

How does the European common lizard, *Lacerta vivipara*, survive the cold of winter?

Claude J. Grenot ^{a,*}, Laurence Garcin ^a, Jérôme Dao ^a, Jean-Pierre Hérold ^b, Bernard Fahys ^c, Huguette Tséré-Pagès ^a

^a Laboratoire d'Ecologie, C.N.R.S. UMR 7625, Ecole Normale Supérieure, F-75005 Paris, France

^b Laboratoire d'Ecophysiologie Animale, Faculté des Sciences, Université de Franche-Comté, F-25030 Besançon, France

^c Laboratoire d'Electrochimie des Solides, Faculté des Sciences, Université de Franche-Comté, F-25030 Besançon, France

Received 16 March 1999; received in revised form 5 June 2000; accepted 8 June 2000

Abstract

Although the European common lizard, *Lacerta vivipara*, is among the most common Eurasian reptile species, we know little about how these lizards cope with very low temperatures. In this study we examined microenvironmental conditions, body temperature, behavior, and cold strategies to see whether strategies of freezing and supercooling, while normally considered to be mutually exclusive, may in fact be adopted simultaneously by the common lizard. Following up on an earlier study of a lowland population, this time we used a mountain population (850 m) to discover differences in overwintering strategies between the two populations. Differential scanning calorimetry conducted during the hibernation period (vs. the activity period) showed that the blood of highland lizards had an increased ability to resist ice formation, confirming an ecophysiological effect most likely mediated by physical properties of the blood. Mean blood glucose level of unfrozen *L. vivipara* in the field increased significantly (about fourfold) from 8.5 ± 0.7 mmol l⁻¹ in September to 33.2 ± 5.6 mmol l⁻¹ in March. The blood glucose level then experienced a significant decline as it fell to 6.2 ± 0.8 mmol l⁻¹ after hibernation in April. Glucose, in conclusion, seems to play a role of cryoprotectant rather than antifreeze. © 2000 Published by Elsevier Science Inc.

Keywords: *Lacerta vivipara*; Hibernation; Hibernaculum; Water balance; Differential scanning calorimetry; Cold tolerance; Supercooling; Blood glucose level

1. Introduction

Most studies on the cold tolerance of ectotherm vertebrates, amphibians and reptiles have been carried out in laboratories (Bauwens, 1981; Bidault, 1982; Claussen et al., 1990; Churchill and Storey, 1991; Costanzo et al., 1995; Costanzo and Lee, 1995; Gregory, 1982; Halpern, 1979). Rela-

tively little is known, however, about the physiology of the European common lizard *Lacerta vivipara*, which is surprising in view of its relative abundance (Bauwens, 1981; Costanzo et al., 1995; Grenot et al., 1996; Grenot and Heulin, 1988, 1990; Grenot et al., 1987; Patterson, and Davies, 1978; Van Damme et al., 1987).

L. vivipara is an adaptable species inhabiting a wide range of habitats and probably has the largest geographical range of all lizards. Its presence extends from the mountains of northwest Spain to Sakhalin on the Pacific coast and from

* Corresponding author. Tel.: +33-1-44323702; fax: +33-1-44323885.

E-mail address: grenot@biologie.ens.fr (C.J. Grenot).

the north of Spain beyond the Arctic Circle. Moreover, it is found in a wide range of humid habitats including meadows, peatbogs, and heathlands from sea level to 3000 m.

This small lacertid lizard has an adult body mass (BM) of 3–4 g, reaching 6 g in gravid females. The size of the lizard allows us to distinguish between three age classes (Grenot et al., 1987; Van Damme et al. 1987). In this long-term study of a highland population in the Jura Mountains, northeastern France, we investigated the cold tolerance of *L. vivipara* in outdoor enclosures. We contrast our results with those from an earlier study of a lowland population in Brittany, western France (Heulin, 1985; Grenot et al., 1987; Grenot and Heulin, 1988).

In the previous lowland study, we discovered overwintering hibernacula sites of *L. vivipara* in a peatbog using ^{22}Na marked animals and calculated the lizards' winter energy budget (Grenot and Heulin, 1988). The lizards were generally found under 2–4 cm of mossy or grassy litter, often in the middle of a large grass clump or a small mammal burrow (Bauwens, 1981; Grenot and Heulin, 1988; Grenot et al., 1996). Observing that the dormant lizards were unfrozen, we speculated that *L. vivipara* survives natural exposure to subfreezing temperature by supercooling. During hibernation, from November to February, the animals' level of ^{22}Na remained stable, suggesting that they were not feeding and that their body mass remained constant.

