Metabolic Adaptations of Overwintering European Common Lizards (*Lacerta vivipara*)

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ABSTRACT

The European common lizard *Lacerta vivipara*, a reptile of cold-temperate climates, provides us an interesting model of low-temperature adaptation. Indeed its unique cold-hardiness strategy, which employs both freeze tolerance and freeze avoidance, may be seen as the primary reason for its large distribution, which extends from Spain to beyond the Arctic circle. To study the metabolism supporting this capacity, we used three techniques: two techniques of calorimetry (oxygen consumption and thermogenesis) and nuclear magnetic resonance spectroscopy. These techniques were used to examine the metabolic balance and the different molecular pathways used between three different periods through the year (September, January, and May). The results show a significant 20% augmentation of winter anaerobic metabolism compared to other periods of the year. This is mainly because of an activation of the lactic fermentation pathway leading to an increase of lactate concentration (>34% in winter). Furthermore, glucose, which increases some 245% in winter, is used as antifreeze and metabolic substrate. Furthermore, this study provides evidence that the physiological adaptations of the common lizard differ from those of other ectotherms such as *Rana sylvatica*. Concentrations of alanine and glycerol, commonly used as antifreeze by many overwintering ectotherms, do not increase during winter.

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Introduction

In amphibians and reptiles, as in mammals, hibernation appears to be a complex adaptive behavior and not only a cold-induced torpor (Joy and Crews 1987). Two cold-hardiness strategies have been reported in invertebrates and a few species of ectothermic vertebrates: freeze tolerance, which involves enduring the freezing of a part of the body fluid, and freeze avoidance, which involves maintaining body fluids in a supercooled state. Supercooling and freeze tolerance are dichotomous strategies for coping with subzero temperatures (Costanzo and Lee 1995), and each of them presents benefits. Freeze avoidance invokes relatively little physiological stress and allows rapid reanimation, whereas for freeze-tolerant animals, the metabolic depression induced during freezing could greatly improve long-term survival (Storey and Storey 1988).

Most of the studies on vertebrates have focused on the wood frog (*Rana sylvatica*), a North American frog with a distribution range extending north to the Arctic Circle. These animals survive freezing for a period of 2 wk (Storey and Storey 1988). In 1994, all known freeze-tolerant vertebrates were endemic to North America. But Costanzo et al. (1995) showed that the European common lizard *Lacerta vivipara*, tolerates freezing (with ice contents ranging from 55% to 65% of total body water) for up to 3 d and supercooling for up to 21 d. This Lacertid from the Old World is a very adaptable species that can inhabit a wide range of environments owing to a remarkable physiological plasticity (Grenot and Heulin 1990). Its distribution extends from the mountains of northwest Spain to Sakhalin on the Pacific coast, a distance of 12,000 km, and from Spain north 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, peat bogs, and heathlands, and from sea level to 3,000 m in altitude.

Because of its unique capacity to endure severe winter by both freeze tolerance and freeze avoidance, overwintering *L. vivipara* provides us with a physiologically relevant model for the study of adaptations to cold environments. However, despite the two studies described above (Costanzo et al. 1995; Grenot et al. 1996), the mechanisms promoting this extraordinary capacity are still poorly understood.

This study focuses on the variations of the metabolic balance throughout a year by comparison of selected metabolic characteristics between three different periods of the lizard's life cycle (September, January, and May). In this manner, a more

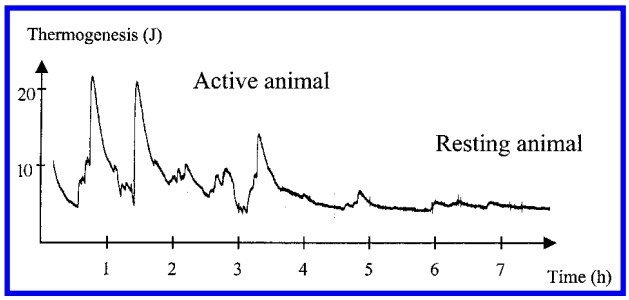

Figure 1. Thermograph of *Lacerta vivipara* obtained with a direct calorimetric technique at 20°C (winter period).

comprehensive view of the metabolic changes necessary for achieving effective overwintering survival can be obtained.

