Terrestrial hibernation in the northern cricket frog, *Acris crepitans*

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Abstract: We used laboratory experiments and field observations to explore overwintering in the northern cricket frog, *Acris crepitans*, in southern Ohio and Indiana. Cricket frogs died within 24 h when submerged in simulated pond water that was anoxic or hypoxic, but lived 8–10 days when the water was oxygenated initially. Habitat selection experiments indicated that cricket frogs prefer a soil substrate to water as temperature decreases from 8 to 2°C. These data suggested that cricket frogs hibernate terrestrially. However, unlike sympatric hylids, this species does not tolerate extensive freezing: only 2 of 15 individuals survived freezing in the –0.8 to –2.6°C range (duration 24–96 h). Cricket frogs supercooled when dry (mean supercooling point –5.5°C; range from –4.3 to –6.8°C), but were easily inoculated by external ice at temperatures between –0.5 and –0.8°C. Our data suggested that cricket frogs hibernate terrestrially but are not freeze tolerant, are not fossorial, and are incapable of supercooling in the presence of external ice. Thus we hypothesized that cricket frogs must hibernate in terrestrial sites that adequately protect against freezing. Indeed, midwinter surveys revealed cricket frogs hibernating in crayfish burrows and cracks of the pond bank, where wet soils buffered against extensive freezing of the soil.

Résumé : Nous avons procédé à des expériences en laboratoire et à des observations sur le terrain pour étudier le sort de la Rainette grillon, *Acris crepitans*, en hiver dans le sud de l'Ohio et de l'Indiana. Les rainettes submergées dans de l'eau d'étang simulée anoxique ou hypoxique sont mortes en moins de 24 heures, mais elles ont survécu de 8 à 10 jours lorsque l'eau avait été oxygénée au départ. Les résultats d'expériences sur le choix d'habitat ont démontré que les rainettes choisissent de préférence un substrat terrestre à un milieu aqueux à mesure que la température descend de 8 à 2°C. Ces données semblent indiquer que l'hibernation se fait en milieu terrestre. Cependant, contrairement aux hylidés qui leur sont sympatriques, les rainettes de cette espèce ne tolèrent pas un gel prolongé: seulement 2 des 15 individus soumis au gel à des températures situées entre –0,8 et –2,6°C ont survécu (durée 24 à 96 h). Les rainettes gardées sèches entraient en surfusion (point moyen de surfusion –5,5°C; étendue de –4,3 à –6,8°C), mais étaient facilement pénétrées par la glace externe entre –0,5 et –0,8°C. Nos données indiquent que l'hibernation de ces rainettes se fait en milieu terrestre, mais que ces rainettes n'ont pas de tolérance au gel, qu'elles ne sont pas fouisseuses et qu'elles sont incapables de surfusion en présence de glace externe; il est donc logique de penser que ces rainettes hivernent dans des milieux terrestres qui les protègent adéquatement du gel. Des inventaires au milieu de l'hiver ont en effet révélé la présence de ces rainettes dans des terriers d'écrevisses et des crevasses sur les rives de l'étang où le sol humide les protège des grands gels.

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Introduction

Temperate-region anurans use several strategies to survive winter temperatures (see review in Pinder et al. 1992). Some, such as aquatic species in the genus *Rana*, hibernate underwater and thus remain unfrozen (Sinsch 1991). One danger of using aquatic hibernation sites is the severe hypoxia that is common in some northern lakes and ponds (Barica and Mathias 1979; Bradford 1983). Therefore, aquatic hibernators typically have some degree of hypoxia tolerance. For example, *Rana temporaria* tolerates at least 4 weeks of submergence in hypoxic water (Boutilier et al. 1997) and *Rana pipiens* survives without oxygen for at least 5 days (Christiansen and Penney 1973; Holden and Storey 1997). Despite the moderate tolerance of hypoxia exhibited

by some frogs, the hypoxic stress associated with severe winters may result in winterkill (e.g., Bradford 1983).

