 20 °C. The results indicate a synergic relationship between changing photoperiod and body temperature selection, resulting in seasonal metabolic adjustment and seasonal adaptation.

Key words: Oxygen consumption – Photoperiod – Circadian rhythm – Temperature – Lizard, *Lacerta viridis*

Introduction

It is now widely recognized that reptiles exhibit diel variations in their metabolic rates (Songdahl and Hutchison 1972; Cragg 1978; Mautz 1979; Feder and Feder 1981; Brownlie and Loveridge 1983). Although not as widely documented, seasonal changes in daily metabolism of reptiles have been reported (Roberts 1968; Heusner and Jameson 1981). The decreased winter O_2 consumption of cool-climate reptiles which hibernate is usually attributed to lower environmental temperatures. This study shows how photoperiod and temperature are

synergic environmental cues which influence reptiles' metabolic rates, reflecting seasonal adaptation. Acute temperature fluctuations can occur at any time of year, whereas the photoperiod remains an unflinching and "noise-free" indication of the past or coming season.

Numerous studies have tested temperature acclimation in both warm and cool climate reptiles; however, few have attempted to correlate diel or seasonal cycles of metabolic rates with this phenomenon. Cool-climate reptiles, which undergo winter dormancy, generally decrease metabolic rates in autumn/winter as a response to cold, and compensate for lower temperatures in spring/summer with an increase in metabolic rate when tested within normal activity range temperatures [reviewed by Tsuji (1988)]. On the other hand, animals which remain active all year round and usually inhabit areas of more moderate temperature change, increase metabolism during cooler times of the year, otherwise showing little compensation of metabolic rates (Dawson 1975).

Low environmental temperatures often correlate with specific times of the year. The role of photoperiodic changes that parallel normal seasonal temperature changes has not often been considered. Some authors have suggested that low T_a s which reptiles experience in autumn to early spring are responsible for seasonal acclimation, i.e., differences found in metabolic rates (Patterson and Davies 1978a, b). The results of this study indicate that in *Lacerta viridis* the photoperiod prompts changes in behavioral thermal functions which in turn results in seasonal metabolic adjustments. Environmental temperature, which is normally but not consistently lower during autumn and winter, may act synergically with photoperiod in supporting these adaptations.

Materials and methods

Lacerta viridis once occurred throughout Europe, from southern Italy, through France and up to northern Germany. Because of depletion of natural populations, animals were bred and maintained in the laboratory under conditions as natural as possible. Parent animals of *Lacerta viridis* used in this 5-year study originated from a mountain population near Bologna, Italy. These were transferred to Marburg, Germany, (50° 48' N) and held in groups in large terrariums. Lighting in the terrarium room was provided by natural spectra fluorescent lamps controlled by a timer which was reset weekly according to the natural photoperiod. Two sets of windows,

Abbreviations: ANOVA, analysis of variance; LD, long day (16 h light); SD, short day (8 h light) T_a , ambient temperature; T_b , body temperature

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facing west and north provided a natural twilight in the room. This room was unheated, which allowed a natural fluctuating thermocycle determined by the outside T_a . Animals not used in the present experiments were overwintered under natural photoperiod at 5 °C. Lizards aroused spontaneously in spring, after which adults mated and produced fertile eggs. These were incubated and raised under conditions mentioned above. *Lacerta viridis* were fed live crickets and mealworms every other day, plus earthworms, slugs and spiders during spring and summer. Water supplemented with vitamin D₃ was available ad libitum.

For experiments in this study, only healthy adult lizards with body masses ranging from 30 to 46 g were used. Six to eight lizards (half males, half females) had temperature-sensitive transmitters (Mini-mitter, model X, 1.2g) implanted intraperitoneally for monitoring deep core body temperature. Experimental animals were housed individually in thigmothermic gradient cages in a constant temperature room at 21 °C. Food and water were available ad libitum and lizards were able to thermoregulate, as the cage floor provided a uniform temperature gradient from 10 to 40 °C at all times. No radiant heat could be gained from the external light source, which was maintained under a natural photoperiod cycle [For details of gradient cage and Mini-Mitter signal retrieval, see Rismiller and Heldmaier (1982, 1988) and Ruf and Heldmaier (1987)]. Individual *Lacerta viridis* were maintained in gradient cages over several years in order to monitor voluntary seasonal changes in T_b under natural photoperiod.

