

# Supercooling and freeze-tolerance in the European wall lizard, *Podarcis muralis*, with a revisional history of the discovery of freeze-tolerance in vertebrates

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**Summary.** Wall lizards were collected in the fall of 1988 from a population introduced in 1951 into Cincinnati, OH. They were acclimated to 5 °C for several weeks prior to testing at sub-zero temperatures. Eleven supercooled lizards were removed from the cooling chamber prior to crystallization after between 15 min and 26 h at body temperatures ranging from -2.2 to -5.9 °C. With the exception of one individual supercooled to -5.0 °C, all lizards recovered fully. The crystallization temperatures of 15 lizards which froze ranged from -0.6 to -6.4 °C. Frozen lizards were stiff with a distinct blue color, which faded upon thawing at 3 °C. The ice contents of frozen lizards were determined calorimetrically and/or estimated from a theoretical model, the two methods being generally in close agreement. Remarkably, five individuals recovered fully from exposures as long as 2 h and with as much as 28% of their body water frozen. Although these animals are not as tolerant as certain other vertebrates they are clearly able to withstand freezing under some circumstances. Failure to survive freezing was attributed either to excessive ice accumulation during a prolonged freeze or to excessive supercooling prior to freezing, which induced a large initial surge of ice formation upon crystallization. Our results accord with those of Weigmann (1929). We accordingly recognize him as the first to demonstrate freeze-tolerance in vertebrates, and we further recognize *P. muralis* as the first vertebrate known to survive freezing.

**Key words:** Freeze-tolerance – Supercooling – Lizards – *Podarcis muralis*

## Introduction

Many ectotherms experience winter temperatures below the freezing point of their body fluids. Several species survive by supercooling, whereas others have the remarkable ability to withstand substantial freezing of their body fluids. Freeze-tolerance has long been known

in terrestrial arthropods and marine mollusks, but until recently this ability was commonly believed to be restricted to invertebrates (Storey and Storey 1988). Several recent studies have added four frogs, a snake, and two turtles to the list of ectothermic species known to tolerate freezing under natural conditions.

Several investigators of freeze-tolerance in vertebrates have been accredited with (e.g., Lotshaw 1977; Schmid 1982) or have claimed (e.g., Costanzo et al. 1988; Storey et al. 1988) various priorities of discovery. A review of the early literature reveals that some of these attributions and claims (including some originating in our laboratory) are unwarranted. The available data suggest that Weigmann (1929) deserves credit as the first to convincingly demonstrate freeze-tolerance in a vertebrate, and that the European wall lizard, *Podarcis* (formerly *Lacerta*) *muralis*, is the first vertebrate shown to be capable of withstanding freezing.

However, before acknowledging Weigmann as the discoverer of freeze-tolerance in vertebrates, it is necessary to verify the existence of such tolerance in *P. muralis*. This is especially important in light of more recent reports indicating the absence of such ability in several lizards (Lowe et al. 1971; Spellerberg 1972). We have, accordingly, reexamined the European wall lizard, *P. muralis*, for supercooling and freeze-tolerance, and, based on our studies coupled with a review of the literature, we present a revisional view of the history of the discovery of freeze-tolerance in vertebrate ectotherms.

## Materials and methods

*Podarcis muralis* were captured in the fall of 1988 from a well-established introduced colony in Cincinnati, OH (see Hedeem 1984) and transported to the laboratory, where they were acclimated gradually to simulated winter conditions (i.e., step-wise reduction of photoperiod and temperature) over a period of several weeks. Food, in the form of crickets, was provided initially (when "day-time" temperatures equalled or exceeded 15 °C) but not during the later stages of acclimatization. Water was available at all times. The final "winter" stage simulated mild winter conditions assumed to exist within a burrow (constant darkness at 5 °C). The lizards

were exposed to this final condition for a minimum of three weeks prior to testing.

Each test animal was pre-cooled to approximately 0 °C to reduce mobility. A fine gauge thermocouple probe was then inserted into its intestine through its cloaca (to a depth of approximately 1 cm) and taped in place. The animal was then carefully placed in the cooling chamber, which consisted of a 19.5 cm diameter, 10.0 cm deep cylindrical plastic tank. The floor and lid of the chamber were insulated with styrofoam (1.5 cm thick), and the lid was further insulated with foam plastic (3.5 cm thick). The chamber walls were lightly insulated with sheet plastic foam. The lizard was placed in a small plastic cage within the cooling chamber to prevent its contacting the chamber walls.

