SEASONAL STATUS AND DISTRIBUTION OF BLANCHARD'S CRICKET FROG IN SOUTH DAKOTA

By

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ABSTRACT

The Blanchard's cricket frog is a historical resident of southeast South Dakota. Prior to this study it was believed to be a very rare resident in, or extirpated from, the state. Archival records document the cricket frog's South Dakota range as extending west along the Keya Paha River to Tripp County and north along the Big Sioux and James rivers to Lincoln and Hanson counties, respectively. Recent studies conducted in the 1990s turned up few frogs throughout the entire state. This study sought to assess levels of cricket frog populations in South Dakota, delineate the frog's current range, and discern the frog's preferred habitat and overwintering strategies. Cricket frogs are seasonally abundant in a number of South Dakota lotic systems. Frogs congregate at hibernacula sites during October and November. The James, Big Sioux, and Missouri rivers are being utilized year-round, though the populations are fragmented along them. Small bodies of water near the rivers are also being used. Of interest is the Vermillion River, which, according to our surveys, is not being used by cricket frogs. Further study will be needed to ascertain the reasons for their absence from this drainage, where they formerly occurred. Large populations are present in Springfield Bottoms, Bon Homme County and the section of the James River below Wolf Creek Colony, Hutchinson County. Habitat, encounter, and mark/recapture surveys were conducted at more populated sites during the summer, but summer abundances were low, except at Springfield Bottoms, where we estimated population size along a 100-meter stretch of shoreline at between 29 and 140 individuals.

Frogs were observed utilizing mud cracks as hibernacula along the James and Big Sioux rivers. Temperature data loggers were placed in potential hibernacula at these sites to monitor overwinter temperature characteristics. Laboratory freezing trials were performed to deduce whether South Dakota cricket frogs were freezing tolerant. Cricket frogs did not tolerate 24-hour freezing bouts at -1.5 to -2.5°C, a freezing exposure that is easily tolerated by freeze tolerant species, but 83% did tolerate 6-hour freezing exposure under these conditions, suggesting only a minor level of freeze-tolerance in this species, similar to cricket frogs from more central portions of their range. During the winter of 2005-2006, hibernaculum temperatures rarely dropped below the freezing point of the body fluids, and in most cases where temperatures lower than the freezing point of the body fluids did occur, the occurred for only a few hours. Thus, the limited freezing tolerance in this species may have some ecological relevance, at least in some winters. However, the single data logger we were able to recover in the winter of 2006-2007 showed extended periods of temperatures below the freezing point of the body fluids, suggesting that frogs would not survive in this hibernaculum.

In summary, two years of auditory and visual surveying demonstrated cricket frog populations extending west to Springfield, Bon Homme County and north to Hanson and Lincoln counties. Further study may be warranted to rule out remnant historical populations outside of this habitat range. Cricket frogs are presently abundant in South Dakota in localized populations. Due to apparent loss of historical range, whether current populations are imperiled is another area requiring further study. Given the absence of freezing survival in these populations and the documentation of hibernaculum

temperatures sometimes dropping below the freezing point of the body fluids, it seems likely the winter temperatures in the hibernacula may act to limit expansion of populations in South Dakota. However, given current trends of warmer winters in this area associated with global climate change, cricket frog populations may expand their range in South Dakota, and at other sites along the northern boundary of their range in the future.

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Chapter 1: Blanchard's Cricket Frog Distribution and Abundance

INTRODUCTION

Blanchard's cricket frogs, *Acris crepitans blanchardi*, were historically found in southeast South Dakota, with past distributional data placing frog populations in Union, Clay, Yankton, Turner, Lincoln, Hutchinson, Bon Homme, Minehaha, McCook, Hanson, Davison, and Charles Mix Counties (Nanjappa and Leininger 2000, M. Gumbert, pers. comm., T. Jessen, pers. comm.). Kiesow (2006, pers. comm.) defines western boundary of the cricket frog range in South Dakota as including Chouteau Creek and Lake Andes in Charles Mix County. South Dakota populations are considered the northwestern limit to cricket frog range (Figure 1), though Michigan populations do occur at higher latitudes (Fischer et al. 1999, Hammerson et al. 1999, Harding 1997). Blanchard's cricket frogs were recently thought to be extremely rare or possibly extirpated within South Dakota by Fischer et al. (1999). More recent surveys, however, found cricket frogs within portions of their historic range in South Dakota (A. Gregor, pers. comm., Kiesow, pers. comm.). Given these conflicting reports, and recent extirpations from neighboring states, we undertook systematic surveys for cricket frogs throughout their historic range in South Dakota.

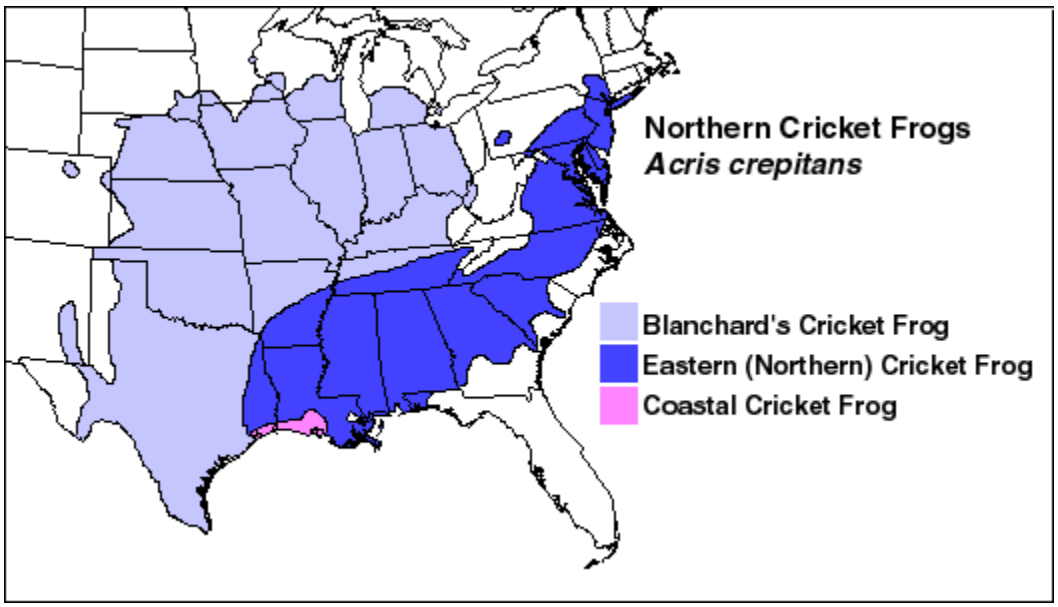

Figure 1: Range estimates for Blanchard's cricket frog by the USGS Amphibian Research and Monitoring Initiative, Midwest Region.