Daily rhythms of oxygen consumption were measured at different times of the year. Each experiment lasted 30–78 h and was conducted at $T_a = 30$ °C under natural photoperiod. The ceiling light source which illuminated the metabolic chamber was shielded with an aluminium plate, reflecting the light upward in order to prevent radiant heat reaching the lizards during metabolic testing. Following measurements in a light-dark cycle, metabolic rates were monitored for 30 h under constant darkness.

Before all metabolic tests, animals were fasted for 2 days. Lizards were placed in 0.5-l metabolic boxes in a Weiss climate chamber which could be regulated within ± 0.1 °C of the set temperature. In an open system, air was pumped through drying tubes before entering the two-channel paramagnetic oxygen analyzer (6-channel Oxytest, Hartmann and Braun), which measured with an accuracy of 0.01 vol% of the full scale of 1 vol%. During long-term experiments (>24 h), zero point drift was monitored using an empty metabolic cuvette.

Metabolic rates were measured at an air flow of 20 l · h⁻¹ over 1-min intervals, where response time of the system was 40 s. All gas volumes were converted to standard temperature and pressure before calculating oxygen consumption according to the method of Heldmaier and Steinlechner (1981). These data, together with time, T_b of each animal, and T_a within the metabolic boxes, were transferred on-line to a desk-top computer and stored on tape for later evaluation.

At two different times of the year, spring (late March) and autumn (early September), daily metabolic rates of lizards were measured at 20, 25, 30, and 35 °C. Experiments at different tem-

peratures were not conducted on consecutive days. Lizards were returned to their gradient cages and allowed to thermoregulate for at least 24 h between each temperature regime to avoid influencing data through temperature acclimation.

Data from each experiment were tested for normal distribution according to the method of Pearson and Stephens (1964). When comparing day/night values of any given group and parameter the two-sample *t*-test was used for determination of significant differences in unrelated samples. The paired *t*-test was used for comparing related samples. ANOVA was used to determine seasonal differences in evaluated parameters. All regressions were calculated using the method of least squares. Values were considered significantly different when $P < 0.05$.

Results

Lacerta viridis show a daily rhythm in their metabolic rate when measured at a constant T_a under natural photoperiod. Figure 1 shows an original registration of O₂ consumption (solid line) and T_b (dotted line) of a single lizard during a summer testing period (16 h light: 8 h dark). Lines connect measurements taken every 6 min throughout the day and night. Lizards were post-absorbant and remained placid, without activity throughout the testing period. Large fluctuations are due to the irregular breathing of the animal. Fluctuations during

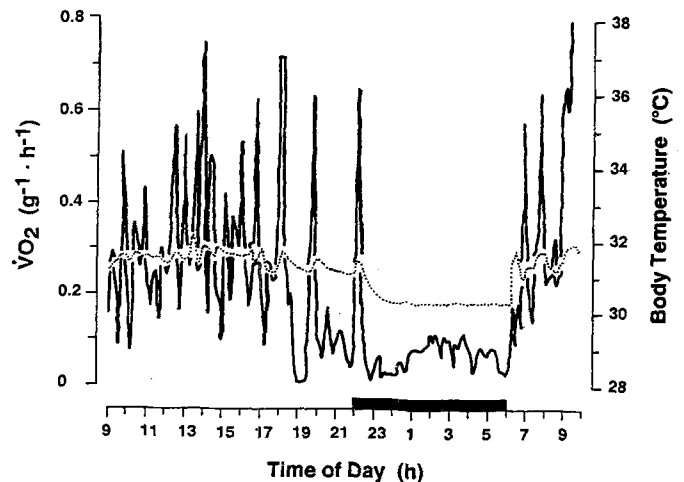


Fig. 1. Original recording of oxygen consumption (solid line) and T_b (dotted line) monitored at 30 °C over 24 h for a single lizard. The dark horizontal bar indicates the scotophase