The cooling chamber was immersed in a Techne RB-12 refrigerated bath preset at -6.0 °C for most tests (but at temperatures as low as -10.0 °C in a few instances). Cooling rates were relatively rapid (0.1-0.3 °C·min<sup>-1</sup>) and varied inversely with body mass and bath temperature. Body temperature was monitored continuously via a Sentsortek BAT-12 thermometer coupled to a chart recorder. All thermometers used in this study were calibrated against a certified mercury thermometer.

Within 10 min of ice nucleation, for lizards in which freezing occurred, bath temperature was elevated to -3 °C to retard subsequent ice formation (cf. Costanzo et al. 1988). Frozen lizards were removed from 15 min to 2 h after ice nucleation and placed in a chamber at 3 °C for thawing. After 24 h, the lizards were transferred to a 5 °C chamber. They were periodically examined for responsiveness and apparent recovery, and where this occurred, they were transferred to a laboratory terrarium at 25 °C containing food and water. Recovery was considered to be complete if the lizards fed and behaved normally at 25 °C for one week. Supercooled lizards were likewise transferred after 45 min to 26 h to 3 °C (24 h), then to 5 °C, and finally to 25 °C upon recovery.

Ice contents were estimated calorimetrically for 13 lizards in a second series of tests using equations which incorporate the properties of wet and dry body mass. For these procedures, known masses of ice or lizards (supercooled or frozen) of known initial temperature were quickly transferred to a heavily insulated vessel containing 400 g of well-stirred water and a thermistor probe attached to a Digitec 5810 thermometer. Water temperature was monitored continuously to the nearest 0.01 °C until equilibrium was obtained. A calorimeter constant ( $F_c$ ) was computed from the data for melting ice and the equation (modified from Lee and Lewis 1985):

$$F_c = \frac{(W_i \cdot S_i \cdot T_s) + (W_i \cdot S_w \cdot T_f) + (W_i \cdot Q)}{S_w(W_w + W_i) \cdot (T_i - T_f)}$$

where  $W_i$  = mass of ice,  $S_i$  = the specific heat of ice,  $T_s$  = the absolute value of the temperature of the ice (or tissue),  $S_w$  = the specific heat of water,  $T_f$  = the final temperature of the water in the calorimeter,  $Q$  = the heat of fusion of water,  $W_w$  = the mass of water in the calorimeter, and  $T_i$  = the initial temperature of the water in the calorimeter.

Seven lizards which failed to survive freezing were oven-dried to a constant weight at 60 °C to determine water content. The dry lizards were subsequently used to determine calorimetrically, as above, the specific heat of dry tissue ( $S_d$ ), using the equation (modified from Lee and Lewis 1985):

$$S_d = \frac{F_c \cdot W_w \cdot S_w (T_i - T_f)}{W_d \cdot (T_f - T_s)}$$

where  $W_d$  = dry mass of lizard tissue.

These collective data were then used to empirically determine ice contents (as % of body water frozen) for supercooled and frozen animals. The mass of ice ( $W_i$ ) in a frozen animal was computed from the equation (modified from Layne and Lee 1989):

$$W_i = \frac{[F_c \cdot W_w \cdot S_w (T_i - T_f)] + (T_s - T_f) [(W_d \cdot S_d) + (W_s \cdot S_w)]}{Q + S_w (T_s - T_f) + S_i (FP_{eq} - T_s) + S_w (T_f - FP_{eq})}$$

where  $W_s$  = mass of water in the lizard body tissues and  $FP_{eq}$  = the equilibrium freezing point of the tissue. For a detailed methodology, see Lee and Lewis (1985).

