# NUTRITIONAL BUDGET AND COLD TOLERANCE, DURING NATURAL HIBERNATION, IN THE EUROPEAN COMMON LIZARD *(LACERTA VIVIPARA)*

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Abstract: The European Common Lizard, *Lacerta vivipara (L.v.)* is one of the most widely distributed reptiles throughout Eurasia and occurs above the Artic Circle, in humid habitats. We have investigated several populations, particularly in a lowland (Brittany), we determined field *hibernaculum* sites, field water flux and metabolic rates of *L.v.* The second, a highland population (Jura Mountains), in which we determined microenvironmental conditions, body temperature, behaviour, cold tolerance, cryoprotectants. We have estimated the daily consumption (DW, dry weight): 13.6 mg DW. $j^{-1}$. $g^{-1}$ for July and 10.6 g DW. $j^{-1}$. $g^{-1}$ during the period from July to September. In freeze tolerance tests, L.*v*. remained supercooled for at least 21 days at - 3.5°C. But it can also survive freezing episodes in the field for about 3 days. *L.v.* is unique because it has both freeze tolerance and freeze avoidance capacities. The mean blood glucose level of *L.v.*, in the field, increased significantly (about 4-6 fold) from September to January, from 4.3 mMol. $L^{-1}$ to 19.8 mMol. $L^{-1}$. These values confirm our previous study during winter 1995-96 in which the level of blood glucose of *L.v.* frozen in captivity was as high as in unfrozen lizard in the field, 33.2 mMol. $L^{-1}$ in March. After the hibernation in April, the glycemia is characterised by a quick fall, the mean blood glucose level is 6.2 mMol. $L^{-1}$. But another a new polyol was found, playing a major role in cell membranes and inside the cells with messenger function. It is 4 fold increase during the winter, this suggests its probably function for the overwintering metabolism. Supercooling can be considered as one of the key adaptations for cold tolerance which enable *L.v.* to resist the coldest winter environments.

KEY WORDS: Cryoproptectants, cold tolerance, ecophysiology, hibernation, *Lacerta vivipara,* nutritional budget, reptile, supercooling.

## INTRODUCTION

The European common lizard, *Lacerta vivipara (L.v.)* is a very adaptable species that can inhabit a wide range of habitats. It has probably the largest geographical range of any living lizard. Its distribution extends from the mountains of northwest Spain to Sakhalin on the Pacific coast, a distance of 12,000 km, and from the north of Spain to beyond the Arctic Circle, a span of nearly 3,000 km in latitude. It is found in a wide range of rather humid habitats such as meadows, peatbogs and heathlands from sea level up to an altitude of 3,000 m.

*L.v.* is a small lacertid lizard, the body mass (BM) for adults is 3 to 4 g reaching 6 g in the case of gravid females. Three age classes can be distinguished according to the size of the lizard (Van Damme *et al*. 1987, Grenot *et al*., 1987).

In the present study in France, we have investigated two populations of *L.v.*, firstly a lowland population in Brittany, with which we previously determined field water flux and metabolic rates, annual nutritional budgets, and field overwintering sites (Grenot. *et al*. 1987, Grenot and Heulin 1988). Then, a second highland population (Jura Mountains, northeast France), with which we determined microenvironmental conditions of overwintering sites, body temperature, behaviour, freeze tolerance and blood cryoprotectants (Grenot *et al*. 1996).

## MATERIAL AND METHODS

### STUDY AREA

The lowland population of *Lacerta vivipara* studied was situated at Paimpont in Brittany, located in the western part of France on the banks of a lake peat bog. The soil was usually saturated with water and the herbaceous vegetation was largely dominated by *Molinia coerulea* (Heulin 1985, Grenot *et al*. 1996) and the climate is generally mild.

The highland population was situated in Bonnevaux (Doubs), at the Biological Station of Franche-Comté University, located in the north-eastern part of France in the Jura Mountains, at an altitude of 850 m (Pernot-Visentin 1978). This is one of coldest parts of France, where the winters are very severe. The average maximal and minimal annual temperatures in this zone are 12.5°C and 2.5°C, respectively, and the mean annual precipitation is between 1,500 and 2,000 mm (Pernot-Visentin 1978; Bidault 1982). Snowfall is between December and March and snowdepth can reach 1m. So it is perfect location to study cold hardiness strategies of living lizards. The Frasne-Bonnevaux peat bog is characterised by *Sphagna, Carex*, moss litter, and other plants (Grenot *et al*. 1996).

### TEMPERATURE RECORDING

Soon after capture, animals were put in enclosures with peat bog vegetation. Temperature and relative humidity (RH) at 30 cm height, were recorded each 30 min intervals with a mechanical thermohygrometer recorder. We followed, in the same time, the temperature and the RH of microclimates with a datalogger ELPRO (Hot Dog) providing with a small external probe for temperature and RH measurements. The sensor probe was buried in the soil, close to overwintering site of *L.v.*.

### NUTRITIONAL BUDGET ESTIMATION

#### 1. LOWLAND POPULATION

In the laboratory, direct estimates of food intake for *L.v.* have been made by establishing a laboratory relationship between food intake and output of faecal or nitrogenous wastes, and then measuring the outup of field-captured animals (Avery, 1971, 1976; Pilorge, 1982; Heulin, 1986; Grenot and Heulin, 1988).