Other anurans hibernate in terrestrial sites. *Scaphiopus* spp., *Bufo* spp., and *Pseudacris streckeri* are fossorial and dig below the frost line to avoid freezing (Pinder et al. 1992; Swanson et al. 1996; Packard et al. 1998). In contrast, other species use superficial overwintering sites under a thin layer of moss or leaf litter. In northern areas, these sites provide little insulation and, thus, the frogs may be exposed to high subzero temperatures (Schmid 1982). To survive winter in such sites, these frogs tolerate ice formation in their tissues and may endure temperatures as low as –6°C and freezing for several days or weeks (Lee and Costanzo 1998). Freeze tolerance requires (*i*) the production of cryoprotectants (glucose or glycerol) upon freezing (Storey 1984); (*ii*) hypoxia tolerance because there is no circulation while a frog is frozen (Holden and Storey 1997); and (*iii*) desiccation tolerance because water leaves the organs to form extracellular ice crystals during freezing (Lee et al. 1992).

Freeze tolerance is present in two families of anurans. Among the ranids, freeze tolerance has been demonstrated only in the most terrestrial species, the wood frog (*Rana*

sylvatica), although it is likely that Palearctic brown frogs with similar habits (e.g., *Rana arvalis*; Kuzmin 1995) are also freeze tolerant. Many hylids with northern distributions, including the gray treefrogs *Hyla chrysoscelis* (Costanzo et al. 1992) and *Hyla versicolor* (Schmid 1982), the striped chorus frog (*Pseudacris triseriata*; Storey and Storey 1986), and the spring peeper (*Pseudacris crucifer*; Schmid 1982), are also freeze tolerant. In contrast, none of the species that burrow or hibernate aquatically in thermally protected sites are freeze-tolerant (Lotshaw 1977; Schmid 1982; Storey and Storey 1986; Layne 1992; Costanzo et al. 1993; Swanson and Graves 1995; Swanson et al. 1996).

Although anurans are typically categorized using these three overwintering strategies (aquatic, fossorial, or freeze tolerant), not all anurans readily fit into this classification (e.g., Sinsch 1991). Our study deals with one such species, the northern cricket frog (*Acris crepitans*). This frog is very small and, unlike sympatric hylids, is highly aquatic, rarely straying far from the edge of permanent ponds and streams (Wright and Wright 1995). Because the overwintering habits of the cricket frog are so poorly known, our investigation began with the simple question of where this species hibernates. We tested the habitat preference (land versus water) of cricket frogs in the laboratory and gauged their ability to tolerate sustained submergence. Next, we searched for hibernating cricket frogs in nature. Having found that cricket frogs hibernate in terrestrial sites, we measured their supercooling ability, degree of freeze tolerance, and physiological responses to freezing. What unfolded was a story of how this unique hylid uses its small size to exploit moist subterranean sites and avoid freezing.

Materials and methods

Frogs were collected at a permanent pond in the Mounds State Recreation Area, Union County, southeastern Indiana, in November of 1995 and 1996. The frogs were initially stored in an incubator with a 10 h light (L) : 14 h dark (D) cycle and the temperature cycling daily between 2 and 8°C (Fig. 1A), to mimic autumnal conditions. Frogs used in experiments in late winter were moved in December into darkness at 2°C to simulate winter conditions. The cages had screen walls and contained a natural soil substrate covered by leaf litter. Each cage included a water bowl. Additional frogs were collected during the prehibernation period (9 October 1997), to compare their physiology with that of animals held in simulated hibernation. The frogs collected on 9 October 1997 were stored on wet paper towels at room temperature (~22°C) until used 5 days later. All experiments were in compliance with the principles and guidelines of the Canadian Council on Animal Care and were approved by Miami University's Institutional Animal Care and Use Committee.

Submergence tolerance

To explore the possibility that cricket frogs hibernate in aquatic sites, we tested their ability to tolerate sustained submergence in cold hypoxic water. Twelve frogs (body mass (mean ± SEM) 0.94 ± 0.06 g) were removed from their cages on 5 March 1996 and placed in a large aquarium containing artificial pond water (5% Holtfretter's solution: 0.7 g $NaCl\cdot L^{-1}$, 10 mg $KCl\cdot L^{-1}$, 20 mg $Ca Cl_2\cdot L^{-1}$, 40 mg $NaHCO_2\cdot L^{-1}$) at 4°C. Frogs were habituated to these conditions for 48 h, during which time they were permitted access to the surface of the water. Each frog was then assigned to one of three treatment groups ($n = 4$ frogs per group) and transferred to a 4-L jar containing 3.7 L of artificial pond water with either a high dissolved oxygen concentration (8 $mg\cdot L^{-1}$), a low dissolved oxygen concen-