We also showed (using tritiated water) that the lowland lizards had among the highest water flux rates (WFR, more than $200 \text{ ml kg}^{-1} \text{ d}^{-1}$ in summer) recorded for a terrestrial lizard, which might be expected in an animal inhabiting humid habitats. All ages and sexes showed decreased water fluxes in autumn ($100 \text{ ml kg}^{-1} \text{ d}^{-1}$). The apparent WFR was $27 \text{ ml kg}^{-1} \text{ d}^{-1}$ during the cold season, about 5–10 times less than the values observed between June and September (Grenot et al., 1996).

To continue and extend our earlier study, this time we studied a highland population in the Jura Mountains, looking at microenvironmental conditions in overwintering sites, behavior, thermal relationships, fluctuation of blood glucose and differential scanning calorimetry (DSC) of the blood. We expected that extreme mountain conditions would be stressful and that the lizards would require specific physiological adaptation to survive overwintering.

2. Study area

The highland population was located in Bonnevaux (Doubs) at the Biological Station of Franche-Comté University at an altitude of 850 m (Pernot-Visentin, 1978), and where winters are very severe. The average maximum and minimum annual temperatures at the site were 12.5 and 2.5°C, respectively, and the mean annual precipitation was between 1500 and 2000 mm (Pernot-Visentin, 1978; Bidault, 1982). Snowfall normally occurs between December and March and with a snowdepth of up to 1 m, creating a perfect location to study the cold-hardiness strategies of lizards. The Frasné-Bonnevaux peatbog is characterized by *Sphagnum*, *Carex*, moss litter, and other plants (Grenot et al., 1996).

3. Material and methods

The experiments were conducted in two outdoor enclosures of about 30 m^2 built at locations where we observed the *L. vivipara* during the prehibernation period. The enclosures were of sheet aluminum 1 m high, buried 10 cm in the earth, and contained undisturbed peat bog substrate. In each case approximately 20 lizards were placed in each enclosure, supplementing the number originally found there. Because the experiments were conducted at the end of the warm weather season, lizard activity was greatly reduced and no interactions among individuals were observed.

The enclosures and sampling regime closely resembled those in the earlier study, however this time we sampled more frequently than in the earlier work. In the present experiments, we sampled the lizards once or twice a week until we were sure they were hibernating, after which we sampled them three times weekly until April (cf. Grenot and Heulin, 1988).

We recorded temperature and relative humidity (RH) at 30 cm height at 30 min intervals with a mechanical thermohygrometer. We simultaneously observed the temperature and the RH of microclimates using a datalogger ELPRO (Hot Dog) equipped with a small external probe for temperature and RH measurements. The sensor probe, which is the size of a pen, was buried in the soil in one of the enclosures.

Each of the *L. vivipara* captured was weighed, measured, and toe-clipped. A blood sample (5–10 μl) was taken from the infraorbital sinus. We collected reference blood samples from several *L. vivipara* in September within 3 min of their first capture. After the initial sampling, additional monthly blood samples were collected in the same way from animals in the enclosures. Blood glucose concentrations were measured using blood glucose test strips (Glucotite) with a Glucometer 4 (Bayer/Ames). The test reaction is based on the hexokinase method using dry reagent technology and provides a quantitative measurement of glucose in blood and plasma (0.5–33.3 mmol l^{-1} with an accuracy of $\pm 0.2 \text{ mmol l}^{-1}$).

In the two enclosures, we studied 18 adult and 20 juvenile lizards, recording their body temperatures and the temperature of their microclimates during and shortly after hibernation. We used a datalogger with five thermocouple inputs and recorded observations continuously throughout the experiment. One probe was buried at the surface of the moss while others were located at depths of 10–20 cm, against the skin underneath the abdomen in overwintering bogsites of *L. vivipara* (Grenot et al., 1996). Temperatures were recorded at 30 min intervals. The lizards may have been disturbed by sampling at the outset of the hibernation period because they sometimes moved slightly when sampled. However, during very cold periods the majority of lizards did not move. We took care at every observation to avoid disturbing the lizards and their hibernacula.