Isotopic methods allow a reliable estimation of the nutritional budget. A close positive correlation exist between food intake and the rate of Na turnover (Buscarlet, 1974). It can be accurately measured using the turnover rate of $^{22}$Na, by following the exponential decline in the specific activity of the isotope, in a manner similar to the use of labelled water to measure water turnover (Lifson and McClintock, 1966; Nagy and Costa, 1980). In those animals whose major source of Na is dietary, reliable estimates of food intake can be established using $^{22}$Na turnover if the Na content of the diet is known. Furthermore food, and hence energy, intake can then be converted to assimilated energy by determining the digestive efficiency of the species. If the animal is in a steady state with respect to energy, assimilated energy is also a good approximation to metabolized, or expended energy (Gallagher *et al*., 1983).

To determine Na turnover, each lizard was labelled with $^{22}$Na by a single intraperitoneal injection of 15 µl of $^{22}$Na Cl with 1.23 Mbq/ml. Each day the lizards were supplied with known quantities of locusts containing a known amount of Na (Grenot and Heulin 1990).

The measurement of Na turnover was not determined by monitoring the specific activity of blood samples, but by a technique for whole body counting of $^{22}$Na activity. The $\gamma$ body activity was measured directly using the probe of solid portable scintillation counter. Counting was carried out every alternate day for 1 week.

When using the portable counter, each lizard was placed in a plastic scintillation vial, allowed to curl up in the bottom, and was held in this position by a soft foam plug to keep it in a constant position relative to the detector below the base of the vial. Each animal was counted in this way for 10 sec., five times. For counting the $^{22}$Na standards, small plastic tubes with solutions were measured in an identical position. Values of k in day$^{-1}$ were calculated from the slope of the least squares linear regression of (Ln) whole body activity versus time for each lizard.

In the field, during hibernation, by radio-tracking with the isotope $^{22}$Na, we determined the overwintering sites of *L.v.* We followed 23 adults in a field enclosure of 100 m$^2$ with natural vegetation of Paimpont peat-bog, to investigate the site of hibernacula and the energy budget of *L.v.* during the winter.

Each of the *L.v.* captured was weighed, measured, toe-clipped, and given a 15 - 30 µl intraperitoneal injection of a solution containing 0.4 MBq of $^3$HHO/ml and 18,5 kBq of $^{22}$Na Cl with a solution 1.23 MBq/ ml. After 3 - 5 h, a blood sample (5 - 10 µl) was taken from the infraorbital sinus and/or urine sample was collected. Lizards were released at the place of their initial capture (Grenot *et al*. 1987, Grenot and Heulin 1988).

### 2. HIGHLAND POPULATION

We collected blood samples from several *L.v.* in September in the field. The sample was taken from the infraorbital sinus of the eye, soon after capture (< 2 min). Blood glucose concentration was measured using blood glucose test strips (Glucotide) with a Glucometer 4 (Bayer/Ames).

In a field enclosure of 15 m$^2$ with natural peat-vegetation of peat-bog near Bonnevaux, we followed 18 adults and 20 juveniles to investigate the body temperature of *L.v.* and lizard skin on the lizard's abdomen and the temperature of microclimates with a Model 50 Datalogger with 5 thermocouple inputs and recorded continuously throughout the period of experiment. One probe was buried at the surface of the moss and others were situated on overwintering sites in bog at 10 to 20 cm in depth. Temperatures were recorded at 30 min intervals. near 2 groups of *L. vivipara* at about 15 cm of depth in the bog (Grenot *et al*. 1996).

We used proton nuclear magnetic resonance (NMR) spectroscopy (Le Moyec *et al*. 1993) to evaluate the major small molecules(polyols) that can play an antifreeze role during hibernation, and to explain the freezing and supercooling capacities of this lizard species.

## Results

### 1. Lowland population

#### Localisation of lizard overwintering sites

23 *L.v.* received a 15-30 µl intraperitoneal injection of a solution containing $^{22}$Na Cl (Grenot and Heulin 1988) to locate the hibernation sites of *Lacerta vivipara* by using a portable scintillation counter. During hibernation, *L.v.* were recaptured in November and in February. They were located mostly in the northern part of the enclosure, the sunniest zone. Two or three lizards were sometimes found close together in the same small overwintering site. Usually they were up to 2 to 4 cm beneath the litter made of moss and grass in grass hummocks or in the middle of a large grass clump, or inside of the burrow of a small mammal (Bauwens 1981; Grenot and Heulin 1988; Grenot *et al*. 1996).

#### Water and nutritional budget.

In the lowland population of Brittany, a high water balance between 170 to 269 ml. kg-1. d-1 was evaluated in *L.v.*, during the activity season from June to September (Grenot *et al*. 1987; Grenot and Heulin 1990). All categories showed decreased water fluxes in autumn (100 ml kg$^{-1}$. d$^{-1}$). During hibernation in winter the lizards maintained a constant BM though not feeding, and showed a very low energy expenditure. *L.v.* still exhibited a significant water exchange rate during hibernation (27 ml kg$^{-1}$ d$^{-1}$). The fact that dormant lizards seem to have wet skin could effectively increase transcutaneous water exchange.

#### Nutritional budget in the laboratory

Twenty lizards were captured in July 1985 and kept in individual terraria in the laboratory (D: 8, N: 16). Room temperature was held between 19.7 ± 2.8°C during the night and 28.0 ± 2.2°C in the daytime. Additional heating was provided for 8h per day by a light bulb (60 W) placed above each terrarium. Lizards were fed on young locusts. In order to estimate the proportion of water provides by food, locusts that were not consumed were weighed and the amount ingested by lizards was calculated from the difference between the mass prey offered and the mass of those not eaten.