tration (2 $mg\cdot L^{-1}$), or nearly no oxygen (0.5 $mg\cdot L^{-1}$). The desired oxygen concentration was achieved by bubbling nitrogen gas through the water while monitoring with a dissolved oxygen electrode (Model 55, YSI). A plastic screen was positioned 2 cm below the water surface to prevent the frog from breathing air, and a 2-cm layer of mineral oil was applied to the surface of the water to reduce gas exchange with the air. All jars were placed in darkness at 4°C. The frogs generally remained stationary in the jars and did not make vigorous attempts to escape.

The viability of each frog was checked daily for up to 10 days with the aid of a small magnetic stir bar that had been placed on the bottom of each jar. When rotated, the stir bar produced a slight current, which stimulated live frogs to move. The stirring also promoted mixing of the water within the jar. In cases where oxygen concentration in the water was 8 $mg\cdot L^{-1}$ initially, dissolved oxygen was measured in the water again, after the death of the frog or when the experiment was concluded.

Dead frogs were removed from their jars and whole body lactate concentration was determined. Frogs were frozen in liquid nitrogen, partially thawed, minced with scissors, and homogenized in 7% $HClO_4$. The acid extracts were then neutralized with KOH and assayed for lactate, using the lactate oxidase procedure (cat. No. 735, Sigma Chemical Co.). Calculations of lactate concentrations were based on initial body mass, because after submergence the frogs were edematous. Data for frogs used in submergence experiments were compared with those for control frogs ($n = 3$) that were sampled directly from the hibernating colony.

Habitat selection

Two tests were performed to identify the preferred habitat of this species when exposed to decreasing temperatures. Following Licht (1991), groups of five or fewer cricket frogs were placed in an aquarium divided into equal areas of land (wet or moist potting soil covered by leaf litter) and water (aged tap water) by a Plexiglas partition. To mimic early winter conditions, this aquarium was held in an incubator with a 10 h L : 14 h D cycle and the temperature cycling daily between a minimum of 2°C (at 08:00) and a peak of 8°C (reached at 18:00) (Fig. 1A). Initially, all frogs were placed on the land–water partition at 08:00, and their position (on land or in water) was noted at 09:00, 13:00, 17:45, 18:15, and 22:00, and at 08:00 the following day. This experiment was performed both with wet soil (1.53 ± 0.05 g water/g dry soil; $n = 47$ frogs) and with moist soil (0.66 ± 0.03 g water/g dry soil; $n = 12$ frogs).

We searched for hibernating cricket frogs in natural habitats where cricket frogs were abundant during the autumn and spring. Aquatic habitats were searched at the Mounds State Recreation Area, Union County, Indiana, using seines and dip nets, on 12 December 1996. The nets were swept through depths between 0 and 40 cm, as well as through sections of the pond bottom and submerged vegetation. Deeper parts of the pond were not searched, because potential predators, such as largemouth bass and bluegill sunfish, were present. Terrestrial sites were searched at Miami-Whitewater State Park, Hamilton County, Ohio, on 2 February 1997. When frogs were found, the depth and physical features of their overwintering sites were observed and photographed. To monitor winter temperature of a typical hibernation site, a temperature data logger (TidbiT, Onset Computer Corp.) was placed under 2 cm of moss in a pre-existing subterranean tunnel that was similar to those used by hibernating cricket frogs. Another data logger (StowAway, Onset Computer Corp.) at the same site measured air temperature 10 cm above the soil surface.