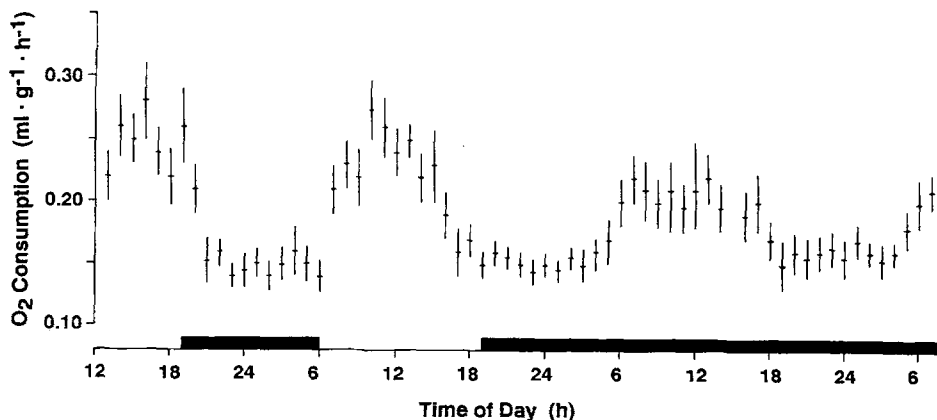


Fig. 2. Mean hourly O₂ consumption \pm SEM of *Lacerta viridis* under natural photoperiod, followed by constant darkness at $T_a = 30$ °C. Black horizontal bar indicates the dark phase ($n = 5$)