The daily consumption was 17 mg (34 mg DW. (dry weight) d$^{-1}$) and 9.4 mg (33 mg DW. g$^{-1}$. d$^{-1}$) rest metabolism for the subadults (BM: 2 g) and adults (BM: 3.5 g) respectively. The dietary water in the locusts consumed by lizards kept in the laboratory made up 16 - 20 % of the total WIF (Grenot *et al*., 1987).

The diet of *L.v.* was also estimated by determing the relationship between Na food intake and $^{22}$Na turnover for 13 lizards eating locusts in the laboratory. The decreasing of radioactivity and the food consumption (locust) were followed each day, during a week (fig. 1). The concentration of sodium in the locusts (Na preys) was controlled and remained relatively constant (2.5 µg. mg-1 DW). The equation Y = (C x Na preys) / BM, is the Na consumption per day (µg /g) of the lizard, (where C = food consumption (g/day).

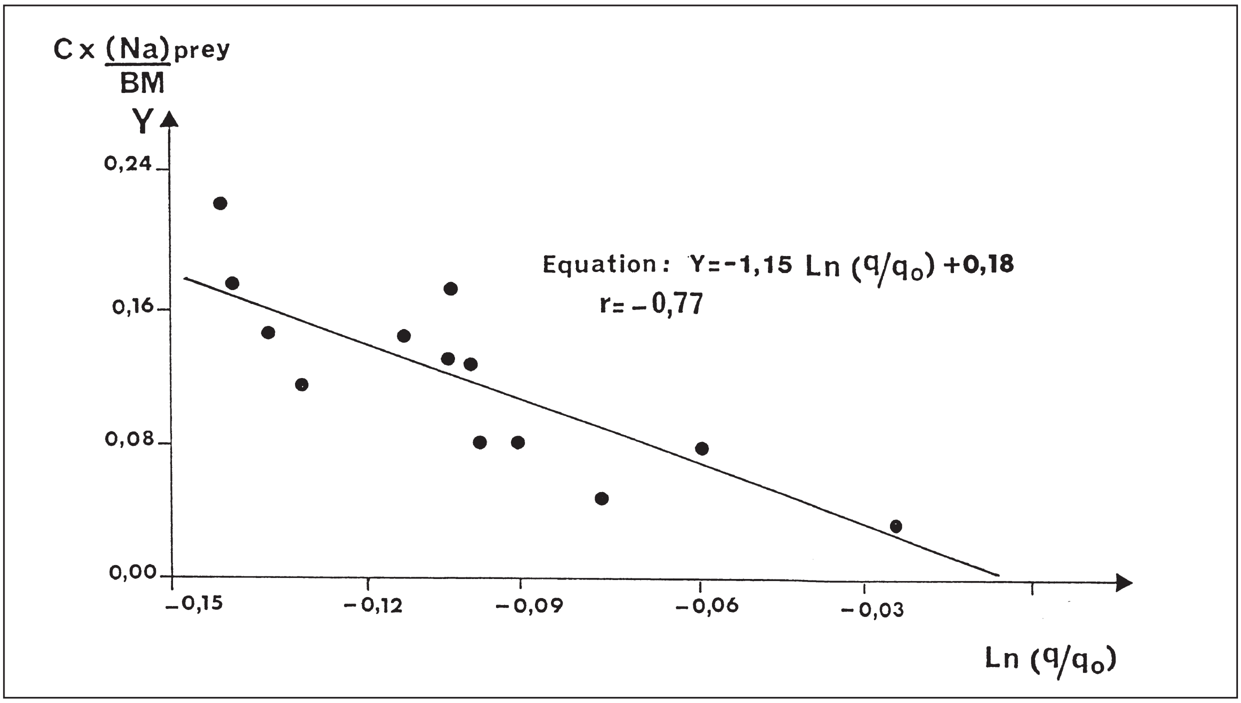
But Y = A. Ln q / q$^0$, where q$^0$ and q = the initial and final quantities of $^{22}$Na, A = Na pool in the lizard. Finally the regression Y = -1.15 Ln q / q$^0$ + 0.18 (correlation coefficient r = - 0.77; so the $p > 0.02$) the regression slope give the value of the body exchangeable Na gives:

A = -1.15 µg. mg$^{-1}$. It is very near of this of the body value 1.18 µg. mg$^{-1}$ LW (live weight). These values allowed us to estimate the daily consumption, when transposed to free-living lizards. So the decreasing of body Na of *L.v.* with a controlled diet is well correlated with the quantity of ingested locusts.

#### Nutritional budget in the field

During activity period, the diet was firstly determined from the analysis of the stomach contents of individuals caught at the same time in the same place. In Brittany, estimates of prey volumes were used to assess daily food intake at 16 mg DW. d$^{-1}$ for juvenile lizards and 51 mg DW. d$^{-1}$ for adults.

Fig. 1: Relation between the consumption of [Na] and the $^{22}$Na To in *Lacerta vivipara* eating locusts. The linear regression relate the mean value of the body Na content of the lizard.
C = consumption (g/day); [Na] preys = [Na] content in locusts; BM = body mass; q° and q = $^{22}$Na initial and final counts respectively.



Secondly, with a $^{22}$Na injection Na turnover can be converted to food intake if the Na content of diet is known. As an estimate of the Na content of the diet, three types of prey (Coleoptera, locusts, spiders) were analysed for Na. The Na content of a known mass of each species was determined by flame photometry.