3.1. Differential scanning calorimetry

Differential scanning calorimetry (DSC) is based on heating (or cooling) a sample and a reference at a pre-set rate, while keeping their temperatures the same, and measuring the compensating heat flux that keeps the temperature of the sample within the limit of a predetermined program. Experimental DSC curves show heat flux (in mJ s^{-1}) or the specific heat capacity C_p (in $\text{J g}^{-1} \text{K}^{-1}$) against temperature (or in isothermal experiments, against time). These temperature measurements are then used to correct the compensating heat flux, which is directly proportional to the change in the internal energy (enthalpy) of the specimen. We used DSC to measure first the equilibrium freezing point and then the crystallization temperature of each lizard's blood (Block,

1994). Thermal analysis was used to measure the heat flow between a sample and a reference pan. This variation in enthalpy indicates the change in state of the sample when it is subjected to crystallization or melting.

We used a Mettler DSC-30 calorimeter with a calibrated scale and a pan containing indium. Each sample was successively frozen at a speed of $-1^\circ\text{C min}^{-1}$, then defrosted at $+1^\circ\text{C min}^{-1}$. Standard aluminum pans of 40 μl were used, with 2–5 μl of blood in the sample pan. The rough values given by the calorimeter were processed by a computer program supplying an analytical thermal curve that identified the freezing temperature (T_{sol}) and the melting point (T_{fus}) for each freezing or heating test. Freezing temperature was defined as the beginning of the exothermic peak of crystallization as temperature decreased, corresponding to the spontaneous conversion of a liquid phase to a solid phase. The melting point, or equilibrium freezing point (T_{fus}), was defined as the maximum of the endothermic curve of melting ice as the temperature increased. According to the manufacturer, the instrument obtains these readings with 4% precision and 1% reproducibility.

Because liquids may be supercooled below their freezing temperatures, a thermodynamically stable phase may not be achieved on account of the kinetic stabilization that occurs in the absence of nucleation centers. The body fluid osmolality then corresponds to the equilibrium freezing point, a thermodynamic parameter. Undercooling capacity, which indicates the capacity of blood to remain liquid below the equilibrium freezing point (T_{fus}), is calculated from the difference between the melting point (T_{fus}) in equilibrium and the freezing temperature (T_{sol}).

Lizard blood was sampled from November–January (hibernating period) and from May–June (activity period). Animals were collected in the enclosure and kept in the laboratory, in an isothermal box with bog, moss and herbs, for less than 24 h before each DSC sampling. Blood was taken from the orbital sinus with a microcapillary treated with heparin and introduced into the calorimeter in < 15 min after sampling. We verified that the presence of anticoagulant did not interfere with the characteristics of the blood as reference Ringer's solution, with and without heparin, showed the same crystallization temperature.

We used unpaired Student's *t*-test for all statistical comparisons. Each result is shown as a mean

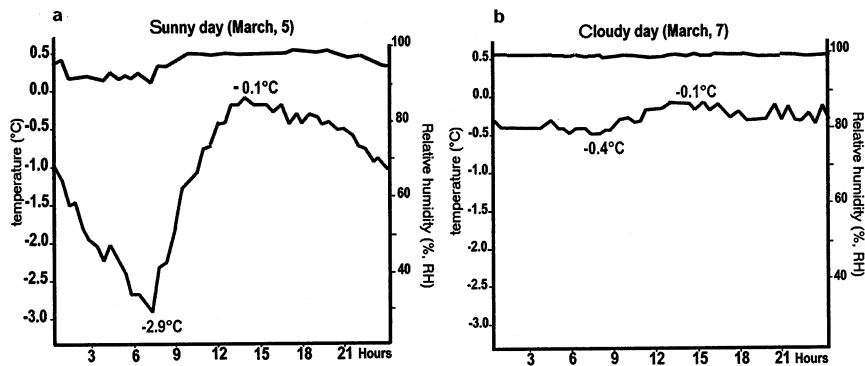


Fig. 1. Temperature and relative humidity (datalogger Hot Dog) inside an hibernaculum (bog) of *L. vivipara*, at the beginning of March: (a) during a sunny day, minimal air temperature at 30 cm above the ground -11°C ; (b) during a cloudy day, minimal air temperature at 30 cm above the ground -4°C .

value with a standard deviation. The number of individuals (n) used for the calculation of each mean is given in brackets.