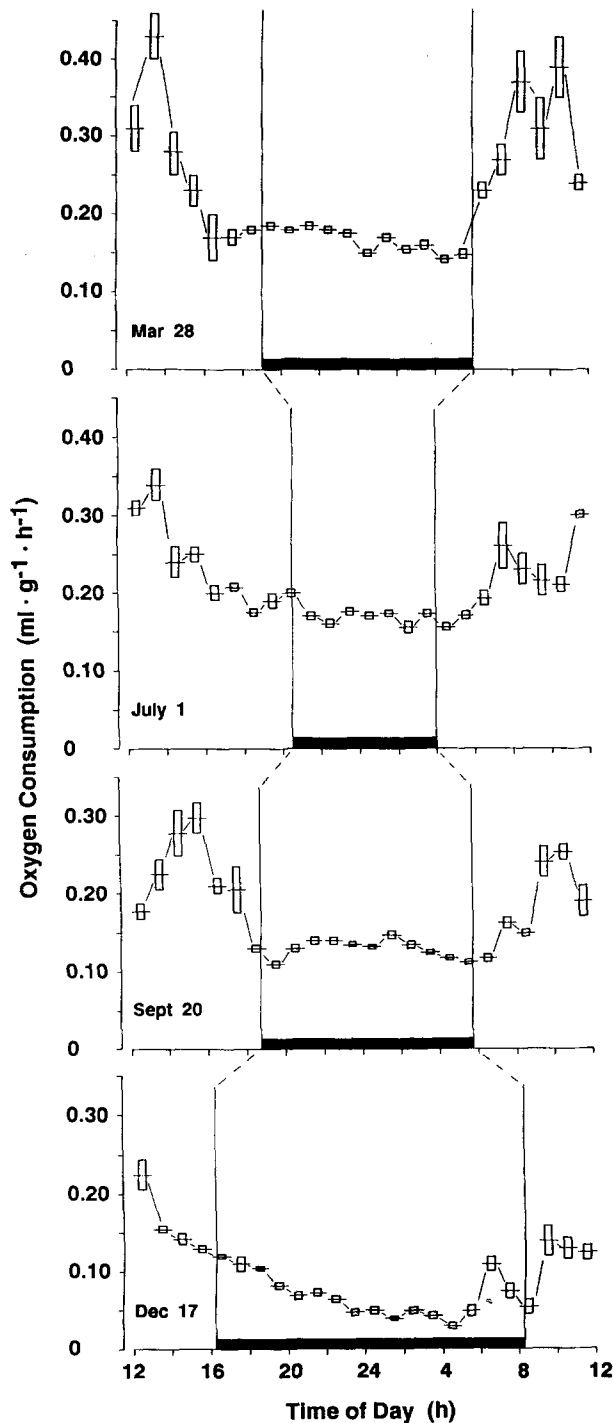


Fig. 3. Daily rhythm in O_2 consumption for different times of the year, shown as the hourly mean (horizontal line) \pm SEM (vertical box). All tests conducted at 30°C and natural photoperiod. Black horizontal bar indicates the scotophase ($n=5-8$)

the day were always greater than at night, as were the absolute levels of metabolic rate.

Monitoring of O_2 consumption under natural photoperiod was followed by measurements in constant darkness (Fig. 2). The daily rhythmicity of O_2 consumption continued with higher metabolic levels coinciding with normal daylight hours, as compared to those during the subjective night. However, metabolic rates were significantly lower ($P < 0.05$) during the subjective day in

constant darkness compared to daytime levels under natural photoperiod.

In order to compare daily patterns of metabolic rate under natural photoperiod, hourly means for groups of 5–8 lizards were calculated for different seasons of the year (Fig. 3). Highest levels of O_2 consumption were always observed several hours after “sunrise” (lights-on) and often in the early afternoon. Peaks were not associated with locomotor activity, as observations throughout the daylight hours proved. Amplitude in mean metabolic fluctuations for any given hour (reflected by the SEM) became less during the late afternoon, and lowest levels of O_2 consumption with minimum fluctuation occurred during the scotophase. Absolute duration of lower level metabolic rates coincide with an increase of the scotophase from spring to winter.

Fluctuations in day/night levels of resting O_2 consumption occurred at all times of the year. Plotted as the mean photophase and scotophase levels (Fig. 4), seasonal changes in both day and nighttime metabolic rates are apparent. The amplitude between mean day and nighttime O_2 consumption levels were significantly different ($P < 0.01$) for every experimental period, except October. March and May had significantly higher ($P < 0.001$) photophase metabolic levels, as compared to all other test periods. These months correlate with mating season and oogenesis. Daytime O_2 consumption levels in February and from June to early September were virtually the same ($0.22 \pm 0.03 \text{ ml} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$). Daytime metabolic levels from winter (December) compared to spring (March/May) were augmented 100% (0.13 and $0.30 \text{ ml} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$, respectively), whereas those at night increased 400% (0.04 and $0.17 \text{ ml} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$).

During late March and September, the effect of temperature on daily metabolic rates of *L. viridis* were measured under natural photoperiod. There was a pronounced daily pattern with significantly higher O_2 consumption during the day compared to night at all temperatures above 20°C . At 20°C , the amplitude between day/night metabolic rates was not significantly different. Indeed, differences in day and nighttime levels of O_2 consumption generally increased with increasing temperatures (Fig. 5). Comparison of metabolic rates at various temperatures and seasons reveals no significant differences at 20 or 35°C for either day or nighttime levels. However both photo- and scotophase O_2 consumption at 25 and 30°C was significantly greater in spring compared to autumn ($P < 0.01$).

Temperature coefficients have been used for decades to express reptilian metabolic sensitivity to temperature change. Hypothetically, the O_2 consumption of an ectothermic reptile conforms to van't Hoff's rule and increases with increasing temperature (Fig. 5). Values of Q_{10} for each temperature interval are presented in Table 1.