Tests of freeze tolerance and physiological responses to freezing

Each frog was individually placed in a small test tube with a thermocouple adjacent to the frog's ventral surface. Temperature

Fig. 1. (A) Diel temperature and light cycle used during habitat-selection experiments. Percent cricket frogs selecting (B) wet soil substrate or (C) only slightly moist substrate versus water.
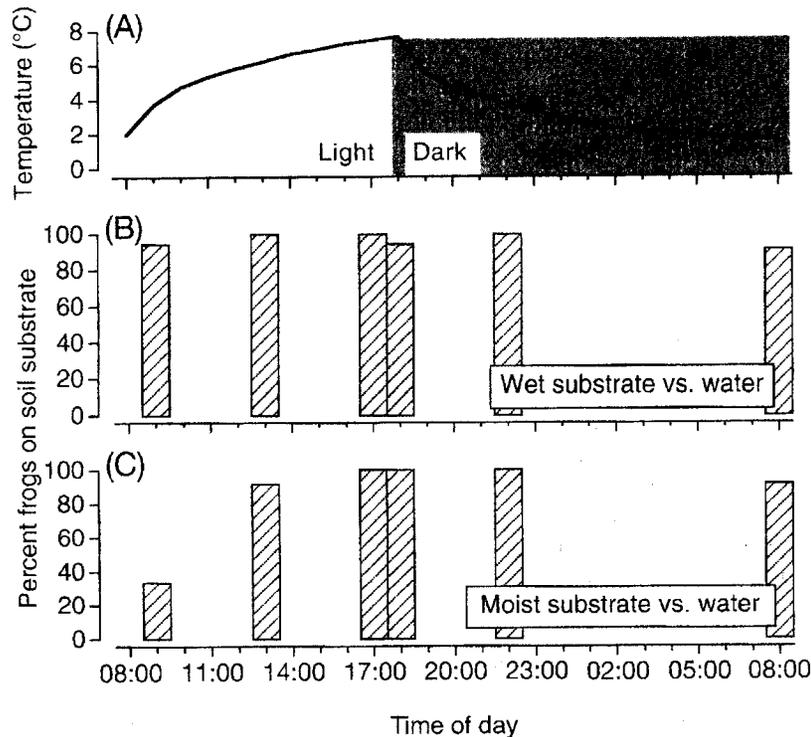
was monitored using a multichannel data logger (RD-3752, Omega Engineering Inc.). The tubes were placed in an insulated beaker that was partially submerged in a cold bath (RTE-140, Neslab Instruments Inc.). To evaluate their degree of freeze tolerance, frogs were initially cooled to between -0.5 and $-1.0°C$, then inoculated to prevent supercooling. Frogs were inoculated by applying aerosol coolant to the outside of the tube, to freeze the condensation inside the tube. Ice spread along the inside of the tube to areas adjacent to the frog's skin, thus stimulating freezing in the tissues of the frog. After inoculation, the frogs were slowly cooled at a constant rate, to reach their final target temperature (between -0.8 and $-2.6°C$) by the end of the trial. Although cooling rate was constant within a trial, cooling rate differed between trials (range $0.05-2.4°C/day$). Cooling rate depended on the duration and final target temperature of each trial (i.e., a higher cooling rate was used for short trials and trials to low final temperatures than for longer and warmer trials). Frogs were thawed at $4°C$ and allowed to recover on wet filter paper. During this time, the recovery of basic physiological functions and behaviors was recorded. A frog was considered to have survived if it had normal posture and could right itself within 2 s of being turned on its back.

In midwinter, additional frogs ($n = 5$) were frozen (duration 24 h; final temperature $-2.3 \pm 0.2°C$), to measure their physiological responses to freezing. An equal number of frogs was given a sham treatment (duration 24 h; final temperature $0°C$), to serve as a

Results are expressed as micromoles per gram of tissue (fresh mass).

Measures of supercooling capacity

To evaluate the supercooling capacity of cricket frogs in the absence of external ice, another trial was performed in which frogs were cooled at a rate of $1°C/h$, while held against a Styrofoam platform in a beaker submerged in a cold bath (as in Swanson et al. 1996). The frogs in this dry chamber were not in contact with ice and thus supercooled maximally.

Results

Submergence tolerance

Because anurans hibernating in northern lakes and ponds generally possess some degree of hypoxia tolerance, we tested the ability of cricket frogs to survive low oxygen conditions. Despite their close association with water, cricket frogs died within 24 h when submerged in simulated pond water that was low in oxygen (2 or 0.5 mg·L^{-1} initially). After 8–10 days, three of the four frogs submerged in water with an initial oxygen concentration of 8 mg·L^{-1} also died, and the frogs had reduced the oxygen level from 8 mg·L^{-1} to 3.8 ± 0.5 mg·L^{-1}. Only one frog survived to the end of the

Table 1. Survival duration and final whole body lactate concentration of cricket frogs submerged in 4°C water, as a function of initial dissolved oxygen concentration.