4. Results

The hibernacula of the highland population in the Jura Mountains were much deeper below the ground than those of the lowland population (Grenot et al., 1996). In January and February, mean daily temperatures at the overwintering sites and at the soil surface were identical, near 0°C . However, thermal fluctuations at the soil surface were more prominent than at depth (9 and 0.9°C , respectively). The thermocouples were protected in winter by the snow pack, and thermal fluctuations were accordingly lower than those registered 30 cm above ground. The overwintering sites were covered by 10 cm of bog and often by several centimeters of snow (up to a maximum depth of 40 cm in mid-February), which are both absorbent substrates. These substrates displayed stable temperatures (about 0°C), which helped keep the RH constant (near the water saturation point) (Fig. 1a). However, temperature variations between -1 and -4°C were observed in the hibernacula during the cold periods when the snow cover was thin and the air temperature low ($< -10^{\circ}\text{C}$) (Fig. 1b). Beginning in March, the temperature at the overwintering sites increased at the same rate as the air temperature. Mean daily temperature in April increased from $+1.5^{\circ}\text{C}$ in March to $+6^{\circ}\text{C}$.

During the experiments, thermal differences increased but stayed lower than those at the soil

surface. The ground (bog) acted as a 'buffer' by keeping temperatures lower during the daytime and warmer at night than those temperatures observed at the soil surface. Given weather conditions such as those that occurred in April, daily thermal differences occasionally climbed as high as 40°C at the surface of dry grass (*Molinia coerulea*) (Fig. 2).

4.1. Freeze tolerance

We used the following criteria to determine whether a lizard was frozen. The general aspect of the animal: frozen animals became hard, their skin color was dark instead of brown and they assumed a characteristic rolled-up position with the snout against the vent. The consistency of the

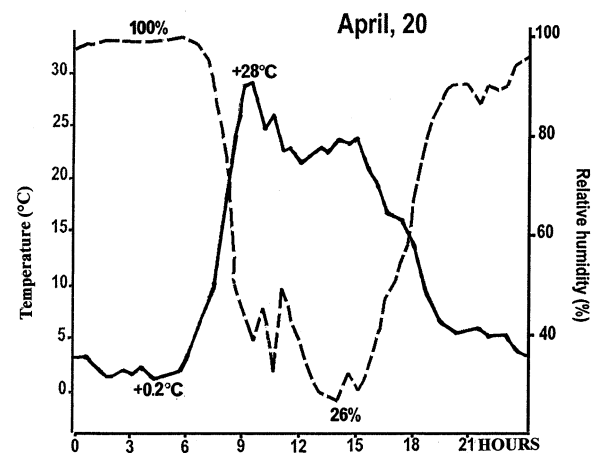


Fig. 2. Temperature and relative humidity (datalogger Hot Dog) near the surface of a tuft of dry Graminea, *Molinia coerulea* in a peatbog at Bonnevaux (Jura Mountains 850 m).



Fig. 3. A frozen and isolated *L. vivipara* (male adult) near the peatbog of Bonnevaux-Frasne in January: (a) in situ, after removing a tuff of moss with bog of about 15 cm deep; dorsal view, the skin is black; (b) ventral view, with layer of ice crystals (the animal seems dead); (c) the lizard just after its thaw, moves very slowly with uncoordinated movements.

body: frozen animals became as hard as ice and were often covered with ice crystals.

Of 15 lizards found in January in the enclosure, three (one juvenile and two adults) were discov-

ered frozen in their overwintering sites. We learned that lizards that froze during the night often began to thaw the next afternoon and that the state of freezing therefore rarely exceeded 48 h

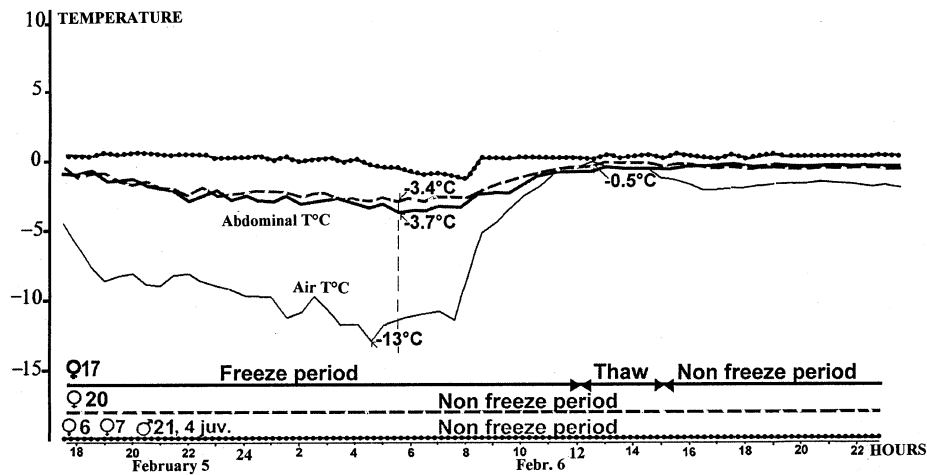


Fig. 4. Ventral temperatures of *L. vivipara* recorded in three different overwintering sites, and air temperature at the surface of the soil, at Bonnevaux (Jura Mountains, 850 m).