Material and Methods

Studied Population

Individuals were collected at the end of summer from the peat bog in the Bonnevaux-Frasnes region in the Jura Mountains of France (latitude, 46°48.7′N; longitude, 6°11.1′E; altitude, 850 m). This region has severe climatic conditions characterized by high rainfall (1,500 mm yr^{-1}) and a strongly seasonal thermal amplitude (means of the warmest month: July, 16°C; the coldest month: January, −1°C; Perno-Visenti 1978).

Captured lizards were kept in 12-m^2 outdoor enclosures (near Bonnevaux-Frasnes), in which the peat bog environment was reconstituted. Each individual was identified and marked by clipping a fingertip.

Measurement of Metabolic Rate

Metabolic rate was used as an index for evaluating the effects of environmental factors and adaptive processes. With the same apparatus, we made direct and indirect calorimetry measurements to estimate, within the same day with same animals, the total metabolism and the aerobic part of this metabolism in resting animals. By measuring these two parameters at the same time, potential variations resulting from diet, dehydration, and starvation level are avoided. The same temperature (20°C) was used for each period of measurements. Before manipulations, animals in active periods were freshly captured from outdoor enclosures, starved 3 d in a box with a substrate of peat moss (*Sphagnum* sp.) and water ad lib. For overwintering individuals, body temperatures were monitored with a model 50 datalogger (Electronic Controls Design) with thermocouple inputs. Because of the relatively warm environmental temperature, animals were always near 0° ± 1°C. They were captured and acclimated to 4°C for 3 d before study. Only nonparasitized lizards were used for these experiments. The presence of a haemogregarine detected in this population (Y. Voituron and Y. Surget-Groba, unpublished data) is known to induce a decrease of O_2 consumption (Opplinger et al. 1996). A smear of blood collected just after clipping a fingertip allowed us to verify the presence/absence of parasites. Smears were air dried, fixed in absolute methanol, and stained with Giemsa stain. For parasite detection, a magnification of 800 × was used.

Microcalorimetric Measurements

The calorimetric apparatus was made from an aluminum cylinder 380 mm in diameter and 260 mm in height. Two 90-mm wells received the two measuring cells made of Plexiglas (60-mm height, 50-mm inner diameter). A Plexiglas stopper fitted with a toric washer covered the lower part of the cells. A captor (partially copper-plated constantan spiral covered with a polyvinyl chloride disc [3-mm thickness, 50-mm diameter]) was inserted in the bottom plate of both calorimetric bases. A heat current of 14.4 kcal h^{-1} produced a thermoelectric power of 1 mV (Technisch Physiche Dienst w.5 32). The ratemeter signals were amplified and recorded (Philips PM 2434 amplifier and a Graphispot-type servo-recorder). This apparatus was kept in a constant temperature unit. The calorimetric unit was totally described by Guyetant et al. (1981). With this device, lizards are not restrained, and long-term heat flow recording is possible. On the thermogram, the deviation reading in millimeters between the baseline and the thermic permanent state represents the total energy production of the lizard in the experimental chamber. In Figures 1 and 2, the baseline corresponds to the *X*-axis.

The recording started approximately 1 h after the thermal balance had been established on the calorimeter. ΔH is defined as

$$\Delta H = D\frac{P}{g},$$

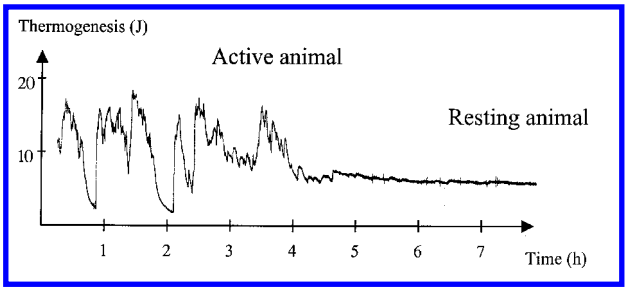

Figure 2. Thermograph of *Lacerta vivipara* obtained with a direct calorimetric technique at 20°C (summer period).