Ice content was also estimated theoretically for all frozen lizards, using a simple model which incorporates measurements of the crystallization temperature ( $T_c$ ), the rebound temperature ( $T_r$ ), a derived estimate of the equilibrium freezing point ( $FP_{eq}$ ), and the final temperature of the lizard upon removal from the freezing chamber (Claussen and Costanzo 1990). Briefly, the abrupt rise in temperature (from  $T_c$  to  $T_r$ ) upon ice nucleation results from the heat of fusion (79.7 cal·g<sup>-1</sup>) of the newly-formed ice. The minimal amount of ice which must be formed in order to generate this initial surge of heat can easily be computed based on the specific heats of the wet and dry masses of the animal. This amount of ice can then be expressed as a % of the body water frozen ( $F$ ). The  $FP_{eq}$ , which will always be at least slightly higher than  $T_r$  if any supercooling has occurred, can then be closely estimated from the equation:

$$FP_{eq} = T_r(100 - F)/100.$$

Once  $FP_{eq}$  has been computed, the ice content of the lizard upon removal from the freezing chamber can be estimated from its final temperature ( $T_f$ ) as:

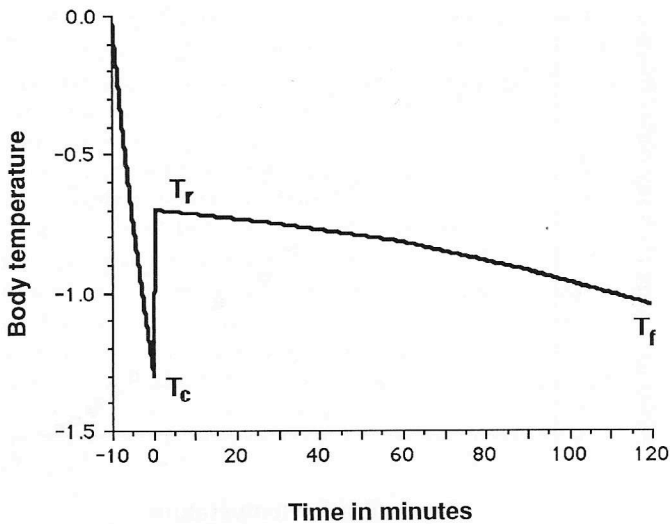
$$F = 100 - 100 (FP_{eq}/T_f).$$

## Results

The 26 lizards used in this study ranged in mass from 1.96 to 7.56 g (mean ± S.D. = 4.69 ± 1.41 g). The mean masses of the supercooled lizards (4.80 ± 1.26 g;  $N=11$ ) and frozen lizards (4.60 ± 1.58 g;  $N=15$ ) did not differ significantly ( $t$ -test;  $P > 0.05$ ).

Eleven animals were removed from the cooling chamber, prior to freezing, at body temperatures ranging from -2.2 to -5.9 °C (mean ± S.D. = -4.0 ± 1.4 °C). With the exception of one individual (mass = 4.78 g) supercooled for 1 h to a temperature as low as -5.0 °C, all of the lizards recovered fully from the treatment (usually within 24 h). The amount of time spent in the supercooled state ranged from as little as 45 min to as long as 26 h (for a 7.10-g individual supercooled to -4.85 °C). Three lizards survived in the supercooled state at temperatures below -5.0 °C (temperatures and exposure times = -5.15 °C for 2 h 15 min, -5.65 °C for 2 h 15 min, and -5.90 °C for 1 h).

Fifteen lizards were allowed to freeze prior to removal from the cooling chamber. These animals showed a typical freezing time-course for a vertebrate ectotherm (Fig. 1). Crystallization temperature was highly variable, ranging from -0.6 to -6.4 °C (mean ± S.D. = -2.9 ± 2.0 °C). There was no significant correlation between body mass and  $T_c$  ( $P > 0.05$ ). The animals were kept in the freezing chamber for 10 min-2 h after ice nucleation. The frozen lizards were stiff upon removal from the chamber and, unlike the supercooled animals, their entire body had a distinct blue color which faded upon thawing at 3 °C. Remarkably, five of the frozen animals fully recovered from the freezing treatment. These survivors had been kept in the frozen state for as long as 2 h (mean ± S.D. = 1.4 ± 0.9 h) and had reached final body temperatures as low as -1.05 °C (mean ± S.D. =



**Fig. 1.** The freezing time-course for a 4.28-g *Podarcis muralis*. Time zero represents ice nucleation at a crystallization temperature ( $T_c$ ) of  $-1.30^\circ\text{C}$ , followed by a rebound temperature ( $T_r$ ) of  $-0.70^\circ\text{C}$ . The lizard was removed after 2 h when it reached a final temperature ( $T_f$ ) of  $-1.05^\circ\text{C}$ . This animal fully recovered from the freezing episode