A. crepitans ranges in size from 1.5 to 4 cm (Conant 1975). The breeding season, during which males participate in advertisement calling extends from late May through July (Harding 1997). *A. crepitans* does not typically engage in male-male combat or agonistic displays, instead modulating call note duration to achieve spatial distribution of individual males (Perrill and Shepherd 1989, Forester and Daniel 1986). Female cricket frogs may lay up to 400 eggs singly or in small clusters, which hatch within days (Wright and Wright 1949). Metamorphosis occurs after five to ten weeks, and takes around two days for completion (Burkett 1984, Harding 1997). Adults in South Dakota appear to survive only one or two breeding seasons, with highest frog abundance occurring in October or November due to surviving adults joining newly metamorphosed frogs in congregations along overwintering sites (McCallum and Trauth 2004). Low abundance occurs in spring due to winter mortality (McCallum and Trauth 2004).

The diet of cricket frogs consists mainly of terrestrial prey acquired from the ground or just above ground, with very little aquatic prey utilized (Labanick 1976). Terrestrial arthropods made up over 97% of stomach contents in a study conducted by Johnson et al. (1976) in two large populations near Des Moines, Iowa. *A. crepitans* also take less prey on average as they increase in size, though the size of prey taken increases (Labanick 1976). This suggests that aquatic habitats are primarily utilized for reproduction and moisture balance.

The purpose of this study was to survey and monitor cricket frog presence and abundance within the species' historic South Dakota range.

Project Methods

Auditory Surveys

We used the South Dakota Atlas & Gazetteer (DeLorme 2001. P.O. Box 298, Yarmouth, Maine, 04096) to identify wetland sites for nocturnal auditory surveys within the historical range of the cricket frog in South Dakota (Figure 1) during the summers of 2005 and 2006 (Table 1). Suitable creeks and lakes within the frog's historic range were chosen based on earlier studies reporting cricket frogs utilizing aquatic/terrestrial edges with low emergent vegetation, and dominated by a rock or mud/rock substrate (Smith et al. 2003, Johnson and Christiansen 1976, Burkett 1984). Surveys were conducted from access points along roads and public lands, but we walked some distance from the access points to get to areas of the site more removed from human presence if frogs were not detected immediately. Surveys were conducted between 1900 and 0100 hours, as these are the hours referenced as cricket frogs' most active period of calling (Bridges and Dorcas

2000). We were primarily interested in surveying aquatic/terrestrial edges and muddy margins along the Big Sioux, Missouri, Vermillion, James, and Keya Paha rivers, and Brule, Chouteau, Ponca, Emanuel, and Spring creeks, as well as wetlands associated with these drainages. Surveys were started early May, but in accordance with the literature we didn't start encountering cricket frogs calling until late May through mid-July (Harding 1997). Cricket frogs were encountered calling from 20 May through 10 July, 2005, and 24 May through 11 July, 2006. We listened for calling for a 10-minute sampling period at each site, and the number of male cricket frogs present was conservatively counted and recorded. Additional frog species were also recorded, though no estimate of their abundance was taken. Auditory surveys were suspended during adverse conditions (cold temperatures or high winds), as these negatively influence frog calling behavior (Heyer et al. 1994).

Visual Encounter Surveys

Visual encounter surveys (VES) for relative abundance were attempted at several sites where we encountered frogs in numbers during auditory surveys (or at sites of fall hibernation congregations). VES transects (100-150m in length) were focused along the shallow water and bank margins at sites bordering the Missouri, Big Sioux and James rivers. VES were conducted in the early evenings (1830 to 2100 hours) for 2005, and late morning/early afternoon (1000 to 1400 hours) during the 2006 surveys. We experienced poor success in locating frogs during VES for both years of the study, as frogs proved difficult to locate when not calling. High vegetation density, the cryptic nature of the frogs, and the early breeding season being a low point in annual cricket frog abundance

conspired to make locating large numbers of frogs difficult (McCallum et al. 2004). When we detected frogs during VES, we marked their location with a flag and subsequently measured microhabitat features at the site where the frog was located according to Smith et al. (2003). Measured features included the frog's distance to water, distance to vegetation, type of microhabitat occupied (mud, vegetation, water, mud/vegetation border, vegetation/mud border), substrate temperature, and a simple index of sun exposure on the patch (full sun, partial shade, full shade). We also used Smith et al.'s method (2003) for random microhabitat sampling measurements at VES study areas where frogs were located so that we could compare microhabitat features of sites occupied by frogs with those at random sites. For random sampling of microhabitats, a meter stick was randomly dropped every 5 to 10 paces along VES transects, and the same variables for occupied sites, sun index, substrate temperature, type of microhabitat, distance to water, and distance to vegetation, were recorded at locations at the two ends of the meter stick. Occupied and random microhabitat variables were measured at the same time, so that results are comparable. Substrate temperatures between random and occupied sites were compared by Student's *t*-test. The Chi Square Goodness of Fit Test was used to compare the frequency of occurrence for sun index categories and microhabitats between random and occupied sites. To allow accurate comparison of temperatures and microhabitat features, statistical analysis was only performed when we had sufficient random and occupied data from the same site and time.

Mark-Recapture Surveys

Again due to cricket frogs' highly cryptic nature and our need to conduct surveys during daylight hours when frogs are not usually calling, mark-recapture surveys proved difficult to undertake. We only found frogs in sufficient density for mark-recapture studies at a single site, Springfield Bottoms, Bon Homme County, during summer 2006. This site had a muddy margin along the upper reaches of Lewis & Clark Lake and frogs were found in good numbers along this margin. Other locations with high abundance during fall congregation months were either obstructed by dense vegetation, or there may have been enough seasonal fluctuation in abundance to make frogs at these sites much less common in summer months (McCallum 2004). We set up a 100-meter mark-recapture transect at the Springfield Bottoms site along a section of the muddy bank and captured all cricket frogs encountered along this transect. Frogs were injected in the footpad with a biologically inert, fluorescent-colored visible injectable elastomer (Northwest Marine Technology, Shane Island, WA) that fluoresces when exposed to UV light. The translucent nature of cricket frog limb structure did not require fluorescent light to detect the presence of the elastomer. Frogs were marked in the left rear foot the first day of marking, and the right rear foot in a different color on the second day of marking to avoid confusion. We used the triple-catch method (Heyer et al. 1994) for determining population size. This method required three visits to the site, which were conducted on 15 July (1215 to 1315), 18 July (0908 to 1008), and 24 July (1028 to 1128) during 2006. The 100-meter transect was walked slowly to capture frogs for the standard amount of one hour each visit. Frogs captured on the second and third visits were classified as new captures or recaptures, and third-day recaptures were classified as marked on 1st visit only, marked on 2nd visit only, or marked on both visits.

RESULTS

Auditory Surveys

Cricket frogs were detected calling in Bon Homme, Clay, and Union counties along the Missouri River, in Union and Lincoln counties along the Big Sioux River, along Brule Creek in Union and Lincoln counties, in Hutchinson, Yankton, and southern Hanson counties along the James River, and along Emanuel Creek in Bon Homme county, but only for the first approximately 7 miles from its confluence with the Missouri River (Figure 2). No cricket frogs were heard along the Vermillion River in Turner, Clay, and McCook counties, along Chouteau, Spring, or Platte creeks in Charles Mix County, along the Keya Paha River in Tripp County, and along the Ponca Creek drainage in Gregory County (Figure 2). The western sites in Charles Mix, Gregory, and Tripp counties were first surveyed rather late in the breeding season (mid July) in 2005, but were intentionally surveyed during mid-June in 2006, which is during the peak of the breeding season. No cricket frogs were heard at these sites during either year, so we are confident that cricket frogs are no longer present in these western regions of the historical range (Ballinger et al. 2000).