In order to determine the relation between metabolic rate and T_b , mean values of O_2 consumption were charted as an Arrhenius plot (Fig. 6). During autumn, the logarithm of O_2 consumption during both day and night is a significant ($P < 0.01$ and 0.05 , respectively) linear function of the reciprocal of absolute temperature, indicating a temperature-dependent reaction. The activation energy over the entire temperature range in autumn was 45.6 and $30.2 \text{ kJ} \cdot \text{mol}^{-1}$ for day and nighttime,

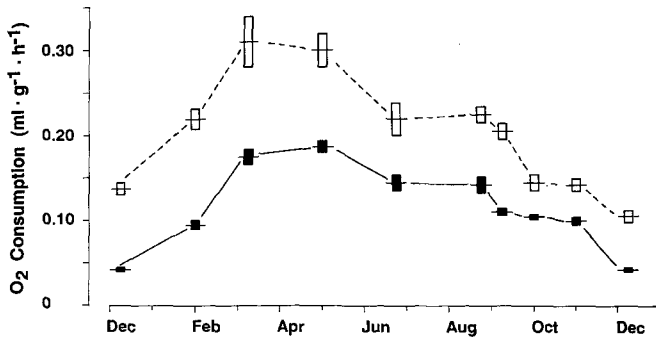


Fig. 4. Annual cycle of mean photophase (open symbols) and scotophase (closed symbols) O₂ consumption levels ± 1 SEM. All tests conducted at 30 °C under natural photoperiod (n = 5–8)

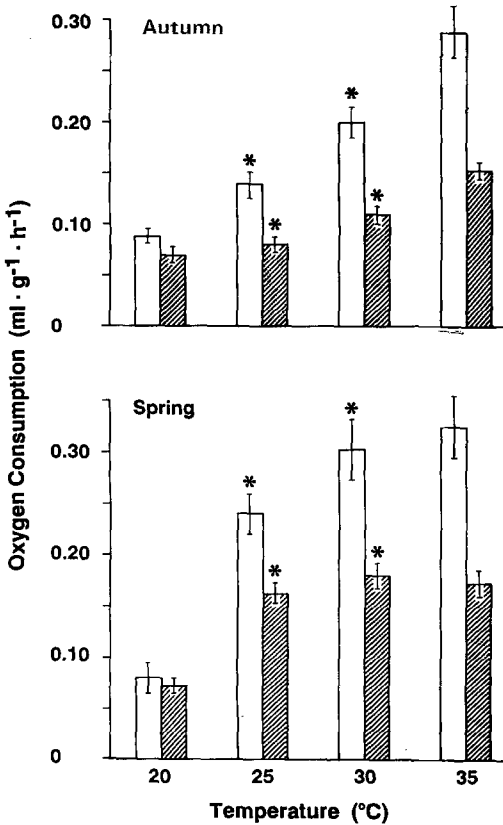


Fig. 5. Mean resting metabolic rates ± 1 SEM at different ambient temperatures during autumn (top) and spring (bottom). Open columns represent photophase and hatched columns scotophase values. All experiments conducted under natural photoperiod (n = 6–8). Day/night differences were significant (P < 0.05) at both times of year at all temperatures except 20 °C. Seasonal differences (P < 0.01) occurred at 25 and 30 °C. Seasonal differences (P < 0.01) occurred at 25 and 30 °C as indicated by *

respectively. However, metabolic rates measured during spring do not describe a temperature effect. There is no significant correlation between metabolic rate and temperature at T_bs of 35–25 °C. This response indicates that in spring, metabolic rate is independent of body temperature.