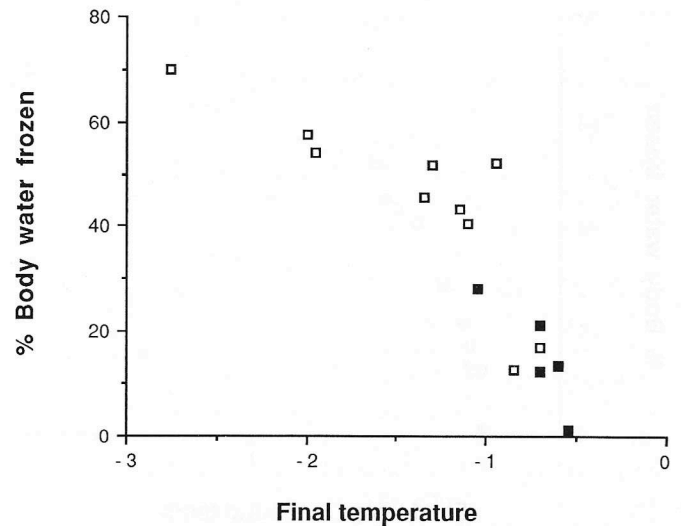
$-0.72 \pm 0.20^\circ\text{C}$ ). A discoloration on the right ventral surface slightly below the forelimbs was noted with some (but not all) of the lizards which failed to revive, but with none of the survivors. Dissection suggested that this was associated with rupture of the gall bladder.

The calorimeter constant ( $F_c$ ) for the apparatus to determine body ice contents, based on melting known masses of ice, was computed as  $1.03 \pm 0.03$  (mean  $\pm$  S.D.;  $N=4$ ), which suggested that the system was adequately insulated. Lizard water content averaged  $68.75 \pm 3.40\%$  (mean  $\pm$  S.D.;  $N=7$ ) of total body mass. The specific heat of dry lizard tissue was determined to be  $0.30 \pm 0.01$  (mean  $\pm$  S.E.M.;  $N=4$ ). The ice contents of four supercooled animals were estimated at  $-2.03\%$ ,  $-0.47\%$ ,  $-0.34\%$ , and  $+0.18\%$  (mean  $\pm$  S.D. =  $-0.67 \pm 0.95\%$ ). These values were all acceptably close to zero (the actual ice content of a supercooled animal).

Empirical estimates of the ice contents of three lizards, frozen solid for several days at  $-15^\circ\text{C}$ , averaged  $81.8 \pm 2.3\%$  (mean  $\pm$  S.E.M.), which is considerably lower than the theoretical ice content of  $96.7\%$  at this temperature, suggesting the presence of bound (i.e., non-freezable) water. The model was accordingly adjusted for the assumption of  $18\%$  bound water as:

$$F = 82 - 82 (FP_{eq}/T_r).$$

The ice content data for eight lizards were used to test the correspondence between the calorimetric method and the theoretical model. The agreement between the two methods was generally good. The mean ice content estimates were  $21.33\%$  and  $20.92\%$  for the calorimetric method and the theoretical method, respectively. The difference between these two methods averaged  $-0.40 \pm 3.98\%$  (mean  $\pm$  S.D.), and never exceeded  $\pm 7.8\%$  even at the highest ice contents. The model was thus judged



**Fig. 2.** The relationship between final temperature and the estimated % of body water frozen for the lizard *Podarcis muralis*. Lizards identified by filled symbols recovered fully from freezing, whereas those identified by open symbols did not

to be acceptable as an estimator of the ice contents of these reptiles.

Model estimation of the ice contents of all frozen lizards revealed a curvilinear relationship between % of body water frozen and the final temperature of the lizard upon removal from the freezing chamber (Fig. 2). The survivors generally had a higher  $T_f$  and as noted above these were never lower than  $-1.05^\circ\text{C}$ .