where D is the deviation read on the thermogram, P is the coefficient of the thermal loss expressed in watts per degree (W deg^{-1}), and g is a constant characterized by the sensitivity used (calorimeter and galvanometer) expressed in meters per degree (m deg^{-1}). Thus, the ratio P/g is expressed in watts per meter (W m^{-1}) and defines the sensitivity of the calorimeter. In this case,

$$\frac{P}{g} = 0.13 \text{ mW mm}^{-1}.$$

The microcalorimetric units of milliwatts (mW) are expressed in millijoules per gram per hour (mJ g^{-1} h^{-1}). Examples of thermograms obtained with this technique are shown in Figures 1 and 2.

O_2 Consumption Measurements

Indirect calorimetry was accomplished by measuring O_2 consumption of lizards in the calorimetric chamber. The chamber was connected to a volumetric apparatus without interrupting the calorimetric recording. CO_2 efflux from the animal was fixed by paper saturated with KOH (8%) that was placed on the inner wall of the chamber. Atmospheric humidity was attained with a few drops of water inside the chamber. During measurements, there were no disturbances on the thermograms, which indicated an absence of stress on the animal. We used a constant-pressure volumetric system in which animal O_2 consumption induced movements of a manometric fluid along a scaled capillary tube.

The control chamber received the same volumetric unit to control possible perturbations resulting from the atmospheric and/or humidity variations. Similar protocol was used by Hérold et al. (1985) with *Rana ridibunda.*

To evaluate CO_2 production, the same protocol was used a second time on lizards but without KOH in the measuring cell. The record of the index movements only began when resting metabolism was equivalent to that found with the preceding measurements with KOH.

Respirometric measurements were made every 15 min for 1 h. The energy equivalent was estimated at 19.67 J mL^{-1} in winter and at 20.2 J mL^{-1} for the other periods. These oxicaloric coefficients were based on the respiratory quotient (RQ) values obtained with the different respirometric measurements (Schmidt-Nielsen 1979).

NMR Spectroscopy

NMR spectroscopy is very useful in the field of comparative physiology (Wasser et al. 1996). Its main advantage in this study is that it allows us to quantify molecules such as glucose, lactate, alanine, glutamate, acetoacetate, and D-3-hydroxybutyrate in a very small sample of plasma (approximately 300–450 μL). The lizards used for this experiment were placed in similar conditions as those described for the calorimetric measurements. Blood was sampled between 2:00 and 4:00 P.M. from the infraorbital sinus of the lizards and collected into heparinized tubes. Blood collection began within 1 min of capture and lasted less than 3 min. Plasma was obtained by centrifugation (1,000 g for 10 min) of the blood samples and was stored at −30°C until assay.

Proton NMR spectra were recorded on a Bruker AM400WB spectrometer (Wissembourg, France) at 400 MHz. The signal from residual water was suppressed by the presaturation technique with an irradiation of 0.08 W for 2 s. Chemical shift resonances were expressed in ppm, with reference to STP (sodium trimethylsilylpropionate, external) assigned to 0 ppm.

One-dimension (1D) experiments were performed at 20°C with a 60° flip angle, and 64 transients were accumulated. Acquisition time was 0.68 s on 8,000 data points corresponding to a sweep width of 6,000 Hz. The Fourier transformation (FT) was performed after a zero-filling to 16,000 data points and exponential multiplication corresponding to 1-Hz line broadening. Two-dimension correlated spectroscopy (COSY) experiments were performed with 1,000 data points in the F2 direction and 256 data points in the F1 direction. The sweep width was reduced to 2,700 Hz, eliminating the aromatic region of the spectrum where no resonance was detected in 1D acquisitions. The 2DFT was applied after zero-filling to 512 data points in the F1 direction and a sine-bell function in both directions.

Each experiment consisted of three 1D acquisitions and three 2D COSY spectra. Peak assignments were made based on data from the literature and by using spectra obtained on standards.