Sites where calling cricket frogs were particularly abundant included the James River south of Maxwell Colony, Hutchinson County, SD, and at a number of small ponds near the juncture of Highways 11 and 46 northwest of Hawarden, IA in Union County, SD, in addition to the Springfield Bottoms site. The James River and Springfield Bottoms sites yielded the highest abundance of frogs. No cricket frogs were detected at

the pond sites near Hawarden, IA, when sampled by visual encounter surveys later in the summer season.

Visual Encounter Surveys

Sufficient frogs were located during VES at two sites for statistical comparisons, Springfield Bottoms, Bon Homme County (42° 50' N, 97° 55' W), and Bolton Game Production Area (GPA), Union Co. (42° 42' N, 96° 48' W). Cricket frogs at Springfield Bottoms were observed along the margin of a pasture, where the emergent grass gave way to a wide muddy flat and a shallow, warm side-channel area of Lewis and Clark Lake. Cricket frogs at Bolton GPA were found along the edges of a pond which had become isolated from the Missouri River by low water levels. This site consisted of a cattail marsh to the northern end, which led down to a wide muddy bank (where the frogs were found) with emergent vegetation. The Springfield Bottoms site was sampled from 1000 to 1300 CST during mid-July, 2006, and the Bolton GPA site was sampled July 12, 2005 from 1830 to 2000 CST. The frogs' mean (±SD) distance from water was 13.1 ± 4.0 cm (n = 56) at Springfield Bottoms and 43.5 ± 9.9 cm (n = 6) at Bolton GPA. Frogs at Bolton GPA occurred significantly farther from water than those at Springfield Bottoms (*t*-test, $P < 0.001$). This could possibly be attributed to differences in sampling time, solar radiation, and temperature, but could also reflect differences in microhabitats between the two sites. Frogs at the two sites did not differ significantly in mean distance to vegetation; the mean distance of frogs from vegetation was 4.3 ± 1.0 cm at Springfield Bottoms and 6.5 ± 4.3 cm at Bolton. There was no significant difference of substrate temperature between random and occupied sites at either Springfield Bottoms or Bolton

GPA, though temperatures were more variable at random sites compared to occupied sites at Springfield Bottoms. This suggests that cricket frogs are selecting microhabitat that avoids particularly high and low temperatures (Table 1). The sample sizes needed for Chi-square comparison of occupied and random sites were only achieved at Springfield Bottoms, and so sunlight index and microhabitat comparisons were limited to this site. Cricket frogs occupied mud and water-vegetation microhabitats higher than their availability, and occupied water and vegetation microhabitats lower than their availability (Table 1; $\chi^2_4 = 16.9$, $P = 0.002$). There was no significant difference in regards to sun exposure among random and occupied sites, suggesting that cricket frogs do not select microhabitat based on sunlight exposure (Table 1). Due to the pasture bordering the Springfield Bottoms wetlands, full shade microhabitat was rare and so further sampling at other sites may be required to rule out a sun exposure preference in cricket frogs, but we were unable to locate cricket frogs in sufficient numbers at other sites for such comparisons.

Mark-Recapture Surveys

For 2006 mark-recapture surveys along the 100-m transect at Springfield Bottoms, Bon Homme County, we captured and marked totals of 22 frogs on 15 July, 12 frogs on 18 July (3 were recaptures from Day 1), and 29 frogs on 24 July (4 were Day 1 recaptures, 7 were Day 2 recaptures, 1 was recaptured from both Day 1 and 2). Population estimates for the Springfield Bottoms study site ranged from 29 - 140 individuals according to the triple-catch method, and from 40-110 individuals according to the Peterson method for the three sampling days.

DISCUSSION

Blanchard's cricket frog is clearly not extirpated from, nor extremely rare within, its southeastern South Dakota range as previously suggested by Fischer et al. (1999). It does, however, appear to have a more restricted range in southeast South Dakota than reported by Kiesow (2006), with this study finding no evidence of frogs using the western edge of the frog's historic western range in South Dakota, the Keya Paha River in Tripp County, Ponca Creek in Gregory County, or Platte Creek in Charles Mix County. More extensive monitoring in this specific area may be needed to fully ascertain if the cricket frog is truly extirpated from that area. However, we spent six nights total during two breeding seasons surveying numerous sites along these drainages without detecting any cricket frogs, so it is unlikely that breeding populations exist in this area. Cricket frogs appear to be concentrated in localized populations along the Big Sioux, Missouri, and James rivers and Emanuel and Brule creeks in varying abundance. No evidence of cricket frog activity was recorded along the Vermillion River, or along Chouteau, or Spring creeks.

Cricket frogs apparently prefer a habitat characterized by slow-moving, clear, waters (Burkett 1984). The presence of a well-saturated bank of mud or mud/rock with low emergent vegetation also seems to be a common factor in cricket frog habitat (Burkett 1984, Smith et al. 2003). Frogs typically occupy the region of bank near the water. Data in our study are consistent with this trend, with an average distance from water in this study of less than 45 cm in our two microhabitat survey sites (Table 1). This is comparable to the 49.8 cm average recorded in a similar study along Rush Creek in

west-central Missouri (Smith et al. 2003). Missouri frogs were reported as preferring a mud or mud-rock microhabitat (Smith et al. 2003). Springfield Bottoms frogs did occupy mud microhabitats, but also utilized mud-vegetation microhabitats as well. It should be noted that the Springfield Bottoms study site is bordered by a pasture used for grazing, and that no mud-rock microhabitats were present. Unlike Missouri cricket frogs that occupied shaded locations at higher proportions than their availability (Smith et al. 2003), Springfield Bottoms cricket frogs showed no preference for sun exposure in their microhabitat choice. Shade was scarce along the water margins at this site, so lack of a preference for shade might be due to a lack of availability of that microhabitat along the edge of the pasture.

Cricket frog abundance varied greatly seasonally and apparently annually at some sites. During breeding-season auditory surveys, 10 or more calling males were recorded only at sites on the Big Sioux River and East Brule Creek in Union County. Springfield Bottoms recorded only 4 or 2 calling males when checked during nocturnal auditory surveys (although higher numbers were calling during daytime mark-recapture surveys), yet yielded a population estimate of up to 140 individuals with mark-recapture analysis. This may suggest that nocturnal auditory surveys are of limited utility for estimating cricket frog abundance, despite its regular use for assessing anuran occurrence data (Heyer et al. 1994, Bridges and Dorcas 2004). Abundance at a number of the James River sites fluctuated greatly, suggesting variations in yearly survival or use of habitat. A fall congregation site around a seep leading into the James River in northern Yankton County yielded too many frogs to count in fall 2005, yet when checked during summer and fall 2006 this site yielded fewer than ten individuals. The emergence of that year's

tadpoles undergoing metamorphosis into adult frogs in late summer probably accounts for much of the seasonal variation of abundance (McCallum et al. 2004), but our survey data suggest that annual variations in winter survival rate or changing habitat conditions likely also influence cricket frog abundance. Vegetation changes and water flow variation may account for the drop in frog detections at the James River site during 2006, as the vegetation had grown beyond emergent stages and water flow was reduced in the seep.