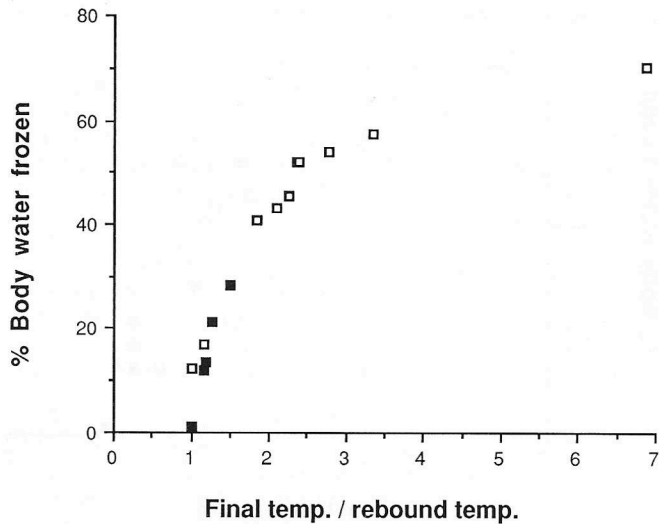
The curvilinear relationship becomes more pronounced if % body water frozen is plotted against the ratio of final temperature divided by rebound temperature (Fig. 3). The relative concentration of the body solutes due to ice formation, and hence the potential for cellular osmotic dehydration, is reflected by this ratio (although more precisely by the ratio  $T_f/FP_{eq}$ ). No lizard with a ratio exceeding 1.5 survived freezing.

Regardless of the final ice content, lizard survival appears to be influenced by the crystallization temperature and the resulting initial surge of body ice formation (Fig. 4). No animal survived a  $T_c$  below  $-2.7^\circ\text{C}$  or an initial surge in excess of  $5\%$  of body water frozen. The relationship between  $T_c$  and initial ice formation is approximately linear.

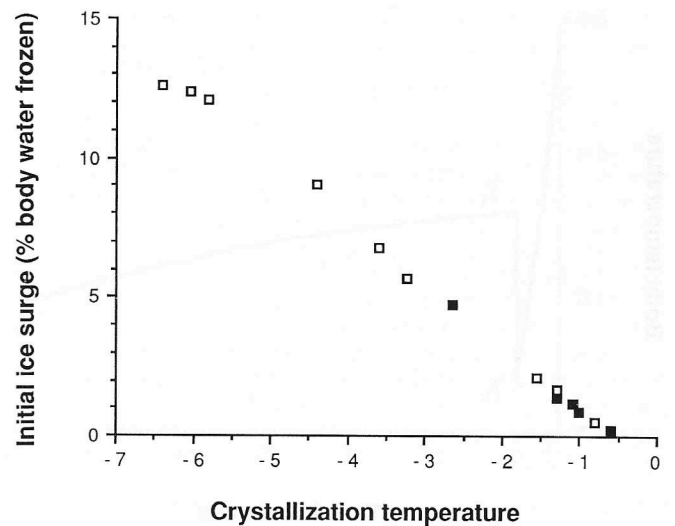
## Discussion

The wall lizard, *Podarcis muralis*, is widely distributed in Europe. Its hibernation period is short, even in the northern part of its range, and it is occasionally active in mid-winter during mild spells (Street 1979). While active on the surface during winter or following spring emergence, these lizards are vulnerable to sudden changes in the weather. Spellerberg (1976) reports a situation in southern England in the early spring of 1975, when a cold snap killed several reptiles including *Lacerta*





**Fig. 3.** The correlation between the estimated % of body water frozen and the ratio of final temperature ( $T_f$ ) to rebound temperature ( $T_r$ ) for the lizard *Podarcis muralis*. This ratio reflects the relative concentration of body solutes resulting from ice formation (see text). Lizards identified by filled symbols recovered fully from freezing, whereas those identified by open symbols did not



**Fig. 4.** The dependence of the estimated initial surge of ice formation (expressed as a % of body water frozen) on the crystallization temperature attained by supercooled *Podarcis muralis*. Lizards identified by filled symbols recovered fully from the cold treatment, whereas those identified by open symbols did not

*vivipara* (a close relative of *P. muralis* with an overlapping distribution).

The Cincinnati, OH, population of *P. muralis* was established with animals introduced from northern Italy in 1951 (Hedeen 1984). Although winter temperatures in Cincinnati are generally mild, the area experienced a prolonged and extreme cold spell as recently as 1981. The soil froze to a depth of 1 m in places in that winter, and it is likely that the lizards experienced subfreezing temperatures (and perhaps contact with ice crystals) even within their hibernacula.