Group	Dissolved oxygen $(mg \cdot L^{-1})$*	Survival duration	Lactate $(\mu mol \cdot g^{-1})$
Control	—	—	4.5±0.7a (3)
Oxygenated	8.4±0.1	8–10 days[†]	5.7±0.3a (3)
Low oxygen	1.8±0.2	<24 h	17.0±2.6b (4)
Near anoxic	0.5±0.01	<24 h	20.5±2.1b (4)

Note: Values are given as the mean ± SEM, with the number of frogs shown in parentheses. Values followed by different letters are statistically distinguishable (ANOVA; Scheffé's, $p < 0.05$). Body mass did not significantly affect lactate concentration (ANCOVA: $F = 1.34$, $p = 0.28$), thus lactate concentration was expressed on a per gram fresh mass basis without complications due to allometry.

*Initial concentration in water at the initiation of the submergence experiment. Values are given as the mean ± SEM and $n = 4$ frogs per treatment group.

[†]Data are for three of the four frogs in this group; one frog remained alive at the end of the 10-day trial.

body lactate concentration of those frogs in initially normoxic water increased only slightly, and not significantly, over that of controls (Table 1).

Habitat selection

When provided a choice between land and water in an experimental apparatus, most cricket frogs moved to terrestrial sites (Figs. 1B and 1C). This pattern held true whether the soil was very wet or only slightly moist. However, frogs took longer (1 to 3 h) to move onto the soil when the choice was between moist soil and water than when the choice was between wet soil and water (<1 h). Habitat preference did not shift within the diel temperature and light cycle. In 80% of all observations, frogs were found under the leaf litter. Some individuals moved into slight depressions in the substrate but none actively burrowed into the soil.

The results of the habitat selection experiments correlated with observations of cricket frogs hibernating in the wild. No cricket frog was captured in the water during winter, although we found many *Rana clamitans* tadpoles and juvenile bluegill sunfish at these sites. In contrast, searches of terrestrial habitats yielded eight frogs, all in either abandoned crayfish burrows ($n = 6$) or natural cracks in the soil of the pond bank ($n = 2$) within 1 m of the edge of the pond. Within these sites the frogs were typically 3–10 cm below the soil surface. The crayfish burrows were 2–4 cm in diameter, but with smaller (<2 cm) external openings, whereas cracks in the bank were of variable size and shape. The openings of the crayfish burrows were insulated by a layer of decomposing leaves ~1 cm thick. The soil in all of the hibernacula was mostly clay and was saturated with water. When cricket frogs were uncovered by widening the openings of their hibernacula, they were found at first to be in a typical hibernation position, with the limbs tucked tightly against the body and eyes closed. Once disturbed, they opened their eyes and some lethargically attempted to escape.

Although otherwise similar to cricket frog hibernacula, the site selected for placement of the data logger was only 2 cm below grade, whereas hibernating cricket frogs were found 3–10 cm below grade. Thus, the temperatures re-

Fig. 2. Temperature recorded 2 cm below the soil surface in a burrow similar to those used by hibernating cricket frogs, and the air temperature recorded 10 cm above the ground at the same site.
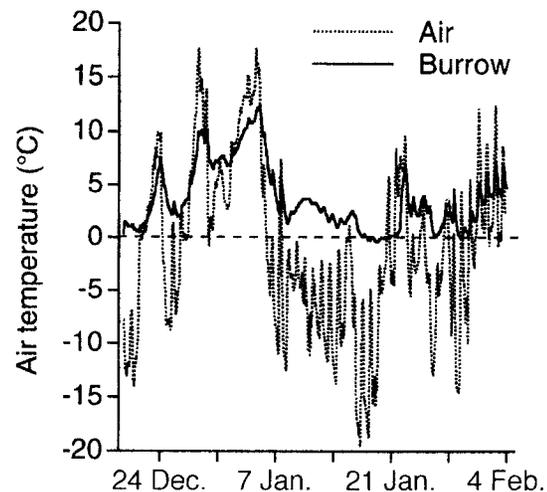
corded for a simulated cricket frog hibernaculum may be slightly lower than those experienced by hibernating cricket frogs. The soil temperatures at this site only fell below 0°C once, despite the external air temperatures falling as low as −19.6°C (Fig. 2). In fact, several weeks of subzero air temperatures in late winter were required to cause the soil to freeze and, even then, the latent heat of fusion slowed cooling of the soil so that it cooled to only −0.5°C.