Chapter 2: Freezing Tolerance and Overwintering Studies

INTRODUCTION

General overwintering strategies in northern climate amphibians include fossorial burrowing below the frost line, aquatic hibernation, and terrestrial hibernation among leaf litter, accompanied freeze tolerance to survive periods of subzero temperatures (Pinder et al. 1992). Burrowing and aquatic overwintering allows amphibians to avoid freezing temperatures, and species using these strategies are not tolerant of freezing. Fossorial hibernation occurs in a number of species, especially toads and spadefoots (Pinder et al. 1992, Swanson et al. 1996). In northern climates, aquatic hibernation can be risky due to hypoxia in ice covered bodies of water (Irwin et al. 1999). While amphibians show tolerance of hypoxic conditions, the stress associated with a hypoxic event can result in winterkill (Bradford 1983). Overwinter hibernation in shallow terrestrial sites (e.g., under leaf litter) is restricted to freeze tolerant species, or occurs in microhabitats in which local conditions do not result in freezing of soils (Irwin et al. 1999).

Overwintering in shallow terrestrial hibernacula routinely exposes the amphibian to temperatures as low as -6˚C for days or weeks at a time (Irwin et al. 1999). Blanchard's cricket frogs are reported to use terrestrial stream bank hibernacula (e.g. cracks in the mud or crayfish burrows) in Ohio, Indiana, and Illinois, where wet soils buffer against extensive freezing of the soil (Gray 1971, Irwin et al. 1999).

Intracellular formation of ice crystals damages intracellular structures, so most animals are killed by freezing (Pough et al. 2001, Storey and Storey 1988). About a dozen amphibian species, however, have adapted to tolerate repeated bouts of freezing and thawing (Pinder et al. 1992). Freezing has three detrimental effects on cells that may affect cell survival (Storey and Storey 2004). First, because water expands upon freezing, freezing of the intracellular fluids may cause the cells to freeze and rupture. Second, cells dehydrate as freezing in extracellular spaces results in osmotic movement of water out of the cell, thus raising the osmolality of remaining intracellular water, which can compromise normal cell function and may result in cell death (Pough et al. 2001, Storey and Storey 1988). Third, freezing causes ischemia due to the cessation of circulation in the animal, and this ischemia disrupts the cellular energy balance. All freeze-tolerant frogs accumulate low molecular weight carbohydrates as cryoprotectants (glucose and/or glycerol) during freezing episodes (Storey and Storey 2004). Cryoprotectant accumulation during freezing is directly triggered by tissue freezing, usually occurring first in the extremities (Layne and Jones 2001, Pough et al. 2001, Storey and Storey 2004). Glycogen in the liver is converted to glucose or glycerol that is rapidly transported throughout the body (Storey and Storey 1985, Pough et al. 2001, Storey and Storey 2004). Maximal levels of glucose are attained in 24-48 hours after the

onset of freezing, corresponding to maximal ice formation (Storey and Storey 1988). Central tissues such as the brain, liver, heart, and kidney show the highest glucose levels (75-100% of blood levels) while lungs, stomach, and intestines have intermediate amounts (~50% of blood levels), and skeletal muscle skin, and gonads accumulate the lowest amounts (<35% of blood level) (Storey and Storey 1988, 2004). Accumulation of glucose in the cells raises cellular osmolality and lowers the freezing point, which reduces the osmotic gradient toward extracellular ice so that cells don't dehydrate as severely or freeze (Storey and Storey 1988, 2004, Pough et al. 2001). *Rana sylvatica*, *Pseudacris triseriata*, and *Pseudacris crucifer* are known to use glucose as a cryoprotectant during freeze tolerance (Pough et al. 2001). *Hyla versicolor* mobilizes glycerol as its main cryoprotectant in response to prolonged cold temperatures, but still initializes glucose mobilization at the first instance of freezing tissues (Layne and Jones 2001). Freeze tolerant frogs can remain with over fifty percent of their body water frozen for a week or two, but survival rates decrease with longer freezing bouts (Pough et al. 2001, Storey and Storey 2004).

Geographic variation in freeze tolerance capacity occurs in *Rana sylvatica*, with northerly populations mobilizing greater quantities of glucose and demonstrating greater freezing survival than southerly populations (Storey and Storey 1988, Costanzo et al. 1994). *Hyla versicolor* and *Hyla chrysoscelis*, however, apparently lack geographic variation in freeze tolerance (Irwin and Lee 2003). Geographic variation of freeze tolerance in freeze-tolerant frogs does not appear to be a general trend, and therefore should be assessed for each individual species.

Northern cricket frogs, *A. crepitans,* from populations near the center of their range are largely freeze-intolerant (Gray 1971, Irwin et al. 1999). However, in the southern Indiana population studied by Irwin et al. (1999), two of 15 individuals frozen for 24-48 hours at -1.6 to -2°C survived this freezing bout, suggesting a minor capacity for freeze tolerance in at least some individuals of this population. Studies of cricket frog populations from the central portion of their range have shown that *A. crepitans* uses mud cracks, crayfish burrows, or burrowing greater than 2 cm deep for hibernacula, where moisture protects against substantial freezing of the soil (Gray 1971, Irwin et al. 1999) so that soil temperatures do not often drop below the freezing point of the body fluids (-0.6˚C). This suggests that the presence of suitable hibernacula buffers cricket frogs from freezing conditions, and the presence of hibernacula providing such conditions may be a major factor limiting their range.

In this study, I investigated freezing tolerance in a population of Blanchard's cricket frog from the northwestern extent of their range in southeastern South Dakota. I hypothesize that Blanchard's cricket frogs from South Dakota populations will show greater freeze tolerance than cricket frogs found in more southerly populations in less severe winter climates. I will measure survival of freezing bouts and prospective hibernaculum temperatures over the winter season. In addition, I will measure tissue levels of glucose, glycogen, and glycogen phosphorylase activity in unfrozen control and 24-hour frozen frogs, as these are major components of cryoprotection pathways in freeze-tolerant species from this same area, such as the chorus frog, *Pseudacris triseriata* (Edwards et al. 2000, Jenkins and Swanson 2005).