In the field, for 12 lizards injected in July and recaptured five days later, the decrease of the radioactivity (i.e Ln $q/q^0$) allowed to calculate this value: Y = 83 µg.Na$^+$/day. The same for 10 other lizards injected in too July and recaptured 60 days after, in september we found a similar value for this long period: Y = 82 µg.Na$^+$/day.

If we take 2.46 µg. mg-1 DW as sodium content mesured for locusts (preferred prey), we can measure a daily consumption of 34 mg$^{-1}$ DW. d$^{-1}$ in July and 33 mg$^{-1}$ DW. d$^{-1}$ for the period from July to September. Refering to the mean BM of lizards (2.5 g in July and 3.1 g from July to October) we obtain consumptions of 13.6 mg DW. g$^{-1}$. d$^{-1}$ and 10.6 mg DW. g$^{-1}$. d$^{-1}$ respectively.

The ingested energy is higher than the one measured in laboratory, because of activity and energetic expenditure of lizards in field.

Earlier, the stomach volumes allowed us to calculate the daily consumption for the Brittany population: 16 mg DW. d$^{-1}$ for yearlings (BM= 0.6 g) and 51 mg DW. d$^{-1}$ for adults (BM= 3.5 g). Refering those values to BM, we obtain daily consumption of 26.7 mg DW.g. d$^{-1}$. and 14.6 mg DW.g. d$^{-1}$ respectively (Heulin, 1986).

Avery (1971) studying a population in Great Britain found for *L.v.* of BM= 0.6 g and 3.5 g daily consumption of 14.9 and 55 mg DW. d$^{-1}$ respectively.

The condition index (length snout-vent, SV) SV/BM of the lizards before and after the hibernation period are the same. The equations are respectively (X = SV mm; Y = BM mg):
$Y = 0.13 X - 3.18, r = 0.96, p < 0.0001$ (N = 46), in September, and
$Y = 0.12 X - 2.86, r = 0.90, p < 0.0001$ (N = 33) in April.

During hivernal period, the $\gamma$ radioactivity decreased slightly between the 30 September and first recapture on November 14, because the lizards continued to be active and to feed up to 10 days prior to the beginning of hibernation (Grenot and Heulin 1988). In comparaison during the following 100 days, from November to February, the $\gamma$ radioactivity decay remained stable and does not vary between February and November, demonstrating their non-feeding, even though their BM stayed constant. Hence, energy expenditure represents about 5 % of annual energy, during the five winter months. It seems to be very low during hibernation, as previously stated (Patterson and Davies 1978).

## 2. Highland population

The hibernacula of the highland population in the Jura Mountains were much deeper below ground than those of the lowland population (Grenot *et al*. 1996). In January and February the mean daily temperatures in the overwintering sites and at the soil surface had the same values, near 0°C. In fact, the thermal fluctuations at the soil surface are more important than at depth (9°C and 0.9°C respectively). The thermocouples are protected in winter by a snow mantle, and the thermal fluctuations are lower than those registered at 30 cm above the soil. The overwintering site is covered by 10 cm of bog and often by several centimeters of snow (up to a maximum of 40 cm depth during mid-February), which are absorbent substrates where the temperatures are stable (about 0°C), and the RH stays constant (near water saturation point) (fig. 2a). However, temperature variation between -1°C and -4°C were observed in the hibernaculum during the cold periods when the snow cover was thin, and the air temperature low < -10°C (fig. 2b). From March, the temperature increased at the overwintering site at the same rate as the increase in air temperature. The mean daily temperature increased from + 1.5°C in March to + 6°C in April.

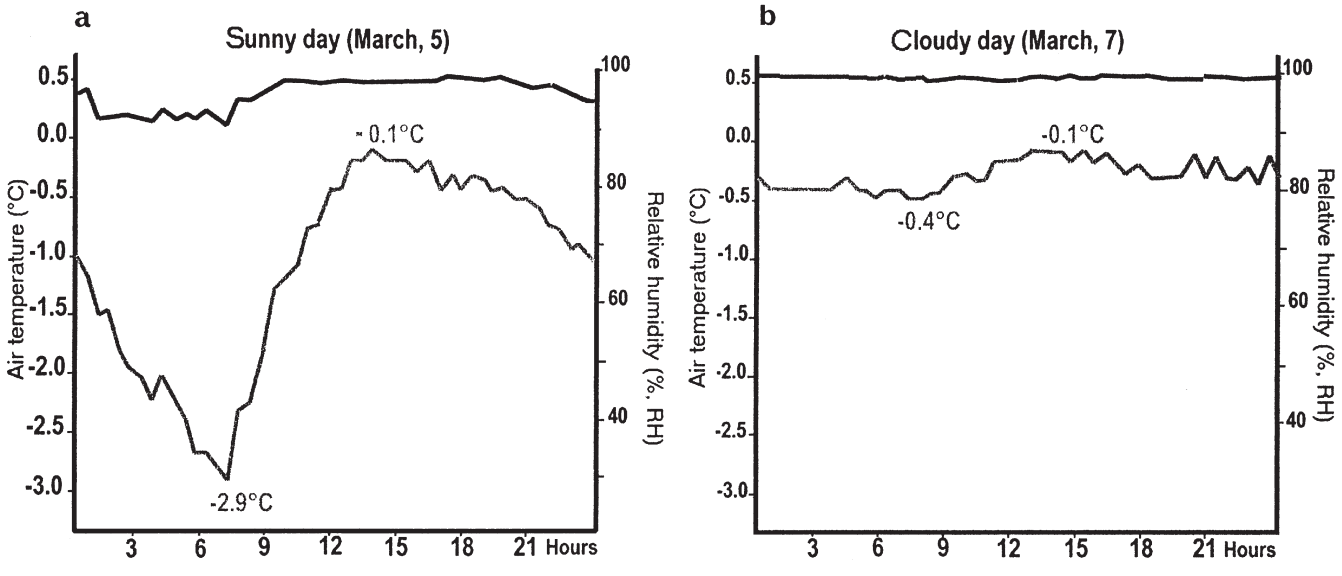


Fig. 2: Temperature and relative humidity (datalogger Hot-dog) inside an hibernaculum (bog) of *Lacerta vivipara,* at the beginning of March:

a - during a sunny day, minimal air temperature at 30 cm above the ground = - 11°C;

b - during a cloudy day, minimal air temperature at 30 cm above the ground = - 4°C.

The thermal differences increased but stayed lower than those on the soil surface. The ground (bog) plays the role of "buffer", with lower temperatures during the day -time and warmer at the night than those observed at the soil surface respectively. With favourable weather, the daily thermal differences were very high, near to 40°C in April at the surface of dry grass, *Molinia coerulea* (fig. 3).

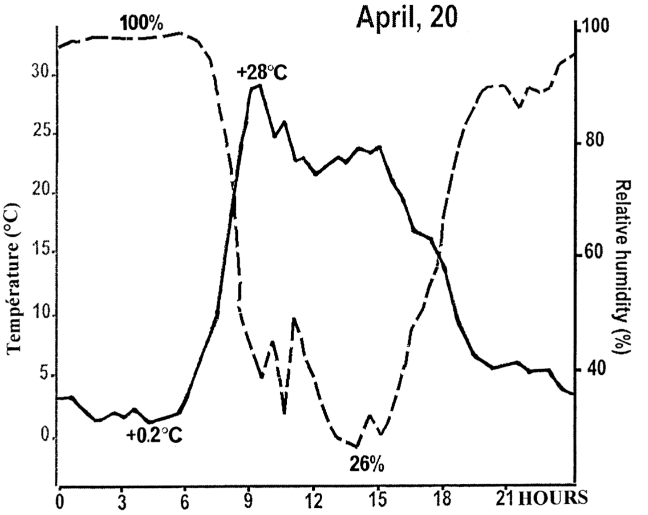


Fig. 3: Temperature and relative humidity (Datalogger Hot-dog) near the surface of a tuff of dry Graminea, *Molinia coerulea* in a peat-bog at Bonnevaux (Jura Mountains 850 m.).

### FREEZE TOLERANCE

Out of 15 lizards found in January in the enclosure, 3 individuals (one young and two adults) were discovered frozen in their overwintering sites.

A frozen lizard during the night, begins to thaw during next afternoon and the freezing state rarely exceeds 48 h. The criteria used to determine the freezing used are:

- the general aspect of the animal: its skin color becomes dark instead of brown and its assumes it characteristic position, rolled up position, with the snout against the vent.
- the consistency of the body is hard, like ice, and often surrounded with ice crystals.

Other individuals (male n° 3) although in contact with snow crystals, do not freeze. It gives an account of the two contrasting strategies of freezing and supercooling which can be produced at the same temperatures, most often between - 0.1 and - 4.0°C (table 1 and fig. 4). This seems to be a rare occurrence, where the same individual can use during the same winter the two strategies. The female n° 20 stay supercooled in January and February, when the temperature of the overwintering site