Tests of freeze tolerance – temperature of crystallization

Other sympatric hylids that hibernate in shallow terrestrial sites tolerate freezing at high subzero temperatures (Layne and Lee 1989; Costanzo et al. 1992), but cricket frogs did not exhibit the same degree of freeze tolerance. Only 2 of 15 frogs survived our freezing treatments (Table 2). The two individuals that did survive freezing were frozen for 24 h to −2°C and for 48 h to −1.6°C.

Despite their poor freeze tolerance, cricket frogs produced glucose (a cryoprotectant used by freeze-tolerant anurans) upon freezing. Freezing increased glucose concentrations 37-fold in the liver and almost 3-fold in the thigh musculature over those in unfrozen control frogs (Table 3). Freezing also increased concentrations of glycerol (another cryoprotectant used by freeze-tolerant hylids) 2-fold in the liver, but no increase occurred in the thigh musculature (Table 3). There were no significant seasonal differences in either glucose or glycerol concentrations between cricket frogs in the autumn (prehibernating) and those collected in midwinter (hibernating) (Table 3).

In the absence of ice, cricket frogs supercooled quite well before spontaneously freezing (mean supercooling point −5.5°C; range −4.3 to −6.8°C). These values are similar to those for striped chorus frogs (*P. triseriata*) of similar size (Swanson et al. 1996). Cricket frogs were, however, easily inoculated by external ice and froze at temperatures between −0.5 and −0.8°C during the freeze-tolerance trials.

Table 2. Survival of cricket frogs (*Acris crepitans*) after freezing.

Month of experiment (1996)	Minimum temperature (°C)	Duration of freeze (h)	Number surviving	Number tested
Mid-November	−0.8 to −1.6	48	1	3
Early February	−1.7 to −2.0	24	1	6
Mid-November	−2.0 to −2.6	24	0	3
Late January	−2.0 to −2.5	96	0	3

Table 3. Glucose concentration (μmol·g^{-1} fresh mass) in prehibernation (October), unfrozen hibernating, and frozen hibernating cricket frogs (*Acris crepitans*).

Metabolite	Prehibernating	Hibernating		F	p
		Unfrozen	Frozen		
Glucose					
Liver	2.4±0.4a	2.9±0.7a	107.5±16.5b	49.83	<0.0001
Thigh	1.2±0.2a	0.7±0.1a	2.0±0.2b	14.29	0.0004
Glycerol					
Liver	1.1±0.2a	2.3±0.5a	5.8±0.6b	44.88	<0.0001
Thigh	1.0±0.6	1.4±0.2	1.2±0.2	2.62	0.108

Note: Values are given as mean ± SEM and $n = 5$. Within a row, values followed by a common letter are not significantly different (Bonferroni multiple comparison). Body mass did not significantly affect metabolite concentration (ANCOVA, $p > 0.13$ in all cases), thus metabolite concentrations are expressed on a per gram fresh tissue mass basis without complications due to allometry.

Discussion

Several lines of evidence indicate that cricket frogs, despite their aquatic nature, do not hibernate in aquatic sites. First, this species is not tolerant of hypoxia and, thus, would not survive the low oxygen concentrations often present in northern lakes and ponds during winter. Cricket frogs submerged in water low in oxygen died in less than 24 h (Table 1) owing to hypoxic stress, as indicated by large increases in tissue lactic acid concentration. This lack of hypoxia tolerance may be due to inadequate bradycardia and metabolic suppression during submergence, without which metabolic acidosis occurs as the by-products of anaerobic metabolism accumulate in the tissues (Pinder et al. 1992).