PROJECT METHODS

Hibernacula Temperatures

During October and early November of 2005 and 2006, we observed large numbers of cricket frogs congregating at several sites along the James and Big Sioux rivers in southeastern South Dakota. Frogs were observed near, or emerging from, numerous deep mud cracks (up to 20-25 cm in depth, Figure 3) in the riverbanks, suggesting that these cracks served as hibernacula. This is consistent with literature descriptions of hibernacula from other locations (Gray 1971, Irwin et al. 1999), so we believe that the deep mud crack microhabitats are also probable hibernacula for South Dakota cricket frogs. Frogs were observed emerging from these cracks during that late fall period, just prior to when frogs entered hibernation. Moreover, during excavation of our 2005-2006 data loggers along the James River in early April 2006, we observed a cricket frog emerging from the soil approximately 15 cm from where a data logger had been placed. This suggests data loggers were placed in appropriate locations to record prospective hibernacula conditions. We placed a number of *iButton* Model 1921Z (Maxim, Sunnyvale, CA) data loggers (14 cm diameter, 5 mm thick, wrapped in self-adhesive cellophane to inhibit moisture damage) at a number of sites where frogs were observed congregating during fall months just prior to hibernation. We placed data loggers (8 during 2005 and 10 during 2006) in mud cracks at sites along the James River (Lat. 43˚12’30” Long. 97˚39’, Lat. 43˚10’30” Long. 97˚37’, Lat. 43˚09’20” Long. 97˚35’50”) and 4 (2005) and 6 data loggers (2006) at two sites along the Big Sioux River (Lat. 42˚50’ Long. 96˚34’, Lat. 42˚49’ Long. 96˚34’30”). Data loggers were placed in cracks from which we observed frogs emerging.

Figure 3: Probable hibernacula site (below the quarter) covered by vegetation along the bank of the James River near Maxwell Colony, SD.

Location of the data loggers at each site was marked by flagging, and an *iButton* data logger (Model 1921G#F50, Maxim, Sunnyvale, CA) was attached to the stem of one flag at each site roughly 0.4 m above a suspected hibernaculum to record ambient air temperature. We placed 4 ambient air temperature loggers at James River sites, and 2 at the Big Sioux River sites. Data loggers used in this study are accurate to 0.25°C, and we programmed them to take temperature readings every 2 hours for the duration of winter (November through early April). We recovered data loggers from hibernation sites in early April 2006, and 9 of 12 total data loggers were recovered. Extensive flooding from March through September 2007 made the recovery of all data loggers deployed during

the 2006 – 2007 winter at the James River impossible, and only one data logger was recovered from Big Sioux River sites in 2007.

Freezing Tolerance Studies

To test freezing-tolerance capacity for cricket frogs from South Dakota, we acclimated cricket frogs to winter conditions according to the protocol of Swanson et al. (1996). 25 cricket frogs were collected for freezing experiments in late October of 2005, and 30 cricket frogs in early November of 2006. Frogs were placed 4 to a plastic terrarium lined with moist paper towels and covered with leaf litter, and then kept in a laboratory incubator at 10°C in complete darkness without food to allow frogs to digest any material within their digestive tracts. In mid-November after two weeks at 10°C, the temperature in the incubator was lowered to 2°C, where it was kept until frogs were used in freezing trials during December-February. Frogs were checked every 2-3 days over the winter period for adequate terrarium moisture, and the moist paper towels replaced every two weeks to avoid molding. In the 2005-2006 winter, 16 frogs were killed due to an incubator malfunction, but all 9 remaining frogs were run through freezing experiments. All 30 frogs in the winter of 2006-2007 were used in freezing experiments. For freezing experiments, single frogs were placed in a freezing chamber consisting of a foam-lined glass jar immersed in a circulating water/ethylene glycol bath (Forma Scientific Inc., Model 2095, Marietta, OH). Body temperature (T_b) was monitored through the trials by a thermocouple (Yellow Springs Instruments 400 series temperature probe, Model 427, YSI Telethermometer, Model 43 TB, Yellow Springs, OH), with the probe held to the cricket frog's abdomen using masking tape. T_b was recorded every 15

seconds throughout the freezing trial. The bath temperature was also continuously monitored by a thermocouple (YSI thermocouple thermometer, Model 4600). Schmidt-Nielsen (1997) lists the freezing point of body fluids in amphibians as approximately -0.6°C. When both frog and freezing chamber had come to equilibrium around -1.0°C, freezing was induced in the cricket frog by placing an ice crystal against its hindlimb. This induced an easily observable freezing exotherm, and indicated that the frogs were indeed freezing. Following freezing, the temperature of the bath was slowly reduced to between -1.5 and -2.5°C (bath cooling rate of approximately 1.0°C/hr). Frogs remained in the frozen state for 24 hours (n = 6 in both 2005-2006 and 2006-2007 trials).

This is a freezing exposure routinely tolerated by known South Dakota freeze-tolerant species like the chorus frog, *Pseudacris triseriata*, but not by freeze-intolerant species like the Woodhouses' toad, *Anaxyrus woodhousei* (Swanson et al. 1996).

During 2006-2007, frogs (n = 6) were also frozen for 6-hour freezing trials, based on our findings from the 2005-2006 data loggers showing that hibernacula temperatures did sometimes drop below the freezing-point of body fluids, but usually not for more than six hours and only for a few nights during the winter. After freezing-bouts, frogs were returned to the incubator and thawed on moist towels at 2°C. They were tested for freezing survival at 1, 3, and 7 days following freezing by using a series of simple tests including righting response, leg retraction, locomotion, and posture retention. Only frogs exhibiting positive responses to all survival criteria were considered to have successfully survived the freezing bout.

Freeze-tolerant anurans routinely accumulate glucose (*Rana sylvatica, Pseudacris triseriata, P. crucifer*)or glycerol (*Hyla versicolor* and *H. chrysoscelis*)as cryoprotectants

to guard against tissue damage (Storey and Storey 2004), and *A. crepitans* did elevate tissue glucose levels during freezing (Irwin et al. 1999). Thus, we conducted a series of tissue assays (liver and leg muscle) on frozen and unfrozen control cricket frogs for glucose and glycogen concentrations, and glycogen phosphorylase (the enzyme splitting glucose from glycogen) activity. The three frogs not frozen for survival experiments in 2005-2006 were double-pithed directly from the incubator as unfrozen controls. In the 2006-2007 winter, 4 to 5 frogs each from 24-hr frozen, and unfrozen control groups were double-pithed at the end of freezing (or without any freezing for controls). After euthanasia, leg muscle and liver tissues were rapidly dissected out over ice. Leg muscle and liver tissues were then frozen in liquid nitrogen and stored at -80°C until later glycogen, glucose, and phosphorylase assays. Assays were conducted according to the procedures outlined in Swanson et al. (1996), Irwin et al. (1999), and Edwards et al. (2000).

RESULTS

Hibernacula Temperatures

During the 2005-2006 winter, temperatures within hibernacula were generally above the freezing point of the frogs' body fluids. However, a few short periods occurred where the hibernacula temperature went below the freezing-point of body fluids, but these only occurred at some hibernacula sites (Figures 4, 5, and 6). Moreover for those sites where temperatures did drop below the freezing-point of the body fluids, this temperature was maintained for only a few hours before warming above the freezing-point temperature. These data suggest that cricket frogs within hibernacula are not

exposed to temperatures that would induce body fluids to freeze except for short periods (i.e., a few hours) at some sites. However, temperatures during the winter of 2005 – 2006 were well above average (3.3˚C higher than the average for the period 1895 – 2008) in southeast South Dakota (South Dakota Office of Climatology). We recovered only one data logger from a Big Sioux River hibernacula site in 2006-2007 which was a colder winter (average temperatures for the winter period were only 0.5˚C above the long-term average). This data logger provided a temperature record from mid-January to mid-March 2007, and temperatures within the putative hibernaculum dipped below –0.6°C for two extended periods. One period lasted eight days with temperatures below -1.4°C, and the other for three days with temperatures dropping to a low of -1.63°C (Figure 6).

