

Freeze Tolerance and Supercooling Ability in the Italian Wall Lizard, *Podarcis sicula*, Introduced to Long Island, New York

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Italian wall lizards (*Podarcis sicula campestris*) were introduced to Long Island, New York, in 1967 and have subsequently spread through many urban and suburban communities. Their ability to spread further may be limited by their ability to tolerate the relatively cold winters of New York. We found that these lizards were able to survive cold temperatures by supercooling if they were kept dry. However, if freezing was initiated as would be expected under shallow hibernation conditions, these lizards froze and died rapidly. We speculate that *Podarcis sicula* must hibernate below soil freezing depth, estimated at greater than 24 cm deep, to survive.

WE investigated freeze tolerance in a Long Island, New York, population of the Italian wall lizard *Podarcis sicula campestris*. Freezing survival by another member of this genus, *Podarcis muralis*, was first reported by Weigmann (1929), and *Podarcis muralis muralis* introduced to Cincinnati, Ohio, has been reported to be moderately freeze tolerant (Claussen et al., 1990). This formed the basis for the suggestion that *P. muralis* may be able to expand its range considerably northward, even into regions that have substantial winter soil freezing. Similar predictions might be made for *Podarcis sicula* if it too is freeze tolerant.

The origin of the New York population is somewhat unclear, but apparently they are the result of a release at a pet shop in Garden City, New York, in 1967 (Gossweiler, 1975). The lizards have since become common in several urban areas of Long Island and are spreading rapidly through Long Island both accidentally and with deliberate human assistance (RLB, pers. obs.). They have also been reported in two locations in New York City (J. L. Behler, pers. comm.), showing there are no major barriers to their dispersal through more of the northeastern United States. A better understanding of the environmental tolerances of this species may allow us to predict the eventual limits to its range.

Some indication of the freeze tolerance of this population may be inferred from the climate of their origin. The subspecific identification of the Long Island lizards was confirmed as *P. s. campestris*, both morphologically using the criteria described by Arnold and Burton (1978) and by using patterns of 12S rDNA variation (Oliverio et al., 2001). This subspecies is the most northern occurring of any *P. sicula* subspecies. It ranges from northern Italy, where minimum winter temperatures can be as low as -14.8 C, to southern Italy, where minimum win-

ter temperatures are only as low as -4.3 C (Cantú, 1977; Henle and Klaver, 1986). However, its range in the north is “scattered” and “determined by thermal conditions” (Oliverio et al., 2000), and its much greater abundance in the southern portion of its range indicates that this subspecies may not be highly cold tolerant. The fact that the Long Island population was started with pet trade animals suggests their origin more specifically as the vicinity of Rome, Italy (41.88°N , 12.50°E), because collectors and exporters of reptiles for the pet trade are concentrated there (C. Bertolucci, pers. comm.; G. Deichsel, pers. comm.). Rome’s minimum winter surface temperatures reach -7.4 C (Cantú, 1977). Although surface temperatures as low as -20 C have been recorded within its current range on Long Island (NOAA, 2001), *P. sicula* is clearly flourishing there. We investigated the freeze tolerance of New York *P. sicula* to determine how it might survive the significantly colder climate of New York relative to its native Italy and to make informed predictions as to whether the species is likely to continue to expand into even colder areas in North America.

METHODS AND RESULTS

The research reported here was carried out in two stages, the first in 1997 and the second in 2000–2001. In both cases, *P. sicula* were caught in West Hempstead and Garden City, New York (40.4°N , 73.4°W). We also collected *P. muralis* from Cincinnati, Ohio in the last week of October 1997. No individual lizard was used for more than one trial except where noted below. *Podarcis sicula* collected in late July 1997 were kept indoors at 27 C for seven weeks on a 12:12 h light:dark cycle. Subsequently they were housed at 15–16 C for another three weeks. During both of these periods, they had access to water, ate crickets, and were active. During

the next 15 days, the temperature was gradually lowered to 5 C. Toward the latter half of this period, the lizards were inactive and made little or no effort to move or eat. The lizards were then held at 5 C for 10 days before testing in October 1997.

For experiments conducted in 1997, our test equipment consisted of a refrigerated water bath with antifreeze added and a thermister taped to the lizard's abdomen (with digital read-out accurate to 0.1 C). Lizards were placed individually in plastic bags with wet paper towels while still at 5 C, and the bags were submerged in the bath at a set temperature and for a set time depending on the particular protocol. Temperature data during cooling and freezing were recorded on a chart recorder. After removal, the lizards were returned to 5 C for 24 h. At the end of each trial, the lizard was warmed to 15–16 C and observed periodically over at least 24 h, and often longer, to assess survival. Survival criteria used included the resumption of breathing, response to stimuli, righting reflex, normal posture, and locomotion.