(Fig. 3). At least several frozen lizards were observed alive a week later.

Some individuals (e.g. male number 3) did not seem to freeze even though they were in contact with snow crystals. We thus saw two contrasting strategies, freezing and supercooling, occurring at the same temperatures, most often between -0.5 and -4.0°C (Fig. 4).

We observed only rarely a single individual using both strategies in the same winter. Female number 20 nevertheless did so. She stayed supercooled in January and February when the temperature of the overwintering site stayed at about -4°C , and was then frozen in March when the temperature of the hibernaculum varied between -1.2 and -1.5°C .

Variations in the temperature and the RH at an overwintering site often depended on weather conditions in the same period (beginning of March, Fig. 1). On one sunny day (March 5) when the minimum ambient temperature was -11°C , the hibernaculum temperature rose from -2.9°C to -0.1°C (between 7:00 h and 13:00 h), an increase of almost 3°C in 5 h. Two days later, even though the minimum ambient temperature was -4°C and the weather cloudy, the thermal range of the hibernaculum was almost constant from -0.1 to -0.5°C .

We also followed temperature and RH of the hibernacula during the emergence of the lizards from hibernation, which normally occurs at the end of March (between the 18th and 25th). The determining factor for emergence seemed to be a

period of sunshine in early spring that warmed the soil in depth. For example, female lizard (number 15) left her hibernaculum on March 23 (Fig. 5). At that time, the temperature of the ground deepened to the level of the overwintering sites. Beginning on 19 March, the daily maximum temperature began to cycle between $+2^{\circ}\text{C}$ (March 19) and $+8^{\circ}\text{C}$ (March 23), the latter corresponding to the date when the first lizard emerged. During this time, the RH did not vary much and stayed above 95%.

Mean cloacal temperatures of active lizards in the field were 32°C in April, and the time range of surface activity was from 07:00 h to 17:00 h

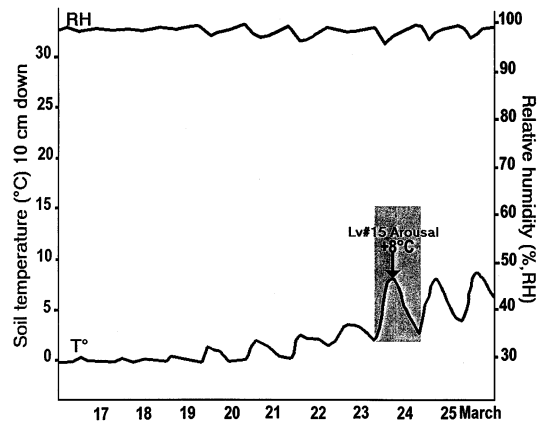


Fig. 5. Temperature and relative humidity (Hot Dog) inside a hibernaculum (bog) of *Lacerta vivipara*, during a period of sunny days. The female number 15 placed in its hibernaculum went out, on March 23 (the maximal temperature of the bog was $+8^{\circ}\text{C}$).

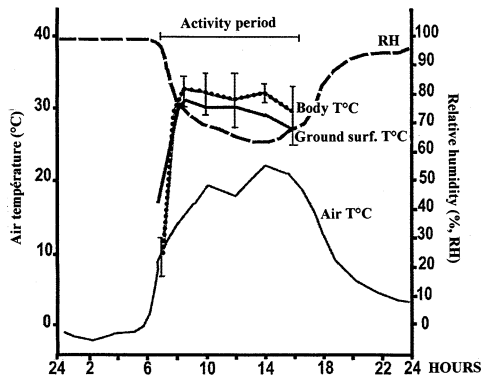


Fig. 6. Mean daily cloacal temperatures ($n = 7$), during three consecutive sunny days in April 19, 20 and 21; after emergence from the bog (Jura Mountains, 850 m).

(Fig. 6). Some of the animals 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 ($< -2^{\circ}\text{C}$) by maintaining a state of supercooling for several days. In another study Costanzo et al. (1995) observed supercooling lasting more than 21 days at -3.5°C . When they were uncovered in December (with a daylight temperature of 1.5 – 2.0°C), their eyes were closed but they were able to make uncoordinated movements and could dig into the peat.