Freezing Tolerance Studies

None of the 12 frogs subjected to 24-hour freezing bouts survived. The 24-hour freeze period used in this study is usually easily tolerated by other freeze-tolerant species in South Dakota (Swanson et al. 1996), provided the frogs are able to mobilize sufficient glucose stores from liver glycogen (Jenkins and Swanson 2005). For the 6-hour freezing bouts, 5 of 6 frogs survived. This suggests a slight level of freezing tolerance that may be ecologically relevant given the short duration of hibernacula temperatures blow the freezing-point of body fluids during the winter of 2005-2006.

The difference in mean liver glucose levels between 24-hour frozen frogs and unfrozen controls was nearly significant ($t = 2.179$, $P = 0.057$), with frozen frogs displaying glucose levels in excess of five times that of unfrozen controls (Table 2). Liver glycogen levels did not vary significantly between controls and 24-hr frozen frogs

(U= 7.00, P = 0.154, Table 2). Liver active phosphorylase levels were significantly lower in controls than in 24-hr frozen frogs (U = 24.00, P = 0.010, Table 2). Total phosphorylase activity in liver, however, was not significantly different between frozen and control frogs (t_8 = -1.328, P = 0.221, Table 2). Leg muscle glucose levels were not significantly different between 24-hr frozen frogs and unfrozen controls (t_{10} = -0.617, P = 0.551, Table 2), yet differences in active-form phosphorylase activity were nearly significant (U = 20.50, P = 0.067, Table 2). Muscle levels of total phosphorylase (t_8 = 0.049, P = 0.962) and glycogen (t_{10} = -0.763, P = 0.463) showed no significant difference among 24-hour frozen and unfrozen controls.

DISCUSSION

Cricket frogs in South Dakota appear to be using mud cracks of up to 25 cm deep for overwintering hibernacula, as we found abundant frogs in several sites with such conditions in late Fall. This is consistent with observations reported in previous studies (Gray 1971, Irwin et al. 1999), wherein the cricket frogs were utilizing saturated cracks or crayfish burrows along banks to avoid bodily contact with freezing soils that could initiate ice crystal formation. Temperatures within some putative hibernacula measured by our data loggers dropped to levels sufficient to freeze body fluids in cricket frogs, but such conditions did not occur at all hibernacula sites. When temperatures below the freezing-point of the body fluids were recorded during the winter of 2005-2006, they remained below the freezing-point for only about six hours at most. The winter of 2006-2007 was a harsher winter more typical of the region, and temperature data for the one data logger we were able to recover showed freezing durations in this putative hibernaculum of up to several days. Cricket frogs would not likely

survive these conditions given the poor freezing tolerance documented in this study. The cricket frog's observed intolerance to 24-hour freezing suggests that temperature may be a factor limiting their range. The limited (6-hour) freeze-tolerance that we documented for South Dakota cricket frogs may have adaptive significance, at least during some winters, by allowing South Dakota cricket frog populations to persist if enough breeding adults manage to survive the winter without exposure to a prolonged freezing event. Cricket frogs appeared to favor a sloped mud bank for hibernacula, as they seldom occupied flat mud banks in this area. Further testing of occupied versus random habitat will be needed to verify if degree of slope of the bank, aspect (i.e. north or south-facing banks), and angle of insolation are important habitat factors for hibernacula sites, as our hibernacula and microhabitat surveys did not critically evaluate these factors. A particular orientation and degree of slope may limit exposure to predators, or increase incident solar radiation, which could serve to moderate temperatures within the hibernacula. Addressing these questions will require future research.

South Dakota cricket frogs did accumulate liver glucose (5.1-fold increase over unfrozen controls) during a 24-hour freeze, but no noticeable amount in muscle tissues. Cricket frogs from Indiana also accumulated liver glucose during freezing, but in much greater concentrations (37-fold) for a total mean hepatic glucose accumulation of 107.5 μmol·g fresh weight^{-1} (Irwin et al. 1999) compared to only 8.8 μmol·g fresh weight^{-1} in this study. Freezing tolerant anurans often accumulate hepatic glucose to levels in excess of 200 μmol·g fresh weight^{-1} under similar 24-hour freezing exposures (Swanson et al. 1996, Edwards et al. 2000, Storey and Storey 2004). Jenkins and Swanson (2005) documented lower hepatic glucose mobilization (89 μmol·g fresh weight^{-1}) and lower freezing survival in *Pseudacris triseriata* in some winters. Similarly, only 2 of 15 Indiana cricket frogs survived 24 to 48-hour freezing bouts

at -0.8°C to -2.6°C despite the 37-fold increase in hepatic glucose mobilization during freezing bouts (Irwin et al. 1999). The reason for the disparity between the two hepatic glucose levels following bouts of freezing in Indiana and South Dakota cricket frogs may be differing levels of hepatic glycogen going into the freezing trials. Anurans rely on glycogen stores to mobilize via the glycogen phosphorylase pathway into glucose, and so frogs with low glycogen stores will likely have low tissue glucose levels following a freezing bout. Such a relationship has been documented for chorus frogs (Jenkins and Swanson 2005). Mean liver glycogen stores were also lower in cricket frogs than in the similar sized chorus frog *P. triseriata* (143 µmol glucosyl units·g fresh weight^{-1} in cricket frogs vs. 325-1019 µmol glucosyl units·g fresh weight^{-1} in chorus frogs; Edwards et al. 2000, Jenkins and Swanson 2005). The mobilized hepatic glucose levels of 24-hour frozen cricket frogs in this study only approximated unfrozen control glucose levels in *P. triseriata* which range from 5 – 20 µmol·g fresh weight^{-1} (Edwards et al. 2000, Jenkins and Swanson 2005). However, post-freeze hepatic glucose levels are similar to post-freeze levels in live of freeze-intolerant toads (Swanson et al. 1996). This suggests that cricket frogs are more similar to the freeze-intolerant toads in regards to regulation of low molecular weight carbohydrate metabolism during freezing (Swanson et al. 1996).

The apparent freezing intolerance of cricket frogs in South Dakota compared to other freeze-tolerant species coupled with hibernacula temperatures below the freezing-point of the body fluids suggest that freezing temperatures within the hibernacula are a main limiting factor on the extent of their northwestern range. That cricket frogs in South Dakota appear to successfully tolerate some short bouts of freezing (6-hours was tested) may allow them to survive over winter in some hibernacula, at least some winters, but evidence suggests that avoidance of freezing by occupation of thermally buffered hibernacula is of primary importance to overwintering survival. In this regard, South Dakota populations are similar to cricket frog populations in other portions of their range (Irwin et al. 1999), so geographic differences in capacity for freezing tolerance associated with differences in winter climate are absent for cricket

frogs. Due to their freezing intolerance of long-duration freezing episodes (24-hours was invariably fatal), the presence of suitable hibernacula that remain unfrozen seems to be a necessity for maintaining cricket frog populations. Future studies should carefully describe cricket frog hibernaculum microhabitats to determine what microhabitat features are characteristic of occupied sites and if these characteristics correlate with enhanced overwinter survival. Despite their poor freezing survival and putative hibernaculum microhabitats that drop below the freezing point of the body fluids, sometimes for extended periods, Blanchard's cricket frogs are not extirpated nor extremely rare within southeastern South Dakota, which indicates that frogs are capable of locating hibernacula that confer overwinter survival. Documentation of what hibernacula microhabitat features are important to cricket frogs will allow preservation of suitable habitats. With suitable habitat protection and monitoring it is my belief that healthy populations of cricket frogs may be maintained in South Dakota indefinitely. In addition, warmer winters associated with global climate change may potentially allow cricket frogs to expand their range northward in South Dakota and elsewhere along their current northern range boundary, given available suitable habitat for breeding and overwintering.