The ability of wall lizards to survive supercooling is not unusual. The iguanid lizard *Sceloporus jarrovi* can survive in a supercooled state for over 30 h at  $-3^{\circ}\text{C}$  (Lowe et al. 1971), and several Australian lizards can withstand supercooling, some to temperatures as low as  $-8.5^{\circ}\text{C}$  (Spellerberg 1972). This latter author reports interspecific ranges in  $T_c$  as great as  $5.4^{\circ}\text{C}$ , which is similar to the  $5.8^{\circ}\text{C}$  range obtained in the present study. Weigmann (1929) reported that *P. muralis* can fully recover from supercooling to temperatures as low as  $-4.75^{\circ}\text{C}$  and can withstand supercooling exposures in excess of 8 h. Five of his seven supercooled lizards showed complete recovery from the treatment. One lizard recovered initially, but died overnight. The crystallization temperatures listed by Weigmann (1929) range from  $-0.97$  to  $-5.50^{\circ}\text{C}$  (mean  $\pm$  S.D. =  $-2.51 \pm 1.67$ ;  $N=18$ ) and are similar to those we report above.

Verification of freeze-tolerance requires (1) convincing evidence that internal ice formation has actually occurred, and (2) documentation that the organism has fully recovered from the treatment. Three categories of evidence have been presented in the literature to support contentions of body freezing: (a) prolonged exposure of the ectotherm to freezing conditions such that internal ice formation is likely (e.g., 5 h ice entombment of a

grass frog in air temperatures of  $-6$ – $-8.7^{\circ}\text{C}$  as described by Müller-Erzbach (1891), or the exposure of hatchling painted turtles to extreme cold in the field (Packard et al. 1989) or laboratory (Paukstis et al. 1989); (b) the stiffness of the limbs or body of the experimental animal, perhaps also with the apparent presence of abdominal ice [e.g., the recovery of some frogs and toads with "frozen" stiff limbs, though not bodies, as described by Knauthe (1891), or the apparent freezing and subsequent recovery of turtles (Musacchia and Sievers 1956) and of an alligator (Lowe et al. 1971)]; and (c) the occurrence of a pronounced exotherm as documented by the direct measurement of body temperature during a cooling regimen (and/or the direct quantification of body ice content). Although the (usually) anecdotal evidence in category (a) is certainly suggestive and worthy of reporting, it is not convincing [see Knauthe (1891) and Weigmann (1929) for critiques of such evidence]. Evidence in category (b), though also primarily anecdotal, is more persuasive, yet demands verification before it can be accepted. We consider evidence appropriate to category (c) to be both necessary and sufficient for documentation of internal ice formation.

Freeze-tolerance, from one perspective, is an "all or none" phenomenon. An individual ectotherm will either recover fully after the formation of internal body ice or it will not. However, the probability of an individual surviving freezing will vary with the population or species (some appear to be more freeze-tolerant than others) and with the freezing regimen (under certain conditions, there will be few or no survivors regardless of the species). Although the complete recovery, following unequivocal internal ice formation, of even one individual ectotherm is adequate in theory to document freeze-tolerance in that species, verification by repetition is highly desirable.

Recovery from freezing is not always adequately defined. Some authors simply state that a given animal "recovered fully" (e.g., Lowe et al. 1971) or "survived undamaged" (Paukstis et al. 1989). However, animals can show an apparent full recovery and yet be dead 1 or 2 days later (Weigmann 1929). Ideally, verification of complete recovery would provide evidence that all functions and behaviors, including reproductive activities, are normal after the freezing episode. This degree of certainty is difficult to obtain, however, and has been provided for only one species, i.e., the garter snake by Costanzo et al. 1988. Nevertheless, at the very least the investigator should monitor the experimental animals for several days and should provide evidence that the animal behaves normally and is capable of feeding and processing food.

Although 10 out of 15 of our lizards failed to survive freezing, five animals clearly recovered fully from freezing, based on the criteria established above. Freeze-tolerance is modest in this species in comparison with other ectotherms. The maximum tolerated ice content of 28% is below the corresponding value of at least 36% for the garter snake (Costanzo et al. 1988), which in turn is below the values of 53–58% reported for turtles (Storey et al. 1988; Costanzo and Claussen 1990). Tolerable ice contents of frogs are also markedly higher, with reported values ranging from 36% to 62% (Schmid 1982; Storey 1984; Layne and Lee 1989). Freeze-tolerance in *P. muralis* may appear to be unnecessary, since some lizards can supercool to an appreciable degree. However, supercooling is highly variable in small lizards and its effectiveness may be limited by both the passage of time and the presence of environmental ice crystals (Kodis 1898). The ability to survive some degree of body freezing may indeed be of adaptive value because, given the habits and distribution of the species, some individuals may encounter subfreezing conditions.