Weight loss of *L. viridis* during metabolic testing differs seasonally. A mean weight loss of 0.46 and 0.58 g occurred at 20 °C for autumn and spring, respectively. Mean losses at 35 °C were 2.02 and 1.35 g, which account for 4–10% of total body mass. Hence, autumn lizards had

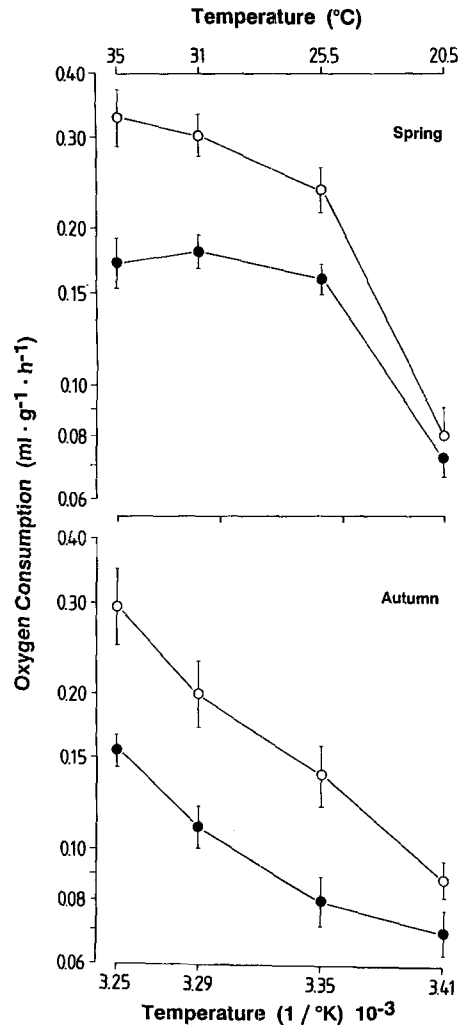


Fig. 6. Arrhenius plot for mean resting day ○ and nighttime ● metabolic values ± SEM measured at four different ambient temperatures during spring and autumn (n = 5–8). For details of significant differences see legend to Fig. 5

Table 1. Q₁₀s for different temperature intervals during spring and autumn

Temperature interval (°C)	Spring Q ₁₀		Autumn Q ₁₀	
	Day	Night	Day	Night
20–25	8.92	4.94	2.49	1.31
20–30	3.79	2.50	2.27	1.57
20–35	2.54	1.66	2.19	1.68
25–30	1.61	1.26	2.07	1.89
25–35	1.36	1.07	2.07	1.90

lower weight losses at 20 °C and significantly higher weight losses at 35 °C as compared to spring animals. Lizards overwintered at 5 °C from October through mid-March show a mean weight loss of 2.2 g.

Lacerta viridis maintained under natural photoperiod in gradient cages, show seasonal variations in daily patterns of T_b selection. Range and frequency of temperatures selected also vary (Fig. 7). At all times of the year, there were larger fluctuations in daytime T_b selection as compared to night. Greatest day/night amplitudes as well as largest daytime fluctuations occurred in