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	Bolton GPA	Springfield	Random
Distance to Water	43.5 ± 9.9 (6)	13.1 ± 4.0 (56)	---
Distance to Vegetation	6.5 ± 4.3 (6)	4.3 ± 1.0 (56)	---
Substrate Temperature			
Frog sites	28.3 ± 0.6 (6)	31.7 ± 0.5 (56)	
Random sites	27.4 ± 0.5 (16)	29.3 ± 0.8 (14)	
Substrate Proportions			
Mud	50.0	25.0	7.1
Mud-Vegetation	50.0	33.9	35.7
Water-Vegetation	-----	23.2	7.1
Vegetation	-----	16.1	28.6
Water	-----	0.0	14.3
Sunlight Proportions			
Full Sun	50.0	57.1	42.9
Partial Shade	-----	37.5	50.0
Full Shade	50.0	5.4	7.1

Table 1: Mean (±SE) distance to water and vegetation (cm) and substrate temperature (°C) at sites occupied by cricket frogs at Bolton GPA and Springfield Bottoms study sites. Also included are proportions (%) of frogs occupying the various sunlight exposure and substrate microhabitats as well as the proportion of these microhabitats available at the Springfield Bottoms study site, where sufficient numbers of frog sites were available for Chi-square comparisons. Sample sizes for each group are given in parenthesis.

	Control	24-h Frozen
Liver glucose	1.7 ± 1.1 (3)	8.8 ± 5.4 (8)
Liver glycogen	142.9 ± 54.7 (4)	113.9 ± 159.6 (8)
Liver phosphorylase a	0.07 ± 0.01 (4)	2.70 ± 2.56 (6)
Liver total phosphorylase	3.27 ± 1.23 (4)	5.71 ± 3.47 (6)
Muscle glucose	0.9 ± 0.5 (4)	1.3 ± 1.2 (8)
Muscle glycogen	8.0 ± 6.2 (4)	16.8 ± 22.0 (8)
Muscle phosphorylase a	0.16 ± 0.06 (4)	1.10 ± 2.13 (6)
Muscle total phosphorylase	12.64 ± 7.81 (4)	12.42 ± 6.30 (6)

Table 2: Mean (±SD) liver and leg muscle glucose (μmol·g fresh weight^{-1}), glycogen (μmol glucosyl units · g fresh weight^{-1}), and glycogen phosphorylase (active form [a] and total) activity (μmol·min^{-1}·g fresh weight^{-1}) in unfrozen control and 24-h frozen frogs. Sample sizes for each group are given in parenthesis.

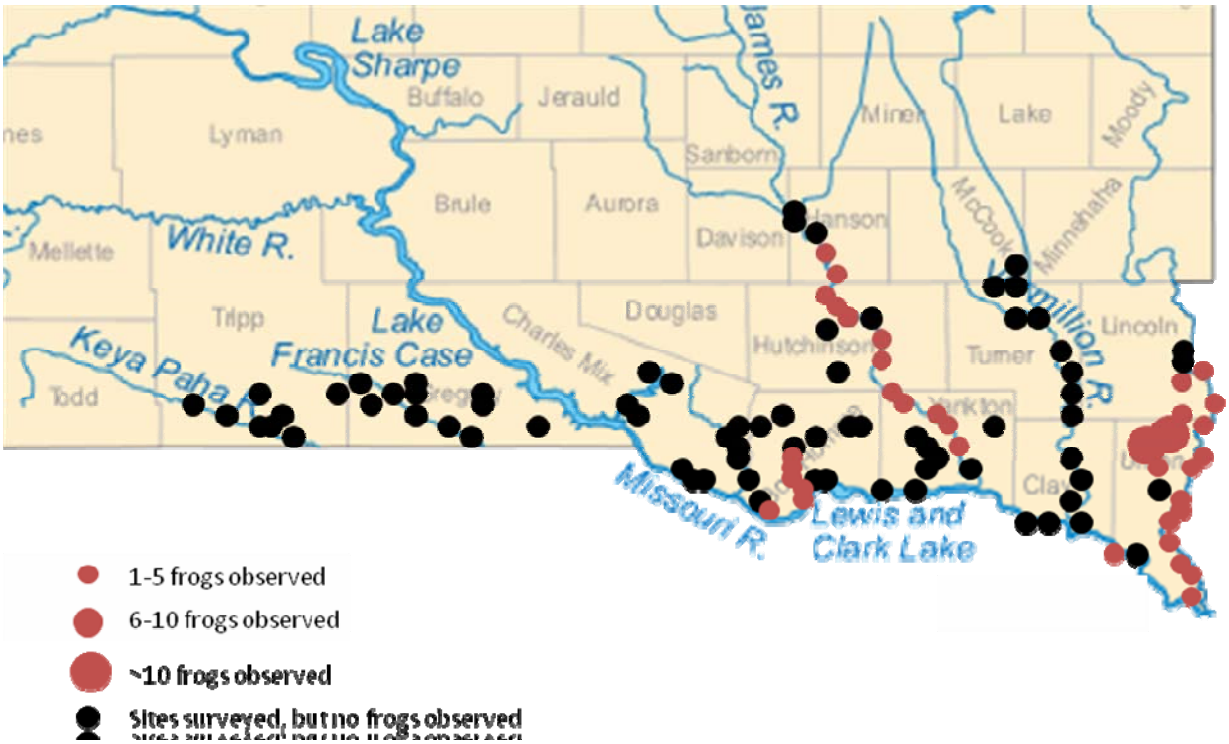

Figure 2: Range map of Blanchard's cricket frog presence or absence in southeastern South Dakota for the field seasons of 2005 and 2006.