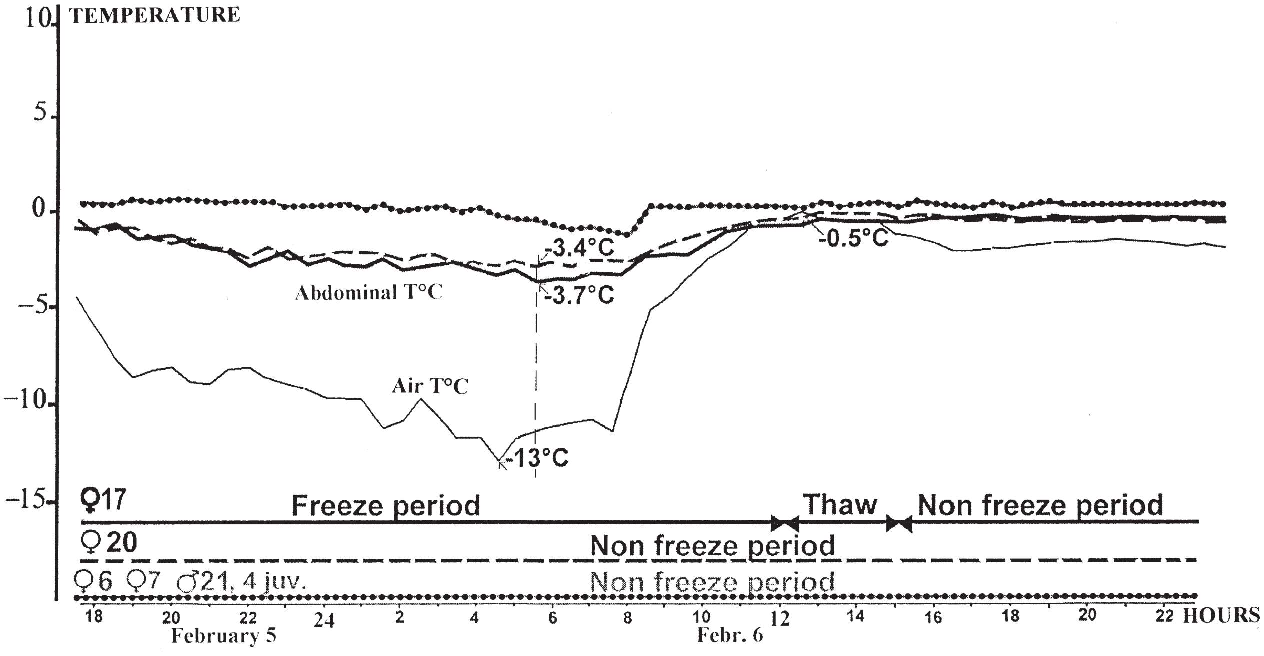


Fig. 4: Ventral temperatures of *Lacerta vivipara* recorded in 4 different overwintering sites, and air temperature at the surface of the soil, at Bonnevaux (Jura Mountains, 850 m.).

stayed at about - 4°C. It was frozen in March when the temperature of the hibernaculum varied between - 1.2 and - 1.5°C.

The variations of the temperature and the RH at an overwintering site could depend on weather conditions in the same period (beginning of March, fig. 2a and b). During a sunny day (March 5) when the minimal ambient temperature was - 11°C, the hibernaculum temperature rose from - 2.9°C to - 0.1°C (between 7:00 am and 1:00 pm), with an increase of almost 3°C in 5 h. Two days later even though the minimal ambient temperature was - 4°C and the weather cloudy, the thermal range of the hibernaculum was almost constant from - 0.1 to - 0.4°C.

The temperature and the RH of the hibernaculum were also followed during emergence from hibernation, which was generally at the end of March (between 18 and 25). The releasing factor for emergence seems to be a period of sunshine at the beginning of the spring. A female lizard n°15 went out of its hibernaculum on the March 23 (fig. 5). The ground warmed up in depth up to the level of the overwintering sites. At 0°C they undergo a nycthemeral cycle which range from + 2°C the March 19 to + 8°C the March 23, corresponding to the date of exit of the first lizard. During this time, the RH does not vary much and stay at more than 95 %.

The mean cloacal temperatures of the active lizard in the field was 32°C in April, and the time range of surface activity was from

Hibernaculum sites

| Date | Air minimal T (°C) | ← 1 | | | | | | 1 → | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | ← 10 | 10 → |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Lacerta vivipara number* | | | | | | | | | | | | | | | | |
| | | F6 | F7 | M21 | J11 | J12 | J18 | J22 | J19 | J23 | F17 | F20 | M16 | M14 | M3 | M4 | M5 | F15 |
| 19/01 | -9 | S<br>(-0.1) | S | S | S | S | S | S | S | F | F | S<br>(-2.1) | S | S | S | F | S | S |
| 20/01 | -10 | S<br>(-2.7) | S | S | S | S | S | S | S<br>(-0.9) | F | F<br>(-4) | S | S | S | S | F | S | S |
| 01/02 | -10 | S<br>(-0.4) | S | S | S | S | S | S | S | F | F<br>(-1.4) | S<br>(-1.5) | S | S | S | F | S | S |
| 06/02 | -10 | S<br>(-1.3) | S | S | S | S | S | S | S | F<br>(-1.6) | F<br>(-3.7) | S<br>(-3) | S | S | S | F | S | S |
| 02/03 | -9 | +/-F | S | S | S | +/-F | S | S | + | + | S<br>(-2.1) | S<br>(-0.1) | S | S | S<br>(-0.1) | + | + | S |
| 03/03 | -12 | F | S | +/-F | S | F | S | S | + | + | S<br>(-3.6) | S<br>(-0.2) | S | S | S<br>(-0.2) | + | + | S |
| 04/03 | -14,5 | F<br>(-1.6) | F | F | F | F | S | F | + | + | F<br>(-1.5) | +/-F<br>(-1.2) | S | S | S<br>(-2.1) | + | + | F<br>(-1.2) |
| 05/03 | -11 | F<br>(-1.6) | F | F | F | F | S | F | + | + | F<br>(-1.7) | F<br>(-1.5) | S | S | +/-F | + | + | F<br>(-2.9) |
| 06/03 | -12 | F<br>(-2) | F | F | F | F | S | F | + | + | F<br>(-4.3) | +/-F<br>(-1.1) | S | S | +/-F | + | + | F<br>(-3) |

| | | | |
|---|---|---|---|
| ( ) | = Minimal ventral temperature of lizards | M | = Male |
| F | = Frozen lizard | F | = Female |
| +/-F | = Partially frozen lizard | J | = Juvenile |
| S | = Unfrozen lizard (Supercooling) | | |
| + | = Dead lizard | | |

Tab. 1: Temperature and cold strategies used by the European common lizard, *Lacerta vivipara,* during the winter at the 3 coldest periods, in 10 hibernaculum sites at Bonnevaux (Jura Mountains, 850 m) .