First, we subjected one lizard to -16 C for 60 min. Next we cooled four lizards in a bath at -5 C. After body temperatures cooled to -5 C, individuals were subsequently held in the bath for 30, 60, 120, or 360 min. After each exposure, lizards were returned to 5 C (Table 1).

For our third set of trials, we cooled three lizards to -5 C, as in the previous trial. After they reached -5 C, we touched them with a probe head that had been cooled to -70 C to initiate nucleation of body fluids. We left these lizards in the ice bath for 15 more min and then returned them to 5 C (Table 1).

Immediately upon removal from the cold temperatures, all lizards were stiff and showed no ability to move. The lizard from the first trial (-16 C exposure) was dead when checked 12 h later. All lizards from the second set of trials survived and behaved normally. The cooling curves recorded by the thermistors showed that the animals had supercooled; body temperatures dropped quickly to 0 C and then dropped more slowly, finally stabilizing near -5 C, where they remained over the entire course of their subzero exposures. No freezing exotherm was detected in any case. These lizards were all alive at least six weeks later.

The pattern of body temperature reduction in the third set of trials was initially similar to that of the second. However, after being touched with the cold probe, the initiation of freezing was seen as an instantaneous jump in body temperature (exotherm) because of the

heat release of crystallization. Body surface temperature remained elevated and virtually constant over the remaining 15 min of freezing exposure. The lizards from this third set of trials were clearly affected by this brief freezing. One was dead within 12 h. The other two were alive but showed abnormal behavior in that they were extremely lethargic, showed very poor righting behavior, and were largely unresponsive to stimuli. We observed them for two days, but they showed no improvement, so they were euthanized. All three *P. muralis* tested under these same freezing conditions also died within 24 h after completion of the tests.

We carried out a second set of studies using *P. sicula* collected in September 2000 from the same source populations; cold and freezing survival was again assessed while also gathering supercooling point and percent of ice data. These lizards were cold acclimated in the manner described above, except they were held for only three weeks at 27 C before being transferred to 15 C for three weeks. Then they were shipped to Carleton University where they were held in ventilated plastic boxes with damp soil at 5 C until experiments were conducted in January 2001. A somewhat different protocol for subzero exposure was used to more closely monitor changes in body temperature during cooling/freezing. Briefly, each lizard was placed on a pad of paper toweling and centered so that its abdomen was in contact with a thermocouple. A band of masking tape was used to secure the animal in place without covering the nares. Lizards were then placed in a variable temperature incubator set to 1.0 C and were allowed to cool for ~ 15 –20 min until body temperature equilibrated with air temperature. Air temperature was then lowered in increments of 0.5 C every 10–15 min, which resulted in a nearly linear cooling of body temperature until an exotherm was noted at the initiation of freezing (Fig. 1). The supercooling point (SCP; also called crystallization temperature) was taken as the lowest body temperature recorded before the exotherm. The length of freezing exposure was timed from the exotherm; animals were then removed and either returned to 5 C to thaw and recover slowly or thawed rapidly in a calorimeter to determine the percentage of body water that was frozen. The calorimeter consisted of an insulated flask sunk into a block of construction-grade Styrofoam insulation and fitted with a Styrofoam plug that filled all but ~ 100 ml at the bottom of the flask. A 50 ml volume of water was placed in the bottom of the flask along with a magnetic stirring bar and a thermister (connected to a digital thermometer); the entire ap-

TABLE 1. SUMMARY OF ALL EXPERIMENTS WITH *P. sicula*.