Statistical Test

All statistical analyses were performed with a SAS computer statistical package (SAS Institute 1989). When necessary, data were log $(X + 1)$ or square-root-square transformed to homogenize variances and normalize residuals. When this was not possible, we used a Kruskal-Wallis nonparametric ANOVA test.

An analysis of covariance (ANCOVA) was used to determine whether or not a thermogenesis effect existed on the O_2 consumption (O_2 consumption as dependent variable and thermogenesis as covariate). In this way, we evaluated the possible differences of the aerobic-metabolism percentages between the seasons. A 5% ($P < 0.05$) level of significance was used in all tests.

Results

Calorimetric Techniques

All values are shown in Table 1. Thermogenesis at rest did not show any significant differences between the three periods ($df = 22$, $F = 2.83$, $P = 0.083$).

Table 1: Values obtained with two calorimetric techniques on resting *Lacerta vivipara* (mountain population) at three different periods

Period	Number of Values (n)	O_2 Consumption ($J\ g^{-1}\ h^{-1}$)	Thermogenesis ($J\ g^{-1}\ h^{-1}$)	Anaerobic Metabolism (%)
September	6	1.53 ± .06	1.85 ± .11	17
January	9	1.26 ± .05	1.99 ± .08	36.5
May	9	1.71 ± .02	2.07 ± .27	17.5

Note. Values are $\bar{X} \pm$ SD.

In contrast, oxygen consumption showed significantly different values for each period (df = 17, $F = 143.4$, $P < 0.001$), with the values being (from highest to lowest) posthibernating period (May), prehibernating period (September), and hibernating period (January). ANCOVA showed a significant effect of season on the O_2 consumption (df = 17, $F = 91.3$, $P < 0.001$).

RQ values calculated from the ratio CO_2 formed/O_2 used are shown in Table 2. A significant difference exists between winter period and other periods (KW = 13.43, $P < 0.001$), but comparison between prehibernating and posthibernating period shows no significant differences.

NMR Spectroscopy

Statistical analyses show a strong seasonal effect on all plasmatic metabolite concentrations (except glutamate). Glucose, lactate, and hydroxybutyrate significantly increase during winter, while alanine shows the inverse response for the same period. The P values (ANOVA test) were always <0.0002 (except for glutamate; $P = 0.29$). Values are presented in Table 3.

Discussion

Metabolic Adaptations

In western Europe, overwintering *Lacerta vivipara* has to endure very low temperatures for 5–6 mo in its shallow terrestrial burrows within grass hummocks (Grenot and Heulin 1993). Patterson and Davies (1978) found that the cost of hibernation represents only 5% of the annual energy budget on an individual *L. vivipara* weighing 3.4 g. But even if biological processes are slowed down at low temperatures, some level of activity is required to maintain vital functions, and so the management of energetic reserves during this period remains important because of the limited quantity of metabolic substrates.

Direct calorimetry, showing no relevant difference between the three periods, indicates that the basal metabolism of *L. vivipara* does not decrease during hibernation more than would be expected from the effects of temperature. However, O_2 consumption values show that the oxidative metabolism fluctuates through the year. All experiments were made at 20°C, so this variation may come from an endogenous cycle linked to the internal clock of the animal. This kind of mechanism has been reported for *Rana temporaria* (Hérold 1992), which also tolerates freezing for at least 24 h (Pasanen and Karhapää 1997) and also increases its anaerobic metabolism by about 20%. The molecular approach by NMR spectroscopy is consistent with the calorimetric results.

Lactate concentration, which usually provides an index of anaerobic metabolism in reptiles (McDonald 1976), increased about 34% during winter. This low value could be explained by the recycling of lactate to glucose via the Cori cycle in the liver. This regeneration pathway is costly (1 ATP and 1 GTP per lactate molecule) but could have the advantage of helping to provide the increase of glucose, which is known to be key to this ectotherm's overwintering survival. The lizards probably face a trade-off between antifreeze production and energy production via fermentation.