The ecological relevance of freeze-tolerance in this species must nonetheless remain conjectural. Little is known about the environmental conditions they actually experience during winter in nature, and nothing is known about their ability to survive freezing exposures in excess of 2 h. Additional study is needed to verify the ecological significance of such tolerance in *P. muralis* (and in many other freeze-tolerant species).

The distinct blue color associated with freezing in *P. muralis* was noted earlier by Weigmann (1929) in many, though not all, of his lizards. The skin discoloration and apparent rupture of the gall bladder in several lizards is similar to that reported by Costanzo (1988) for garter snakes succumbing to ice entombment.

Although the abrupt rise in body temperature at ice nucleation is almost universally utilized as an indicator of the onset of freezing, the accompanying ice formation has received little attention. Most workers have assumed this initial surge of ice to be negligible, either explicitly (e.g., Layne and Lee 1987) or implicitly [by assuming that  $T_r = FP_{eq}$ ; e.g., Weigmann (1929); Paukstis et al. (1989) and many others]. This assumption is reasonable, however, only when the degree of supercooling is minimal. Consider the hypothetical example of a 5-g lizard

which supercools to, and undergoes ice nucleation at,  $-5\text{ }^\circ\text{C}$  ( $=T_c$ ) before rebounding to  $-0.5\text{ }^\circ\text{C}$  ( $=T_r$ ). If, for the sake of simplicity, we assume a specific heat for the entire lizard of  $1.0\text{ cal}\cdot\text{g}^{-1}$ , then about 22.5 cal is needed to raise the body temperature of this animal the necessary  $4.5\text{ }^\circ\text{C}$ . Because the formation of one gram of ice generates 79.7 cal, at least 0.28 g ice must form to generate the required heat. If the 5-g animal has a 68% water content (i.e., contains 3.4 g water), this 0.28 g ice thus represents 8.2% of the lizard's body water ( $F=8.2\%$ ). While relatively small, this amount of ice is far from negligible. Furthermore, the removal of this amount of water as ice will concentrate the remaining solute, thereby producing an equilibrium temperature below the true freezing point ( $FP_{eq}$ ) of the original unfrozen animal. For the example given,  $FP_{eq}$  would equal  $-0.46\text{ }^\circ\text{C}$ , which is close to, yet above,  $-0.5\text{ }^\circ\text{C}$  ( $T_r$ ). This example is oversimplified in that the average specific heat of the freezing lizard would be close to 0.75 rather than 1.0 due to its dry mass and the presence of forming ice; however, some of the heat of fusion is dissipated to the cooling environment and does not directly contribute to the temperature surge, so values based on a specific heat of 1.0 may actually slightly underestimate the amount of surge ice formed.

The total amount of ice formed seems to determine the ability of *P. muralis* to withstand freezing (Figs. 2 and 3). However, the very rapid formation of ice immediately following nucleation may be especially deleterious (Fig. 4). Fortunately, exposure conditions during freezing episodes in nature (slow cooling rates and presence of ice crystals) would tend to limit supercooling and thus promote freezing with minimal surge ice.

#### *The history of freeze-tolerance in vertebrates*

In spite of methodological differences, the results of the present study are similar to those of Weigmann (1929). He froze 18 lizards for as long as 64 min, obtaining a clear exotherm for each individual. Eight lizards failed to recover. Five additional animals, though showing some signs of initial recovery, were dead the next day. Five lizards recovered, including one that was frozen for as long as 38 min, but one these (which supercooled to  $-4.97\text{ }^\circ\text{C}$  prior to nucleation) required more than 2 days for full recovery. Although Weigmann (1929) did not measure ice contents, application of our freezing model suggests that this lizard experienced a surge ice content of at least 8.0% and a final body ice content of 21.4%. One of his lizards recovered fully when removed within 1 min of attaining an estimated surge ice content of 6.1%, but three other lizards, similarly removed within 1 or 2 min, failed to recover from surge ice contents estimated to range from 7.1 to 9.0%. One individual recovered from a final ice content estimated at 37.7%, but all other lizards with estimated ice contents of 35.5% or higher ( $N=5$ ) failed to recover. Although Weigmann's lizards may have been slightly more tolerant to freezing than were ours, it appears that failure of his animals to recover can similarly be attributed