ID	Cooling procedure	Subsequent procedure	Result
1997 #1	-16 C for 60 min on wet towel		Died
1997 #2	Cooled to -5 C on wet towel	Held at -5 C 30 more min	Lived
1997 #3	Cooled to -5 C on wet towel	Held at -5 C 60 more min	Lived
1997 #4	Cooled to -5 C on wet towel	Held at -5 C 120 more min	Lived
1997 #5	Cooled to -5 C on wet towel	Held at -5 C 360 more min	Lived
1997 #6-8	Cooled to -5 C on wet towel	Initiated freezing, held at -5 C 15 more min	Died
2000 #1-2	1 C for 15-20 min, lowered -0.5 C every 10-15	Held at -2.4 & -2.7 C for 2.5 h	Lived
2000 #3	1 C for 15-20 min, lowered -0.5 C every 10-15	Held at -3.7 C for 30 min	Lived
2000 #4-7	Lowered -0.042 ± 0.002 C/min to exotherm on dry towel	Removed 2-21 min postexotherm	Mean SP -4.88 ± 0.13 SE, died
2000 #8-9	Lowered -0.0380 ± 0.004 C/min to exotherm on dry towel	Removed 3 and 8 min postexotherm	Mean SCP -4.30 ± 0.07 SE (for #8-11), lived
2000 #10	Lowered -0.0380 ± 0.004 C/min to exotherm on dry towel	Raised air temperature to -3.0 C, held at -3.0 C for 40 min	20.7% of total body water as ice, lived
2000 #11	Lowered -0.0380 ± 0.004 C/min to exotherm on dry towel	Raised air temperature to -3.0 C, held at -3.0 C for 90 min	32.9% of total body water as ice, died
2000 #12	Lowered -0.0336 C/min to exotherm on wet towel	Held at exotherm temperature 129 min postexotherm ^{erm}	SCP: -3.5 C, 30.7% ice, died
2000 #13	Lowered -0.0287 C/min to exotherm on wet towel	Held at exotherm temperature 133 min postexotherm ^{erm}	SCP: -3.7 C, 2.98% ice, died
2000 #14	Cooled to -3.2 C on dry paper towel	Nucleation initiated, temperature raised to -2.2 C, held for 60 min	7.7% ice, alive

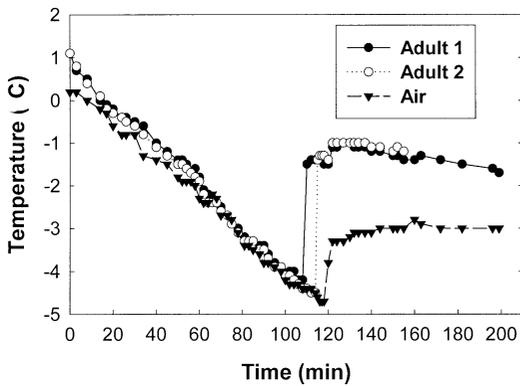


Fig. 1. Changes in body surface temperature for two adult *Podarcis sicula* over the course of cooling to the supercooling point and then holding for 90 min (Adult 1 was 2000–11; filled circles) or 40 min (Adult 2 was 2000–10; open circles) postexotherm. Air temperature (triangles) was raised to -3 C after nucleation of both animals.

paratus was then placed on top of a magnetic stirrer to continuously stir the water. After recording the initial temperature of the water, a frozen lizard was quickly added, the Styrofoam plug was fitted and water temperature was recorded as the lizard melted until temperature stabilized at a consistent low value after 2–3 min. The lizard was then retrieved, assessed for immediate signs of life, and then replaced at 5 C. Survival was reassessed after 24 h and again after 2–3 weeks for those animals that survived the first 24 h. The percentage of body water converted to ice was calculated as outlined by Layne and Lee (1991) using experimentally determined values for our system: *F*-factor for the calorimeter = 1.06, percentage of body mass that is water for *P. sicula* = $68.0 \pm 1.6\%$ (from measurements of wet and dry mass of adult and subadult lizards that died), and specific heat of the dry mass (SD) = 0.232. The melting point of body fluids was estimated as -0.5 C, in line with the typical osmolality of reptile body fluids.

We began this second set of studies by first testing whether subzero temperature exposure itself could be injurious. Two juveniles and 1 subadult (body masses 1.06, 1.72, and 3.67 g) were cooled to body temperatures of -2.4 , -2.7 , and -3.7 C, respectively, and held at these temperatures for 2.5 h for the juveniles and 30 min for the subadult. All individuals were immediately active when removed from the subzero incubator. The subadult was still healthy when assessed three weeks later and the juveniles appeared normal when reused for freezing tests two days later (Table 1).

Next, supercooling capacity was assessed.

Four juvenile lizards (mean mass \pm SE = 1.39 ± 0.14 g) were cooled at an average rate of 0.042 ± 0.002 C/min. The mean SCP \pm SE was -4.83 ± 0.13 with a range from -4.6 to -5.2 for lizards cooled on dry paper toweling. These were removed from the subzero incubator at times ranging from 2–21 min postexotherm. None showed immediate signs of life and all were confirmed dead the next day. Four adults cooled in the same manner showed a significantly higher mean SCP \pm SE of -4.30 ± 0.07 ($P < 0.025$; mean mass \pm SE = 4.48 ± 0.58 g; mean cooling rate \pm SE = -0.0380 ± 0.0041 C/min; Table 1).