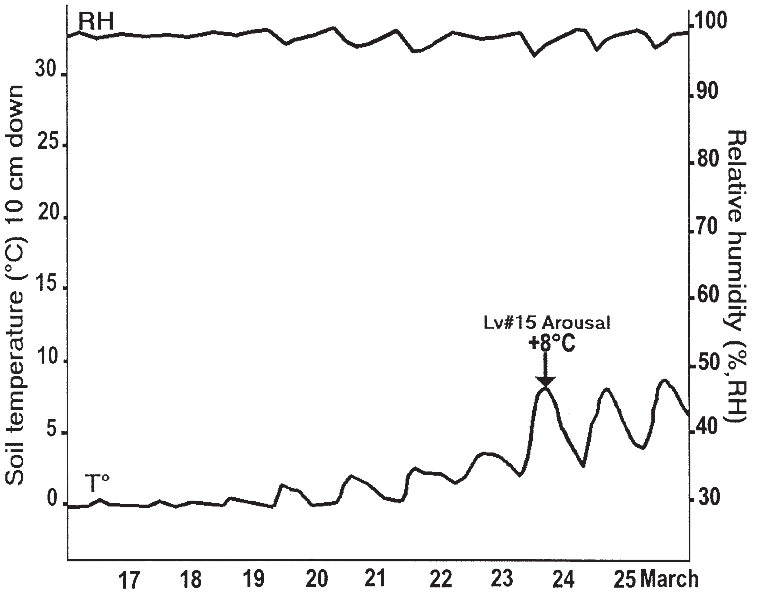


Fig. 5: Temperatures and relative humidity (Hot-dog) inside an hibernaculum (bog) of *Lacerta vivipara,* during a period of sunny days. The female n°15 placed in this hibernaculum went out, on the March 23 (the maximal temperature of the bog was + 8°C).

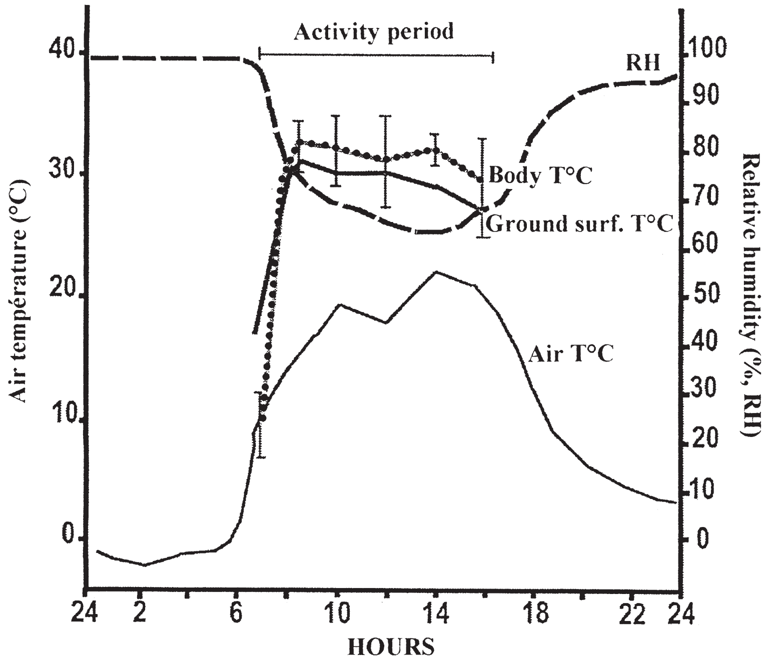


Fig. 6: Mean diary cloacal temperatures (N = 7), during 3 consecutive sunny days in April 19, 20 and 21; after the emergence of the bog (Jura Mountains, 850 m.).

7:00 am to 5:00 pm (fig. 6).

Some of the animals, that were enclosed in the frozen bog and cooled to about - 2°C, froze when they came in contact with ice crystals. Most of the lizards tolerated sub zero temperatures (<-2°C), by maintaining a state of supercooling. When they were uncovered in December (temperature 1.5-2.0°C, daylight), their eyes were closed but they were able to make uncoordinated movements and could bury into the peat.

CRYOPROTECTANTS

Glycemia showed a considerable individual heterogeneity, 5.8 to 28.6 mMol. L$^{-1}$ (for 2 adults of 3.6 and 2.5 g respectively) in the field, a few weeks before hibernation (October) and during hibernation, for example in January 13.8 and 37.9 mMol. L$^{-1}$ (for 2 adults of the same BM). The blood glucose increases in November (18.0 mMol. L$^{-1}$, $p < 0.0001$) and in January (25.8 mMol. L$^{-1}$, $p < 0.01$).

The mean blood glucose level of *L. vivipara* held in a field enclosure increased significantly (about 4 - fold) from September to March, from 8.5 ± 0.7 mMol. L-1 to 33.2 ± 5.6 mMol. L$^{-1}$ (fig. 7). During the winter, the level of blood glucose of *L. vivipara* frozen in captivity was as high as in unfrozen lizard. The maximum value was in January (37.9 mMol. L$^{-1}$), found in an unfrozen lizard. In the same month, glycemia for one of the frozen *L. vivipara* was 32.8 mMol. L-1. In this case, the blood sample was collected when the lizard was thawed before a new daily freeze-thaw cycle.

The maximal and the minimal glucose values of *L. vivipara* were 50.1 mMol. L$^{-1}$ in March and 4.5 mMol. L$^{-1}$ in September. After the hibernation in April, the glycemia is characterised a fall in the mean blood glucose level by 11 mMol. L$^{-1}$ ($p>0.001$), but was almost egual to the value in September.

However, another small molecule, a polyol, was found recently by RMN, in significant concentrations in plasma (Grenot and Voituron, 1999; Grenot, Voituron and Eugene, in press). With a low molecular mass (the same as glucose), it plays a major role in cell membranes and inside cells with a messenger function and growth factor. The increasing blood concentration of this new polyol (approximately 200 %) during the winter which follows the increasing glycemia.