During hibernation, most energy is derived from lipids (Avery 1970, 1974) stored in triacylglycerol form, and this energy source explains why we used a different value of the oxicaloric coefficient to convert oxygen consumption in joules per gram per hour ($J\ g^{-1}\ h^{-1}$) during this season. The yield from the complete oxidation of these compounds is about 9 kcal g^{-1} (Stryer 1988). This high caloric yield is possible because they are reduced and anhydrous. These molecules are degraded by the β-oxidation into acetyl-CoA, which enters the citric acid cycle only if fat and carbohydrate degradation are balanced (Stryer 1988). Because overwintering lizards prefer to burn fatty acids accumulated before winter, acetyl-CoA is diverted to the formation of acetoacetate and D-3-hydroxybutyrate. This ketone body formation permits the regeneration of free coenzyme A for β-oxidation and may explain the extraordinary jump (+216%) of D-3-hydroxybutyrate. Moreover, the ratio of hydroxybutyrate to acetoacetate depends on the $NADH/NAD^+$

Table 2: RQ of resting *Lacerta vivipara*

Period	RQ
Prehibernating	.93 ± .01
Hibernating	.76 ± .03
Posthibernating	.94 ± .01

Note. Values are $\bar{X} \pm$ SD; for all values, $n = 6$.

Table 3: Concentration variations (mmol L^{-1}) of molecules detected by NMR spectroscopy in plasma samples of *Lacerta vivipara*

	Glucose	Lactate	Hydroxybutyrate	Alanine	Glutamate
September	4.26 ± .09	4.39 ± .09	.59 ± .04	.57 ± .03	1.19 ± .22
January	19.77 ± .12	7.78 ± .12	2.46 ± .05	.27 ± .03	.69 ± .19
May	6.20 ± .10	5.10 ± .10	.50 ± .04	.18 ± .03	.99 ± .16
KW	7.2	7.2	6.5	6.4	2.8
P	.003	.003	.01	.01	.29

Note. Values are $\overline{X}$ ± SEM.

ratio. So it is possible to consider this last transformation as a balance to generate the NAD^+ involved in the Cori cycle. After release in the blood, hydroxybutyrate can become an energetic substrate for the brain, a further possible advantage for lizards by preserving glucose for antifreeze function. The cryoprotectant system is, thus, linked to the catabolism of lipid reserve (Patterson et al. 1978).

These metabolic reactions occurring in the liver illustrate its essential role for overwintering individuals. This conclusion is in complete agreement with results from several experiments on *Rana sylvatica* (Pinder et al. 1984; Rubinsky et al. 1994) that show that the liver is the last organ to freeze when ice has propagated throughout the bodies of frogs.

Cryoprotective Molecule

Glucose deserves special analysis because of its possible role as both a metabolic substrate for tissues and as a cryoprotectant. With regard to the absolute values, active *L. vivipara* glycemic state is comparable to various lizard species (Dessauer 1970), and basal blood-glucose levels for hibernating lizards are similar to those found by Costanzo et al. (1995).

This molecule and other polyols protect the lactate dehydrogenase during hibernation (Carpenter and Crowe 1988), which is in complete agreement with the activation of the lactic fermentation pathway. This phenomenon is well known in other vertebrates such as *R. sylvatica* (Storey 1991, 1996; Costanzo et al. 1993). When a frog freezes, rapid glucose mobilization from the liver glycogen reserve and its distribution by the blood permit survival. But *L. vivipara* seems to increase its glucose concentration (about 200%) for the entire winter period and not only during the freezing episode. Decreases were observed in glucose precursors such as alanine (−63% in winter period) and glutamate (−42% in winter period). In this way, the animal depresses its freezing point and increases its freeze tolerance (Costanzo et al. 1993; Swanson et al. 1996).

It is quite interesting to note this drop of alanine concentration because this molecule is used as an antifreeze in a few species of insects (Somme 1967) and in amphibians such as *R. sylvatica* (Storey et al. 1986). A production and, thus, an increase of this end-product would indicate anaerobic amino acid or protein catabolism coupled to carbohydrate fermentation. Alanine function seems to be different in the European common lizard.