4.2. Differential scanning calorimetry (DSC)

DSC measurements on total blood of *L. vivipara* did not show significant differences between males and females (Table 1). The mean freezing onset T_{sol} (kinetic parameter) was lower during the overwintering period than during the lizards' period of activity. The mean melt or equilibrium freezing point onset (T_{fus}) (thermodynamic parameter) was also lower during the overwintering period than during the period of activity. The supercooling capacities ($T_{\text{fus}} - T_{\text{sol}}$) were quite different ($T = 2.3^{\circ}\text{C}$) between the two periods and were higher during the overwintering period (Table 1).

4.3. Blood glucose

The mean blood glucose level of *L. vivipara* held in our enclosures increased significantly from September to March, from 8.5 ± 0.7 to 33.2 ± 5.6 mmol l^{-1} ($P > 0.001$) (Fig. 7). During winter, the

Table 1

Comparison among physical parameters of the blood of *Lacerta vivipara* between two periods of the life cycle ($n = 7$)^a

	December–March	May–October
Freezing temperature T_{sol} ($^{\circ}\text{C}$)	-12.7 ± 0.2	-8.1 ± 0.2
Equilibrium freezing point T_{fus} ($^{\circ}\text{C}$)	-3.8 ± 0.2	-1.5 ± 0.2
Supercooling capacity ($^{\circ}\text{C}$)	8.9	6.6

^a Relevant test T : $P < 0.005$.

blood glucose level of frozen lizards was as high as that in unfrozen lizards. The maximum value found in an unfrozen lizard in January was 37.9 mmol l^{-1} . The blood glucose level for one of the frozen *L. vivipara* was 32.8 mmol l^{-1} the same month. In this case, the blood sample was collected when the lizard was thawed before a new daily freeze–thaw cycle.

We felt that hyperglycemia that we observed was not a stress response to frequent handling because blood was taken at most once a month in the enclosures. Moreover, blood glucose levels indicated considerable individual heterogeneity. In the field, a few weeks before hibernation in October, for example two adults of 3.6 and 2.5 g, respectively measured 5.8 and 28.6 mmol l^{-1} . Later, during hibernation in January, two adults of the same BM measured 13.8 and 37.9 mmol l^{-1} . Blood glucose levels did not differ between adults and juveniles nor between sexes.

The maximum and minimum glucose values of *L. vivipara* were 50.1 mmol l^{-1} in March and 4.5 mmol l^{-1} in September. After hibernation in April, blood glucose levels were characterized by

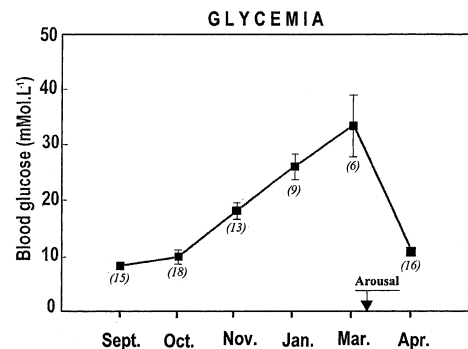


Fig. 7. Seasonal field blood glucose level in a highland population (Jura Mountains, 850 m) of unfrozen *L. vivipara* (n), \pm S.E.M.

a drop in the mean blood glucose level of 11 mmol l⁻¹ ($P > 0.001$), the level was close to the value observed in September.

5. Discussion

In the past, winter dormancy in reptiles was considered simply a cold-induced torpor resulting from the animal's inability to maintain a high body temperature at low ambient temperatures. Today we realize that dormancy may be accompanied by important changes in body composition comparable, but not identical to, those observed in hibernating mammals (Gregory, 1982). Weigmann (1929) showed that the wall lizard, *Podarcis muralis*, avoids freezing during winter. 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). Today we also know that many animals with reduced freezing points in winter create antifreeze substances which prevent the growth of deleterious ice crystals within their bodies (Grenot and Heulin, 1990).

The values for the freezing points we obtained in these experiments are significantly lower than those measured for amphibians, for example such temperatures for frogs range from -0.5 to -1.5°C (Héroid et al., 1994; Somme, 1995).

Laboratory studies by Costanzo et al. (1995) showed the importance of hyperglycemia to sur-

vival of frozen lizards. In the present study, the two-to-fivefold higher levels of blood glucose in both frozen and unfrozen *L. vivipara* indicate that this species uses glucose both during supercooling and during freezing. Elevated glucose levels persist long after thawing and begin before freezing. This persistence may reflect a much stronger freeze-induced elevation of intracellular glucose levels. The cryoprotective system of *L. vivipara* seems to be very different from that of *Rana sylvatica* (Storey and Storey, 1986), which rapidly increases its glucose level to more than 300 mmol l⁻¹ at the beginning of freezing.