**Table 1.** A revisional history of important discoveries concerning cold tolerance in ectothermic vertebrates

Contribution	Source
First convincing report of freeze-tolerance in any animal (an insect)	Réaumur 1737
First report of supercooling in vertebrates	Kodis 1898
First convincing evidence of freeze-tolerance in vertebrates.	Weigmann 1929
First evidence for freeze-tolerance in reptiles and in lizards ( <i>Podarcis muralis</i> )	
The most comprehensive study of supercooling in vertebrates	Lowe et al. 1971
First report of freeze-tolerance in the wood frog, <i>Rana sylvatica</i>	Lotshaw 1977
First electrocardiogram study of a freezing vertebrate	
First evidence for freeze-tolerance in the chorus frog, <i>Pseudacris triseriata</i>	MacArthur and Dandy 1982
First report of freeze-tolerance in the tree frogs, <i>Hyla versicolor</i> and <i>H. crucifer</i> .	Schmid 1982
First measurements of body ice content and first evidence of a cryoprotectant (glycerol in <i>H. versicolor</i> )	
First report of glucose as a cryoprotectant of vertebrates (in <i>R. sylvatica</i> ) First organ specific study of cryoprotectant accumulation	Storey 1984
First enzymatic studies of frozen vertebrates ( <i>R. sylvatica</i> ).	Storey 1985
First report of the time course and trigger for cryoprotectant synthesis	
First report of the time course of ice formation in a vertebrate ( <i>R. sylvatica</i> )	Layne and Lee 1987
First report of freeze-tolerance in a snake ( <i>Thamnophis sirtalis</i> ).	Costanzo et al. 1988
First report of successful reproduction in a previously frozen vertebrate	
First report of freeze-tolerance in a hatchling turtle ( <i>Chrysemys picta</i> )	Storey et al. 1988
First report of freeze-tolerance in an adult turtle ( <i>Terrapene carolina</i> ), the largest vertebrate thus far known to withstand freezing	Costanzo and Claussen 1990

to a combination of surge ice exposure and total ice content.

Although verification is desirable, the studies of Weigmann (1929) suggest that *Lacerta agilis* also exhibits freeze tolerance. One of three lizards frozen (to a final temperature of  $-1.12^{\circ}\text{C}$  after supercooling to  $-1.21^{\circ}\text{C}$  and rebounding to  $-0.78^{\circ}\text{C}$ ) recovered fully. Weigmann (1929) also examined cold tolerance in the

frog, *Rana fusca*. Six out of 12 animals recovered from exposure to subfreezing temperatures. At least two of these had apparently been frozen; however, no exotherm was obtained for any animal (although body temperature was monitored continuously) and evidence for freezing is based only on the appearance (i.e., stiffness; category (b) above) of the animals upon removal. As noted above, we do not consider this to be conclusive.

Anecdotal reports of freeze-tolerance date back at least 1900 years to the Roman Empire and the *Natural History* of Pliny, who reported recovery of apparently frozen fishes from the Black Sea [see Knauthe (1891) for this citation, and Cameron and Brownlee (1913) for a review of other early reports]. Numerous authors prior to Weigmann (1929) examined cold or freezing tolerance in fishes, amphibians or reptiles. Although evidence for freeze-tolerance is presented in some of these studies (e.g., Müller-Erzbach 1891), but not in all (see Knauthe 1891), the data are inconclusive [see Weigmann (1929) for an excellent review]. Most authors failed to monitor body temperature adequately and continuously or to check for more than immediate recovery. The most persuasive study prior to 1929 appears to be that of Cameron and Brownlee (1913) who monitored body temperatures in the frog, *Rana pipiens*, and who found some suggestion of supercooling and some evidence (category (b) above) for freeze-tolerance. Their criteria for "recovery", however, are unspecified and freeze-tolerance has not been verified in this amphibian. We accordingly accept Weigmann (1929) as the first to present convincing evidence for freeze-tolerance in a vertebrate and *P. muralis* as the first vertebrate demonstrated to show such tolerance. The lizard *L. agilis* may well deserve co-recognition with *P. muralis* as the first freeze-tolerant vertebrate, but this should await verification. The history of the discovery of freeze-tolerance in vertebrates must thus be revised to reflect these past accomplishments (Table 1).

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