On the assumption that lizards in nature would probably hibernate in damp surroundings and therefore be susceptible to inoculative freezing caused by contact with environmental ice, we also assessed supercooling of lizards that were in contact with wet paper toweling. Supercooling was somewhat reduced in this case; SCP values for one adult (6.2 g) and 1 subadult (3.7 g) were -3.5 and -3.7 C, respectively.

To determine whether survival would be better at a higher subzero temperature, we tried additional protocols (Table 1). First, two adult lizards were cooled on dry toweling until freezing began at the SCP (-4.2 and -4.5 C) and then air temperature was raised to -3 C and the lizards were held for either 40 or 90 min (Fig. 1). Ice content of both lizards was measured by calorimetry. The lizard frozen for 40 min survived with 20.7% of total body water as ice (and was still alive two weeks later) whereas the lizard frozen for 90 min died with 32.9% ice. Second, two lizards were cooled on wet toweling until their SCP was reached, held 130 min postexotherm at -3.5 C to -3.7 C, and then percent of ice was measured. Values were 29.8% and 30.7% ice, respectively, and both lizards were dead. In a third trial, another adult (6.0 g) was cooled to -3.2 C on dry paper toweling and nucleation was initiated by touching the skin of the tail with a -70 C metal rod. After nucleation, air temperature was raised to -2.2 C and held for 60 min. This lizard survived but with only 7.7% of the body frozen. Our several attempts to nucleate lizards at higher subzero temperatures (after equilibration at -1 C or -2 C) failed.

DISCUSSION

If *P. sicula* could avoid ice formation, such a capacity would probably allow the animals to endure overnight subzero exposures in their natural environment or perhaps even longer bouts in a supercooled state. A well-developed ability

to supercool is quite common among reptiles; supercooling points as low as -16 C have been reported (Lowe et al., 1971; Costanzo et al., 1999). A close relative, the European common lizard, *Lacerta vivipara*, can survive extended supercooling (Costanzo et al., 1995; Grenot et al., 2000). *Lacerta vivipara* also showed significant freeze tolerance, enduring freezing times as long as 72 h, freezing temperatures as low as -3 C, and as much as 48% of total body water converted to ice (Costanzo et al., 1995; Voituron et al., 2002). This contrasts dramatically with the results of our 1997 studies on *P. sicula* where after only 15 min freezing exposure, one individual was killed outright and the other two were extensively injured. The results of our 2000–2001 studies are consistent with those from our 1997 work. Adults and subadults survived only very short freezing exposures. Adults removed 3- or 8-min postexotherm were alive and showed immediate but weak movement of limbs that had improved considerably when reassessed 18 h later. However, a 2 h freezing was lethal. We found juveniles had no ability to survive even the briefest freezing.

The initial tests of freezing survival for both adults and juveniles in 2000–2001 were made with animals that were cooled to their SCP values and then held at low air temperature (-4.5 C to -5 C) after freezing began. Despite very short exposure times (as little as 2 min), the initial surge of ice formation might have been too high to allow survival at these temperatures, especially for the juveniles. Indeed, Claussen et al. (1990) found that *P. muralis* survived only if they froze at a SCP above -3 C where the initial ice surge was $< 5\%$ of total body water. We also found that survival was higher at higher subzero temperature and low percent of ice.

Overall, then, freezing survival by *P. sicula* was very poor. The best survival statistics for adults were the endurance of 40 min of freezing with $\sim 21\%$ ice. However, freezing for 90–130 min with 30–33% ice was lethal. Significantly, body ice content had not reached its maximum in any of these cases. This was apparent because body temperature was still significantly above ambient at the end of each trial because of the continuous heat release of ice formation (Fig. 1). Thus, if adult *P. sicula* froze in nature as a result of a fall in ambient temperature to the SCP, the body ice content would quickly rise within only a couple of hours to a lethal value. The only hope for survival in these circumstances would be quick reversal of ambient temperature to a value that was at or above the melting point of body fluids (about -0.5 C). It is highly unlikely that ambient temperature in the hiber-

naculum would normally change so quickly. However, the possibility exists that the initiation of freezing might immediately stimulate lizards to actively seek out a warmer place where internal ice would then melt. This possibility remains problematic, because we don't know whether these lizards have much freedom to move within their hibernacula.