Supercooling and freeze tolerance were formerly considered alternate strategies for coping with subzero temperatures (Costanzo and Lee, 1995). Our study suggests that *L. vivipara* has developed a capacity for both supercooling and freeze tolerance. We were lucky to observe, on March 4, three lizards located next to each other under the same mound of bog: the first frozen, the second supercooled, and a third which was half and half. The posterior part of its body was frozen, whereas the anterior part was unfrozen with the head driven into the bog. The two strategies of freezing and supercooling were thus encountered in the same microclimate. Moreover these observations suggest that the strategy used by an animal depends above all on its physiological capability. Our results also suggest that further study should be directed to discovering additional antifreezes or absence of ice nucleators to expand our knowledge of freeze avoidance strategies.

Table 2
Cold tolerance of some species of ectotherm vertebrates

Species	Supercooling capacity		Freeze tolerance	
	Abdominal temperature ($^{\circ}\text{C}$)	Duration	Abdominal temperature ($^{\circ}\text{C}$)	Duration
<i>Chrysemys picta</i> juveniles	-12.0 (1) ^a	about 12 days	-4.0 (2)	>24 h
<i>Chrysemys picta</i>			-2.5 (3)	3 days
<i>Terrapene carolina</i>			-0.83 to -3.60 (4)	72 h
<i>Sceloporus jarrovi</i>	-3.0 (5)	about 30 h		
<i>Lacerta vivipara</i>	-3.5 (6)	≥ 21 days	< -3.0	>3 days
<i>Thamnophis sirtalis</i>			-0.75 to -1.20 (7)	48 h
<i>Thamnophis sirtalis</i>			-2.5 (8)	3 to 5 h
<i>Rana sylvatica</i>			-2.5 to -3.0 (3)	3 to 13 days
<i>Rana sylvatica</i>			-1.5 (9)	>11 days
<i>Hyla versicolor</i>			-2.5 (10)	13 days

^a (1) Packard and Packard, 1993; (2) Storey et al., 1988; (3) Storey and Storey, 1986; (4) Costanzo and Claussen, 1990; (5) Halpern, 1979; (6) Costanzo et al., 1995; (7) Costanzo and Claussen and Lee, 1988; (8) Churchill and Storey, 1991; (9) Laynes et al., 1998; (10) Storey and Storey, 1985.

One of the most striking characteristics of overwintering lizards during cold-hardiness is their capacity to accumulate a high concentration of glucose. Our data for *L. vivipara*, which represent the longest record of supercooling for any vertebrate, suggest that they can maintain a body temperature of -3.5°C for at least 3 weeks (Table 2). However, it does not mean that lizards remained supercooled throughout the whole winter.

Acknowledgements

We are particularly grateful to the staff of the C.P.I.E. at Bonnevaux Station (Haut-Doubs) for providing us with a welcoming environment, and to F. Sottile for valuable assistance. We wish to thank Dr Jerry Freilich for comments and for improving our manuscript. The authors also wish to thank the reviewers for constructive and helpful criticism. Studies performed here were not supported by any grant from our laboratory (C.G.).