The exact cause of death of the three frogs in the high oxygen (8 mg·L^{-1}) treatment group is less clear. These frogs may also have succumbed to the hypoxia induced through consumption of the oxygen in the container, but our data suggest that this was not the case. First, these three frogs did not have significantly elevated levels of lactate following treatment (Table 1), which suggests that they were not relying heavily on anaerobic metabolism. In addition, the one individual that survived the entire 10-day treatment had less oxygen available in its container (only 3.0 mg·L^{-1}) than those that died (3.8 ± 0.5 mg·L^{-1} (mean ± SEM)), suggesting that oxygen may not have been the limiting factor in the normoxic treatment group.

An alternative explanation is that the frogs in the high oxygen (8 mg·L^{-1}) treatment group died from complications owing to osmotic stress. Because hylids are generally terrestrial, few studies of their submergence tolerance have been performed. *Hyla arborea* submerged in aerated water but without access to air live only 4–24 h (Czopek 1962). Other hylids held in water but given access to air die within 10 days (Schmid 1965). Both of these studies suggest that

the short duration of submergence tolerated by hylids may be due to their higher rate of water uptake, possibly compounded by a poor ability of the cutaneous vasculature to extract oxygen from the aquatic medium (Czopek 1962). Regardless of whether these frogs died of hypoxic stress or osmotic stress, aquatic overwintering is not a viable strategy for this species for physiological reasons. Indeed, no cricket frog was found in an aquatic hibernation site within the study area.

Our laboratory evidence suggests that cricket frogs hibernate in terrestrial sites. In habitat-selection experiments, cricket frogs strongly preferred terrestrial sites regardless of temperature or soil-moisture level. These observations are similar to those for another terrestrial hibernator, *R. sylvatica*, under the same experimental conditions (Licht 1991). Unlike *R. sylvatica*, however, cricket frogs did not burrow into the substrate to create forms (Licht 1991), but they did occupy pre-existing depressions and crevices in the soil substrate.

Our observations of cricket frogs hibernating in terrestrial sites support earlier anecdotal observations of cricket frogs hibernating in cracks in the soil of the pond bank (Walker 1946; Gray 1971). Other terrestrial hibernation sites used by this species are under logs (Neill 1948) and beneath shoreline vegetation (Walker 1946). The use of terrestrial hibernacula coupled with our observation that this species did not burrow when exposed to cold, led us to ask whether cricket frogs may tolerate freezing like other northern hylids. For example, sympatric Cope's gray treefrogs (*H. chrysoscelis*) tolerate freezing for at least 24 h at temperatures as low as −2.9°C (Costanzo et al. 1992). Cricket frogs did not tolerate this degree of freezing. However, the survival of two frogs at mild subzero temperatures indicates that cricket frogs may have a minor degree of freeze tolerance. This limited freeze tolerance is comparable with that observed in another freeze-

intolerant species, *R. pipiens*, which survives for up to 8 h at –2°C (Layne 1992).

Cricket frogs, although lacking a high degree of freeze tolerance, produce substantial quantities of glucose upon freezing, especially in the liver. A similar observation has been made in the freeze-intolerant *R. pipiens* and is believed to be a generalized stress response. Indeed, it has been hypothesized that very high production of glucose in freeze-tolerant species has evolved through enhancement of this generalized stress response (Costanzo et al. 1993). Thus, although the 108 μmol·g^{-1} of glucose in the liver of frozen cricket frogs falls within the range seen in freeze-tolerant wood frogs (40 to >300 μmol·g^{-1}; Storey 1985; Costanzo et al. 1991), this cryoprotective glucose is not adequate to confer freeze tolerance on cricket frogs. This lends additional support to the conclusion that glucose itself is not adequate to confer freeze tolerance and that other physiological adaptations are required (Costanzo et al. 1993).

Unlike the freeze-tolerant gray treefrogs, cricket frogs did not have high levels of glycerol in their tissues, either in the early fall or after freezing (Table 3). Our unfrozen cricket frogs had less than 2.3 μmol·g^{-1} glycerol in the liver or thigh musculature (Table 3); unfrozen gray treefrogs (*H. versicolor*) have higher concentrations (12.5 ± 5.9 mM) of glycerol in the plasma (Layne 1999). Although we observed a statistically significant increase in hepatic glycerol with freezing (Table 3), even these concentrations were well below the glycerol levels observed in unfrozen gray treefrogs (Layne 1999).