Another interesting element has to be noted: NMR spectroscopy did not detect glycerol in any season. From this, we may conclude that *L. vivipara* does not use this triol as an antifreeze. This also makes *L. vivipara* different from other animals who are well adapted to the cold, such as *Rhabdophga strobiloïdes* (Miller 1982), mites (Block and Convey 1995), fishes (De Vries et al. 1977), and frogs (Schmid 1982).

This study provides conclusive evidence that the physiological adaptations of the common lizard *L. vivipara* differ from those of other ectotherms such as *R. sylvatica* (alanine and glycerol are not used as antifreeze). These adaptations help to explain the exceptional survival rates of *L. vivipara* (88%–100%, all age classes) during the extreme cold of winter (Bauwens 1981).

But we must be aware that our data are difficult to extrapolate to lowland populations of *L. vivipara*, on which tests of freeze tolerance have been made, because the degree of cold tolerance strongly depends on geographic origin (Spellerberg 1976). Furthermore, it seems reasonable to infer that the relative contribution of anaerobic metabolism could be greater in frozen individuals because the lungs are not functional. In this state, oxygen transport is stopped and lactic fermentation is then the only source of energy. But the Cori cycle is also stopped because of the frozen state of the liver, and the resulting accumulation of lactate could explain the limited survival time (3 d) of freezing in this species.

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Literature Cited

Avery R.A. 1970. Utilization of caudal fat by hibernating common lizard, *Lacerta vivipara*. Comp Biochem Physiol B 37: 119–121.

———. 1974. Storage lipids in the lizard *Lacerta vivipara*: a quantitative sudy. J Zool (Lond) 173:419–425.

Bauwens D. 1981. Survival during hibernation in the European common lizard, *Lacerta vivipara*. Copeia 1981:741–744.

Block W. and P. Convey. 1995. The biology, life cycle and ecophysiology of the Antartic mite *Alaskozetes antarticus*. J Zool (Lond) 236:431–449.

Carpenter J.F. and J.H. Crowe. 1988. The mechanism of cryoprotection of proteins by solutes. Cryobiology 25:244–255.

Costanzo J.P., C. Grenot, and R.E. Lee. 1995. Supercooling, ice inoculation and freeze tolerance in the European common lizard, *Lacerta vivipara*. J Comp Physiol B 165:238–244.

Costanzo J.P. and R.E. Lee. 1995. Supercooling and ice nucleation in vertebrate ectotherms. In R.E. Lee, G.J. Warren, and L.V. Gusta, eds. Biological Ice Nucleation and Its Applications. American Phytopathological Society, St. Paul, Minn.

Costanzo J.P., R.E. Lee, and H. Lortz. 1993. Glucose concentration regulates freeze tolerance in the wood frog *Rana sylvatica*. J Exp Biol 181:245–255.

Dessauer H.C. 1970. Blood chemistry of reptiles: physiological and evolutionary aspects. Pp. 1–72 in C. Gans and T. Parsons, eds. Biology of the Reptilia. Vol. 3. Academic Press, New York.

De Vries A.L. and Y. Lin. 1977. The role of glycoprotein antifreezes in the survival of Antarctic fishes. P. 439 in G.A. Llano, ed. Adaptations within Antarctic Ecosystems. Gulf, Houston.

Grenot C., L. Garcin, and H. Tséré-Pages. 1996. Cold-hardiness and behavior of the European common lizard, *Lacerta vivipara*, from French populations during the winter. Pp. 115–121 in F. Geiser, A.J. Hulbert, and S.C. Nicol, eds. Adaptations to the Cold: Tenth International Hibernation Symposium. University of New England Press, Armidale.

Grenot C. and B. Heulin. 1990. Sur la plasticité ecophysiologique du lézard vivipare *Lacerta vivipara* (Reptilia, Lacertidae). Bull Soc Herpétol France 54:1–22.

———. 1993. 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 3:307–310.

Guyetant R., J.P. Hérold, and G. Cudey. 1981. Microcalorimetric measurements of energy flux in *Rana temporaria* L. and *Bufo bufo* L. tadpoles during development. Comp Biochem Physiol 69A:705–708.

Hérold J.P. 1992. Enquête thermodynamique sur les échanges énergétiques chez quelques ectothermes (Amphibien). Bull Soc Ecophysiol 17(suppl.):43–50.