Hence, it is apparent that, although *P. sicula* may endure subzero exposures by supercooling, they cannot survive freezing temperatures for long if ice nucleation occurs. This would make their endurance of prolonged subzero exposures in the natural environment unlikely unless permanently dry hibernacula could be chosen where individuals would not come in contact with environmental ice. Ice is a potent nucleator and ice propagation across skin can rapidly seed crystallization in body fluids. This can happen within as little as 30 sec in supercooled frogs that come into contact with environmental ice (Layne et al., 1990). However, the failure of our several attempts to nucleate lizards at higher subzero temperatures (after equilibration at -1 C or -2 C) suggests that the skin of *P. sicula* may be somewhat resistant to inoculation. Nevertheless, 17 of 18 *L. vivipara* still froze, most within 3 h, when they were in contact with ice, even though their body temperatures (-1.2 C to -1.8 C) were well above their supercooling points (Costanzo et al., 1995). If similar statistics apply to *P. sicula*, then even at very mild subzero temperatures, the prospect of long-term freezing survival is very low because a lethal ice content (our data suggest 20% is survivable, 30% is not) would be reached within just a few hours. Hence, the use of freeze tolerance as an ecologically relevant part of the hibernation strategy of this species seems unlikely. It is probable that prolonged survival by *P. sicula* at subzero temperatures depends on the maintenance of a supercooled state and the avoidance of contact with environmental ice or other nucleators.

Our studies found that neither *P. sicula* nor *P. muralis* survived a 15-min freezing exposure at -5 C. These data for *P. muralis* contrast somewhat with those of Claussen et al. (1990) who documented recovery by five (of 15 frozen) specimens of *P. muralis* after freezing for 10–120 min and with a maximum of 28% of total body water as ice. In our study, we adopted similar procedures to those used by Claussen et al. (1990) but with one difference. In Claussen et al. (1990), the ice bath temperature was raised 10 min postnucleation from the -6 C used for cooling the lizards to -3 C where it was held for the duration of the freezing exposure. Although such a procedure will retard the rate of

ice growth and hence improve survival potential (i.e., eight lizards with greater than 30% ice did not survive freezing in their study), we could not find an ecologically sound reason to raise the ice bath temperature in this way after nucleation because a similar temperature change would be highly unlikely to occur in the natural environment. If animals began freezing in their hibernacula when temperature dropped to their SCP (-4 C or lower in the present studies), it would be unreasonable to expect that environmental temperature would quickly rise very soon thereafter.

Both our results and those of Claussen et al. (1990) indicate that the ability to tolerate any significant amount of internal ice formation is quite limited in *Podarcis* species and suggest that freeze tolerance is not ecologically relevant for either *P. muralis* or *P. sicula*. Once freezing of body water begins, these lizards would have only a very short time (probably well less than an hour) to move to a more favorable microhabitat before rising ice content impeded further muscle movement. Thus, attempting to overwinter in places where ambient temperature routinely drops below the freezing point of body fluids would be extremely risky.

The poor ability of the Ohio population of *Podarcis muralis muralis* to withstand cold temperatures is surprising because this subspecies occurs well into central Europe, including southern parts of Austria and Hungary, and at high altitudes in the southern parts of its range. Furthermore, Deichsel and Gist (2001) have identified the origin of the Ohio population of *P. m. muralis* as Lake Garda (northern Italy), where nearby winter temperatures as low as -15.6 C have been recorded (Cantú, 1977).

Our results suggest ways in which overwintering in *Podarcis* species is limited: either they must stay below the soil freeze depth or supercool without freezing. If they avoid freezing by staying below freezing soil, we can speculate how deep their hibernacula are. We examined data on soil freezing depths predicted by a model developed by DeGaetano et al. (1996, 1997). This model was used to predict soil freezing depth at John F. Kennedy airport over 30 of the years (1967–1997) since *P. sicula* were released on Long Island. Data from that location were used because it is the closest weather station (40 km from our study site) with sufficient data to run the model. The deepest freezing depth for bare soil over this time period was predicted to be 43.8 cm in 1994, whereas for soil with grass the deepest was predicted to be 24.6 cm the same night. Even under grass, soil stayed frozen to 24.6 cm for 3–5 days. Very likely, any lizards

in this freezing soil would be frozen, and supercooling would be impossible. *Podarcis sicula* may not have to be freeze tolerant on Long Island if they routinely bury themselves more than 24 cm deep to avoid being frozen in the winter. Unfortunately no field data are available concerning the overwintering sites used by any *Podarcis*. *Podarcis sicula* are apparently active year round in Italy, although they are above ground considerably less often from November through February (Foà et al., 1992; Rugiero, 1995). R. L. Burke and S. E. Ner (unpubl.) have observed that *P. sicula* on Long Island are not active above ground for the same period. We conclude that *P. sicula* on Long Island probably use hibernacula deep underground, which precludes above ground activity during any occasional warm periods but minimizes their exposure to freezing temperatures. Their continued range expansion into areas with colder climates may be limited by access to appropriate hibernacula.

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