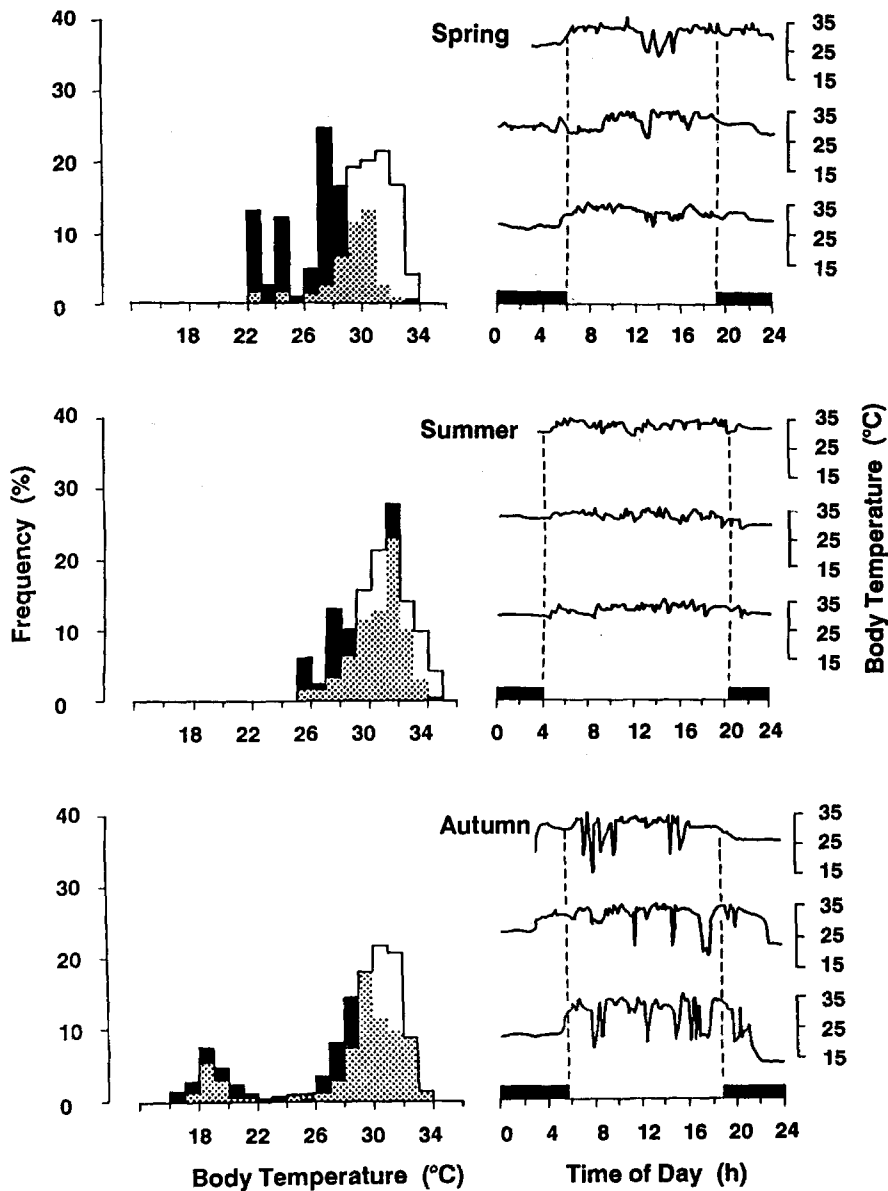


Fig. 7. Frequency distribution (left) of selected T_b s for *Lacerta viridis* at different times of the year, with original T_b data over three consecutive days shown at the right. Open areas of histogram represent photophase T_b s, closed areas scotophase T_b s, and dotted areas indicate overlapping. Each plot represents data from three animals over 5 days (= 7000 data points)

autumn. In summer, mean day and nighttime levels of T_b differed by only 1 $^{\circ}\text{C}$, whereas differences of 4 and 11.5 $^{\circ}\text{C}$, respectively, were noted in the spring and autumn.

Discussion

In this study, *Lacerta viridis* show seasonal adjustments of metabolic rates and T_b selection when maintained under natural photoperiodic conditions. Mean level and duration of photo-/scotophase metabolic rates depend on time of year, and also correlate with seasonal variation in daily T_b patterns. Day/night metabolic rates are greatest in spring, corresponding to periods of mating and oogenesis, and decrease throughout the summer and autumn months. Seasonal changes in response to different ambient temperatures, together with adjustments in O_2 consumption and T_b selection, reflect changes which may be essential to survival in the natural environment of a cool-climate reptile.

Results of other studies also indicate a metabolic response to photoperiod, with possible photoperiod acclimation. Cragg (1978) obtained *L. viridis* from impor-

ters during summer months and maintained them under a light: dark cycle of 12:12 h. Metabolic rates of these animals during both photo- and scotophase are similar to the present measurements under natural photoperiod in September. Ragland et al. (1981) found summer/winter differences in the metabolic rates of the lizard *Anolis carolinensis* acclimated during the summer to 10 and 30 $^{\circ}\text{C}$ in total darkness for 14 days. In the summer, lizards acclimated to 10 $^{\circ}\text{C}$ showed the expected upward shift in metabolic rate compared to the 30 $^{\circ}\text{C}$ group. However, O_2 consumption of *A. carolinensis* captured in the winter was lower than either laboratory group.