Hérold J.P., R. Guyetant, and G. Cudey. 1985. Direct and indirect calorimetry measurements during the annual growth cycle of juvenile frogs *Rana ridibunda* P. Comp Biochem Physiol 81A:465–468.

Joy J.E. and D. Crews. 1987. Hibernation in garter snakes (*Thamnophis sirtalis parietalis*): seasonal cycle of cold tolerance. Comp Biochem Physiol A 87:1097–1101.

McDonald H.S. 1976. Methods for the physiological study of reptiles. Pp. 19–126 in C. Gans and W.R. Dawson, eds. Biology of the Reptilia. Vol. 5. Academic Press, New York.

Miller K. 1982. Cold-hardiness strategies of some adult and immature insects overwintering in interior Alaska. Comp Biochem Physiol 73A:595–604.

Opplinger A., M.L. Celerier, and J. Clobert. 1996. Physiological and behavioural changes in common lizards parasitized by Haemogregarines. Parasitology 113:433–438.

Pasanen S. and M. Karhapää. 1997. Can boreal common frog (*Rana temporaria* L.) survive in frost? Ann Zool Fenn 34: 247–250.

Patterson J.W. and P.M.C. Davies. 1978. Energy expenditure and metabolic adaptation during winter dormancy in lizard *Lacerta vivipara* Jacquin. J Therm Biol 3:183–186.

Patterson J.W., P.M.C. Davies, D.A. Veasey, and J.R. Griffiths. 1978. The influence of season on glycogen levels in the lizard *Lacerta vivipara* Jacquin. Comp Biochem Physiol 60B: 491–494.

Pernot-Visentin O. 1978. Climatologie et microclimatologie de quelques biotopes caractéristiques de la région de Bonnevaux-Frasne-Bouverans. Pub CUER 2:67–107.

Pinder A.W., K.B. Storey, and G.R. Ultsh. 1984. Estivation and hibernation. Pp. 250–274 in M.E. Feder and W.W. Burggren, eds. Environmental Physiology of the Amphibians. University of Chicago Press, Chicago.

Rubinsky B., S.T.S. Wong, J.S. Hong, J. Gilbert, M. Roos, and K.B. Storey. 1994. 1H magnetic resonance imaging of freezing and thawing freeze-tolerant frogs. Am J Physiol 266: R1771–R1777.

SAS Institute. 1989. SAS/STAT User's Guide, Version 6. 4th ed. SAS Institute, Cary, N.C.

Schmid W.D. 1982. Survival of frogs in low temperature. Science 215:697–698.

Schmidt-Nielsen K. 1979. Animal Physiology: Adaptation and Environment. 2d ed. Cambridge University Press, Cambridge.

Somme L. 1967. The effect of temperature and anoxia on haemolymph composition and supercooling in three overwintering insects. J Insect Physiol 13:805–814.

Spellerberg I.F. 1976. Adaptations of reptiles to cold. Pp. 261–285 in A. d'A Bellaires and C.B. Cox, eds. Morphology and Biology of Reptiles. Linn Soc Symp Ser 3:261–285.

Storey K.B. and J.M. Storey. 1986. Freeze tolerant frogs: cryoprotectant and tissue metabolism during freeze/thaw cycles. Can J Zool 64:49–56.

———. 1988. Freeze tolerance in animals. Physiol Rev 68: 27–84.

———. 1991. Gelés mais vivant. Pour la Science 160:40–45.

———. 1996. Natural freezing survival in animals. Annu Rev Ecol Syst 27:365–387.

Stryer L. 1988. Biochemistry. 3d ed. W. H. Freeman, New York.

Swanson D.L., B.M. Graves, and K.L. Koster. 1996. Freezing

tolerance/intolerance and cryoprotectant synthesis in terrestrially overwintering anurans in the Great Plains, USA. J Comp Physiol B 166:110–119.

Wasser S.J., R.G. Lawler, and D.C. Jackson. 1996. Nuclear magnetic resonance spectroscopy and its applications in comparative physiology. Physiol Zool 69:1–34.