References

- Bauwens, D., 1981. Survivorship during hibernation in the European common lizard *Lacerta vivipara*. *Copeia* 3, 741–744.
- Bidault, M., 1982. Les zones humides du secteur Frasnne-Bonnevaux Doubs. *Bull. Ecol.* 13 (2), 241–263.
- Block, W., 1994. Differential scanning calorimetry in ecophysiological research. *Acta Oecol.* 15, 13–22.
- Churchill, T.A., Storey, K.B., 1991. Metabolic responses to freezing by garter snakes. *Cryo-Lett.* 12, 359–366.
- Claussen, D.L., Townley, M.D., Bausch, R.G., 1990. Supercooling and freeze tolerance in the European wall lizard, *Podarcis muralis*, with a revisional history of the discovery of freeze tolerance in vertebrates. *J. Comp. Physiol. B* 160, 137–142.
- Costanzo, J.P., Claussen, D.L., 1990. Natural freeze tolerance in the terrestrial turtles, *Terrapene carolina*. *J. Exp. Zool.* 254, 228–232.
- Costanzo, J.P., Claussen, D.L., Lee, R.E., 1988. Natural freeze tolerance in a reptile. *Cryo-Lett.* 9, 380–385.
- Costanzo, J.P., Grenot, C., Lee, R.E., 1995. Supercooling and freeze tolerance promote winter survival of the European common Lizard, *Lacerta vivipara*. *J. Comp. Physiol. B* 165, 238–244.
- Costanzo, J.P., Lee, R.E. (1995). Supercooling and ice nucleation in vertebrates ectotherms. In: Lee, R.E., Warren, G.J., Gusta, L.V. (Eds.), *Biological Ice Nucleation and Applications*. Am. Phytopathol. Soc. Press, Minnesota, chapter 12.
- Gregory, P.T. (1982). Reptilian hibernation. In: Gans C., Pough, F.H. (Eds.), *Biology of the Reptilia*, vol. 13. Academic Press, New York, pp. 53–154.
- Grenot, C., Heulin, B., 1990. Sur la plasticité écophysiological du lézard vivipare, *Lacerta vivipara* (Reptilia, Lacertidae). *Bull. Soc. Herp. Fr.* 54, 190–204.
- Grenot, C., Garcin, L., Tséré-Pagès, H. (1996). Cold-hardiness and behavior of the European Common Lizard, *Lacerta vivipara*, from French populations during the winter. In: Geiser, F., Hulbert, A.J., Nicol, S.C. (Eds.), *Adaptations to the Cold: Tenth International Hibernation Symposium*. University of New England Press, Armidale, pp. 115–121.
- Grenot, C., Heulin, B., 1988. Emploi de radioisotopes pour la localisation de *Lacerta vivipara* et l'étude de son métabolisme au cours de l'hivernage. *C.R. Acad. Sci. Paris. Ser. III* 307, 305–310.
- Grenot, C., Heulin, B., Pilorge, T., Khodadoost, M., Ortega, A., Mou, Y.P., 1987. Water budget in some populations of the European common lizard, *Lacerta vivipara* Jacquin. *Funct. Ecol.* 1, 131–138.
- Halpern, E.A., 1979. Supercooling in the iguanid lizard *Sceloporus jarrovi*. *Physiol. Zool.* 52, 190–204.
- Hérould, J.-P., Dao, J., Grenot, C., Fahys, B., 1994. Variations altitudinales du point de congélation du sang et du plasma chez la grenouille rousse (*Rana temporaria* L.). *Bull. Soc. Herp. Fr.* 71–72, 33–37.
- Heulin, B., 1985. Démographie d'une population de *Lacerta vivipara* de basse altitude. *Acta Oecol. Oecol. Gener.* 6, 261–280.
- Laynes, J.R., Costanzo, J.P., Lee, R.E., 1998. Freeze duration influences postfreeze survival in the frog *Rana sylvatica*. *J. Exp. Zool.* 280, 197–201.
- Packard, G.C., Packard, M.J., 1993. Hatchling painted turtles (*Chrysemys picta*) survive exposure to sub-zero temperatures during hibernation by avoiding freezing. *J. Comp. Physiol. B* 163, 147–152.
- Patterson, J.W., Davies, P.M.C., 1978. Energy expenditure and metabolic adaptation during winter dormancy in the lizard *Lacerta vivipara* Jacquin. *J. Thermal. Biol.* 3, 183–186.
- Pernot-Visentin, O., 1978. Climatologie et microclimatologie de quelques biotopes caractéristiques de la région de Bonnevaux-Frasne-Bouverans. *Publication du CUER* 2, 67–107.
- Somme, L. (1995). Invertebrates in hot and cold environments? *Adaptations of Desert Organisms*. Springer.
- Storey, K.B., Storey, J.M., 1985. Adaptation of metabolism for freeze tolerance in the gray tree frog, *Hyla versicolor*. *Can. J. Zool.* 63, 49–54.

- Storey, K.B., Storey, J.M., 1986. Freeze tolerant frogs: cryoprotectant and tissue metabolism during freeze/thaw cycles. *Can. J. Zool.* 64, 49–56.
- Storey, K.B., Storey, J.M., Brooks, S.P.J., Churchill, T.A., Brooks, R.J., 1988. Hatchling turtles survive freezing during winter hibernation. *Proc. Natl. Acad. Sci. USA* 85, 8350–8354.
- Van Damme, R., Bauwens, D., Verheyen, R.F., 1987. Thermoregulatory responses to environmental seasonality by the lizard *Lacerta vivipara*. *Herpetologica* 43, 405–415.
- Weigmann, R., 1929. Die Wirkung starker Abkühlung auf Amphibien und Reptilien. *Z. Wiss. Zool.* 134, 641–692.