The effect of photoperiod on other physiological parameters has also been reported. Licht (1968) studied thermal tolerance of *A. carolinensis* acclimated to different temperatures and photoperiods. At any temperature, lizards under 14 h light were more heat resistant than those maintained at 6 or 0 h light. However, Songdahl and Hutchison (1972) documented a reverse effect of photoperiod in *Sceloporus cyanogenys*. Lizards acclimated to short photoperiod always showed higher metabolic rates than those acclimated to long photoperiod,

irrespective of acclimation temperature. It may be advantageous for *S. cyanogenys*, a warm-climate lizard, to have lower metabolic rates during the hot summer months to help conserve water and energy. These results, taken together with those of the present study, indicate that photoperiod helps to cue seasonal changes in behavior and physiology. Low ambient temperatures appear to be essential for survival of cool-climate lizards throughout winter months, as they subsequently influence reduction of metabolic rates and further suppress energy expenditure. This energy conservation is reflected in the small loss of body mass observed in *L. viridis* during the hibernation period.

Patterson and Davies (1984) stated that T_b selection by a reptile under natural photoperiod must be considered when studying temperature acclimation of metabolic rates. In turn, if metabolic compensation is important to seasonal adaptation in cool-climate lizards, it should be related to seasonal levels of T_b , which are not necessarily directly correlated with seasonal levels of environmental temperature (Mayhew 1963). Few studies show the extent of metabolic compensation at species-characteristic selected T_b s of reptiles. Bennett and Dawson (1976) concluded that there is little, if any, adjustment of resting metabolic rate to levels of T_b . Innumerable discussions on how and what the selected T_b of a reptile represents and how it should be measured (Brattstrom 1965; Dawson 1975) are beyond the scope of this paper. However, the finding that *L. viridis* selects a lower (especially nighttime) T_b with the decreasing photoperiod of autumn suggests that in lizards inhabiting high latitudes selection of lower temperatures are cued by the photoperiod, and that this in turn influences metabolic rates.

Fritzpatrick et al. (1978) documented patterns of partial and complete metabolic independence of temperature coinciding with the selected T_b range for active, thermoregulating *Sceloporus olivaceus*. However, this independence of ambient temperature was not evident during fall testing, whereas *Lacerta viridis* demonstrated greater temperature independence during spring compared to autumn. At the same time, there was greater fluctuation in the daytime pattern of selected T_b in autumn compared to spring. The increased frequency of lower T_b s may be instrumental to the seasonal acclimation process.

Low Q_{10} s are indicative of relative metabolic independence from temperature changes. However, it is not possible to surmise if the underlying mechanisms for such changes are of a chemical or a physiological nature. Hence, the use of Q_{10} to describe biological whole-animal processes is ambiguous and does not encompass an animal's physiological capabilities. This applies particularly to reptiles, where metabolism is usually disregarded when the relationship between temperature and metabolic rates are examined. Arrhenius plots and activation energy more accurately describe temperature effects on biological processes (Christophersen 1973). Arrhenius plots for autumn metabolic rates showed a similar temperature sensitivity during day and night over the entire range of temperatures tested. In spring, a temperature effect was only observed at temperatures below 25 °C, whereas between 25 and 35 °C metabolic

rates remained almost constant, indicating a temperature compensation of the control of O_2 consumption. This abrupt change in the slope of the Arrhenius plot indicates a change in the animal's physiological processes, which only occurs during spring.

Changes of metabolic rates and T_b selection in *L. viridis* represent seasonal physiological adjustments. These are apparently cued by some component of the environment such as photoperiod, and represent regulation, as opposed to passive acclimation, to temperature. Selection of higher T_b s during spring and summer as well as selection of lower T_b s during autumn may further facilitate acclimation to seasonal temperatures. These findings emphasize the synergic action of photoperiod and temperature in the physiology of a reptile.

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