In addition to poor cryoprotectant production, several other physiological characteristics of the cricket frog may limit its tolerance of freezing. The freeze-tolerant *R. sylvatica* has a significant degree of ischemia tolerance and survives for 5 days submerged in anoxic water, likely as a by-product of its ability to tolerate tissue ischemia during freezing (Holden and Storey 1997). In comparison, cricket frogs have a low tolerance of hypoxia (<24 h survival in hypoxic water) and this may limit their ability to survive without oxygen during freezing. Also, because tissue dehydration is a major consequence of ice formation, freeze-tolerant anurans are generally also highly tolerant of desiccation (Costanzo et al. 1992), and the physiological responses to dehydration stress enhance freeze tolerance in these species (Churchill and Storey 1993). In contrast, the cricket frog, being highly aquatic, is the least tolerant of the Nearctic hylids of desiccation (Ralin and Rogers 1972); this too may limit its tolerance to freezing. The absence of other adaptations required for freeze tolerance, such as continued cardiac activity during ice formation (Layne et al. 1989), remains to be explored in cricket frogs.

Although cricket frogs do not survive freezing to the extent observed in other sympatric hylids, they are also unable to rely on supercooling to survive subzero temperatures, because they are susceptible to inoculative freezing. In a dry environment, cricket frogs supercool to –5.5°C, as would be expected for an animal of such small size (Costanzo and Lee 1995). However, when in the presence of ice, cricket frogs do not resist inoculation and freeze between –0.5 and –0.8°C, very close to the freezing point of frog tissues (Layne and Lee 1987). Thus, cricket frogs may be inoculated at subzero temperatures under field conditions (as in *R. sylvatica*; Layne 1991; Costanzo et al. 1999). Because cricket frogs are suscep-

tible to inoculative freezing, they would freeze and die while hibernating in the poorly insulated moist microenvironments used by freeze-tolerant hylids (Schmid 1982).

The cricket frog is unusual among the northern hylids, because it is a terrestrial hibernator and yet is not freeze-tolerant or fossorial. Also, like other anurans, it is also incapable of supercooling in the presence of external ice. Thus, cricket frogs must select microhabitats that remain above the freezing point of their tissues. Indeed, the clayey soils where cricket frogs were found were saturated with water, which, given the high specific heat of water, provide considerable thermal buffering. Even when the soil froze, the latent heat of fusion held the soil temperature stable at 0°C for many days before further cooling occurred (Fig. 2). As a result, after weeks of subzero air temperature, the temperature in a natural cavity 2 cm below the soil surface fell to only –0.5°C, a temperature close to the freezing point of frog tissues (Layne and Lee 1987). In fact, hibernating cricket frogs were found only in cavities that were more than 2 cm below grade, and thus they possibly avoid freezing temperatures altogether. This observation is supported by the cohabitation in cricket frog hibernacula of millipedes and terrestrial isopods, both of which are intolerant of tissue freezing (David et al. 1996; Lavy et al. 1997). The small openings of the crayfish burrows and the layer of leaf litter present also likely help protect and insulate against cold air.

In conclusion, northern cricket frogs hibernate in situations unlike other northern hylids. Although this species does not burrow, it uses its extremely small size to overwinter in existing burrows and natural cracks in the pond bank. The thermal inertia of the wet soil in these sites moderates temperatures enough during the winter to prevent freezing. Although this is a unique overwintering strategy among the hylids, other anurans that do not tolerate extensive freezing also use the buffering capacity of water to protect themselves from freezing. For example, boreal toads (*Bufo boreas*) in Colorado choose shallow terrestrial sites adjacent to streams, where water permeates the soil and prevents freezing even when outside air temperatures fall to –31°C (Campbell 1970).

Although cricket frogs exhibit a low degree of freeze tolerance, on par with that of the freeze-intolerant *R. pipiens*, this degree of freeze tolerance may improve survival under certain conditions. During a drought or an extremely cold winter, freezing may penetrate more than 2 cm into the soil, to the depth of >3 cm at which cricket frogs hibernate. Cricket frogs would freeze under these conditions but, as long as temperatures remained high, some portion of the population may be able to survive. Apparently cricket frogs have considerable success with their physiological–behavioural responses to cold, because overwintering mortality can be extremely low (Gray 1983).

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