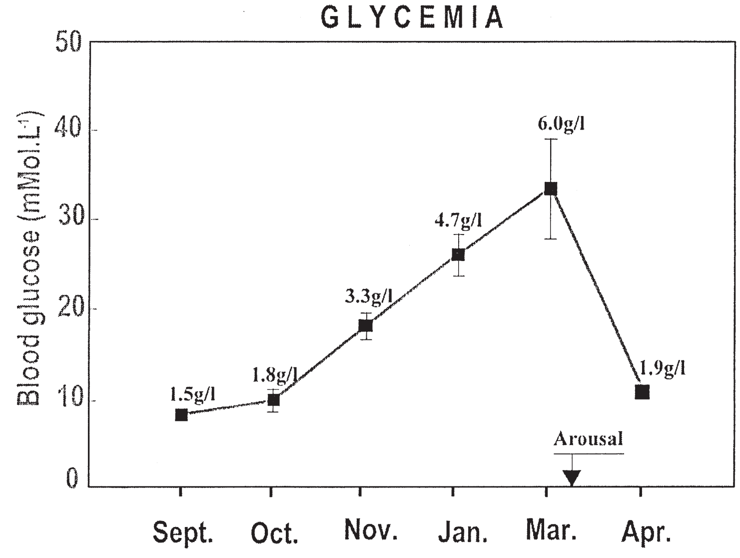


Fig. 7: Seasonal field glycemia in a highland population (Jura Mountains, 850 m.) of unfrozen *Lacerta vivipara* ( +/ - SEM).

## Discussion

*L. vivipara* presents one of the most high exchange of $^{3}$H (H2O) among the terrestrial lizards, because it lives in humid habitat. It drinks about 70 % during the activity period. Between November and February, the apparent water flux rate is 27 ml (about 5 to 10 times less than observed between June and September).

In the past, winter dormancy in reptiles was considered simply as a cold-induced torpor resulting from an inability of the animal to maintain a high body temperature at low ambient temperatures. Nevertheless, it may be accompanied by changes in body composition comparable, but not identical to those observed in hibernating mammals (Gregory 1982). Weigmann (1929) was the first to present evidence for freeze tolerance in a vertebrate, the wall lizard *Podarcis muralis*. Some individuals of an isolated population of *P. muralis* in Ohio could tolerate brief freezing episodes of 10 - 120 min with body temperatures from - 0.6 to -1.0°C (Claussen *et al*. 1990) (table 2).

In the field, the blood glucose level of *L.*

*vivipara* increased regularly from September to March; it is the only reptile known to show this progression, when the lizards become active again, the mean blood glucose decreases to the level of September (10.95 ± 0.73 mMol/L). This species also exhibited the highest value of glucose (33.24 ± 5.63 mMol/L) currently known for reptiles. Blood glucose levels are low early in the winter, and progressive hyperglycemia is increased during the winter.

The two to five fold higher levels of blood glucose, in recently frozen (laboratory tests, Costanzo *et al*. 1995) and unfrozen (in natura, Grenot *et al*. 1996) *L. vivipara*, indicate that this species uses glucose as cryoprotectant. Elevated glucose levels persist long after thawing and before freezing.

Supercooling and freeze tolerance are usually dichotomus strategies for coping with subzero temperatures (Costanzo and Lee 1995). The present study provides conclusive evidence for a well developed capacity for both supercooling and freeze tolerance strategies in the same species, *L. vivipara*. With chance, in the March 4, under the same mound of bog, we observed three lizards next to each other: the first frozen, the second supercooled, and a third whose half part (posterior) of the body was frozen, while the anterior part was unfrozen with the head drive in the bog. So the 2 strategies freezing and supercooling can be encountered in the same microclimates. It seems, according to these observations, that the strategy used by an animal depends first on its own physiological conditions.

One of the most striking characteristics of overwintering lizard during cold hardiness is its capacity to increase concentration of two cryoprotectants of low molecular weight, glucose and a new blood "polyol". *L.v.* in this study, show again its reliability to be a model for animal cold adaptation. It has this suprising capacity to develop supercooling and freezing during winter. Our data for *L.v.*, which represent the longest record of supercooling for any vertebrates, suggest at a body temperature of - 3.5°C for at least 3 weeks.

| | SUPERCOOLING CAPACITY | | FREEZE TOLERANCE | |
|---|---|---|---|---|
| | Abdominal Temperature | Duration | Abdominal Temperature | Duration |
| ***Chrysemys picta***<br>juveniles<br>(Packard and Packard, 1993) | -12°C | about 12 days | | |
| ***Chrysemys picta***<br>(Churchill and Storey, 1991) | | | -2.5°C | 3 days |
| ***Sceloporus jarrovi***<br>(Haalpern, 1979) | -3°C | about 30 hours | | |
| ***Podarcis muralis***<br>(Claussen et al, 1990) | -4.75°C | 8 hours | | |
| ***Lacerta vivipara***<br>(Costanzo et al, 1993) | -3.5°C | over 21 days | under -3.0°C | over 3 days |
| ***Thamnophis sirtalis***<br>(Churchill and Storey, 1991) | | | -2.5°C | 3 to 5 hours |
| ***Rana sylvatica***<br>(Storey and Storey, 1986) | | | -2.5°C to -3.0°C | 3 to 13 days |

Tab. 2: Cold tolerance of some various species of ecthoterms vertebrates.

Acknowledgments: The authors thank Dr. A. Hailey for helpful criticism on the manuscript, M. Ballan and F. Sottile for their valuable assistance.

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