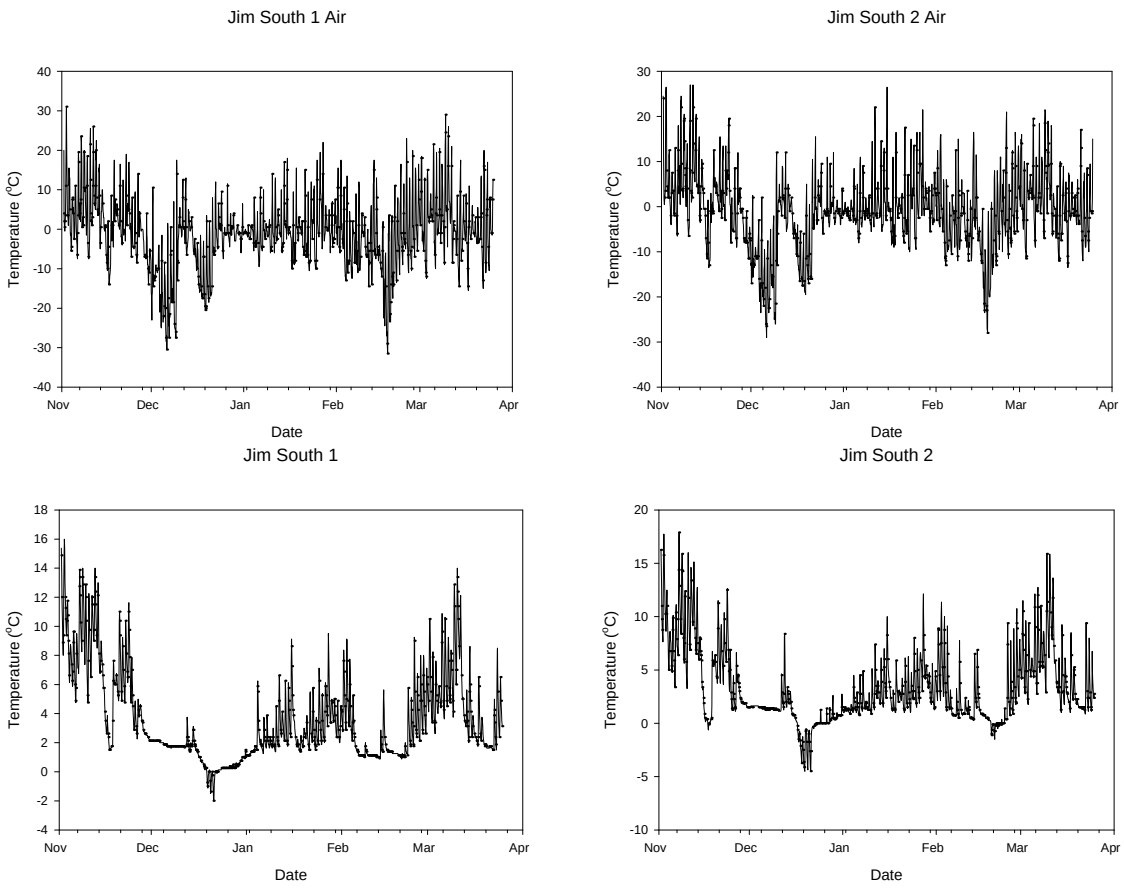

Figure 4: Air and hibernacula temperature profiles for two sites along the James River during winter 2005-2006. Hibernaculum temperatures during the coldest days fell below the body fluid freezing point of cricket frogs (-0.6°C) for a few hours at night, and warmed above freezing temperatures the next day.

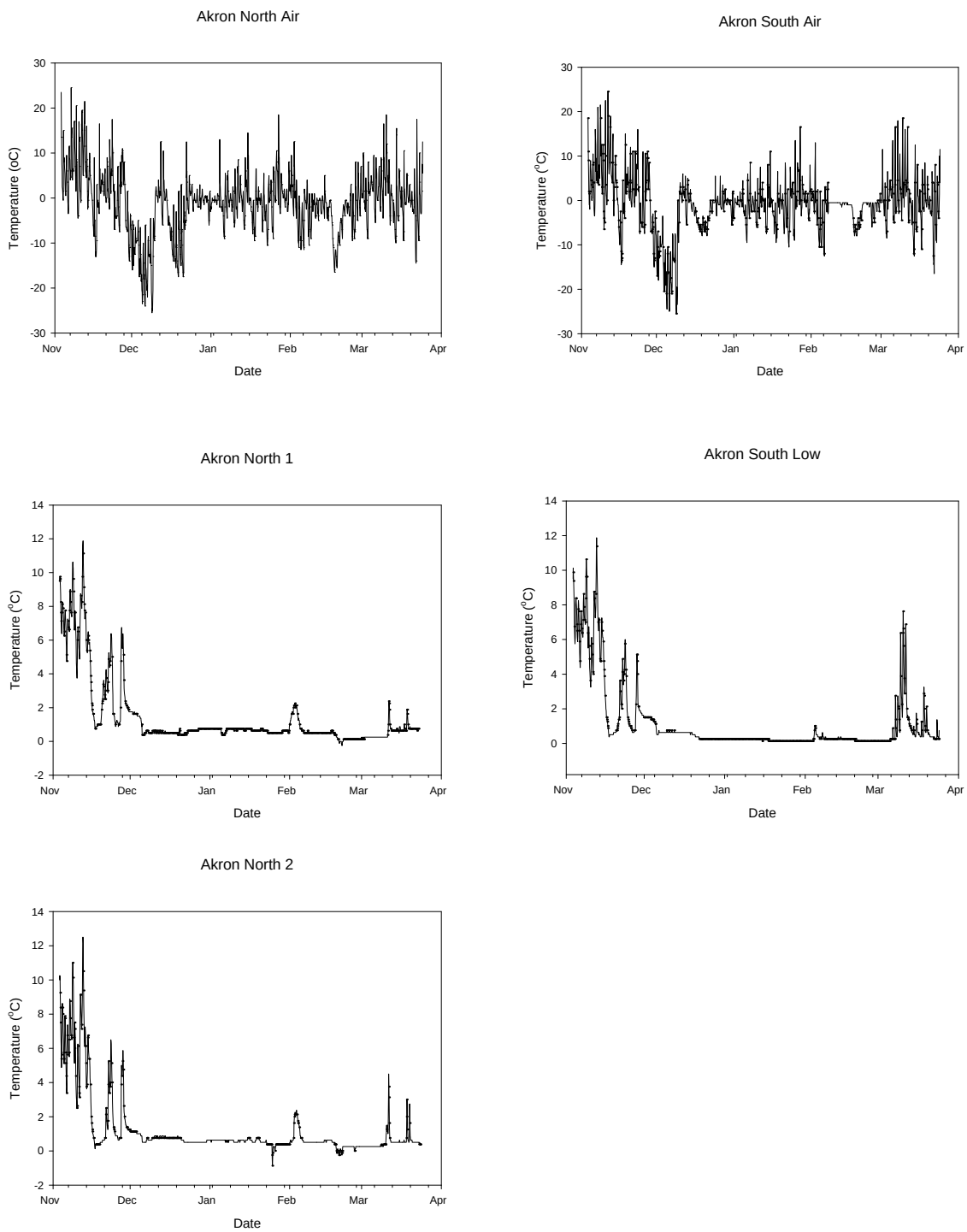

Figure 5: Air and hibernacula temperature profiles for two sites along the Big Sioux River, near Akron, Iowa. Temperatures in hibernacula rarely dipped below body fluid freezing point (-0.6°C), and some sites were probably submerged in water for a portion of the winter (flat portions of the traces. The air temperature data logger for Akron South was probably under water mid-February as well, as denoted by the flat temperature line.

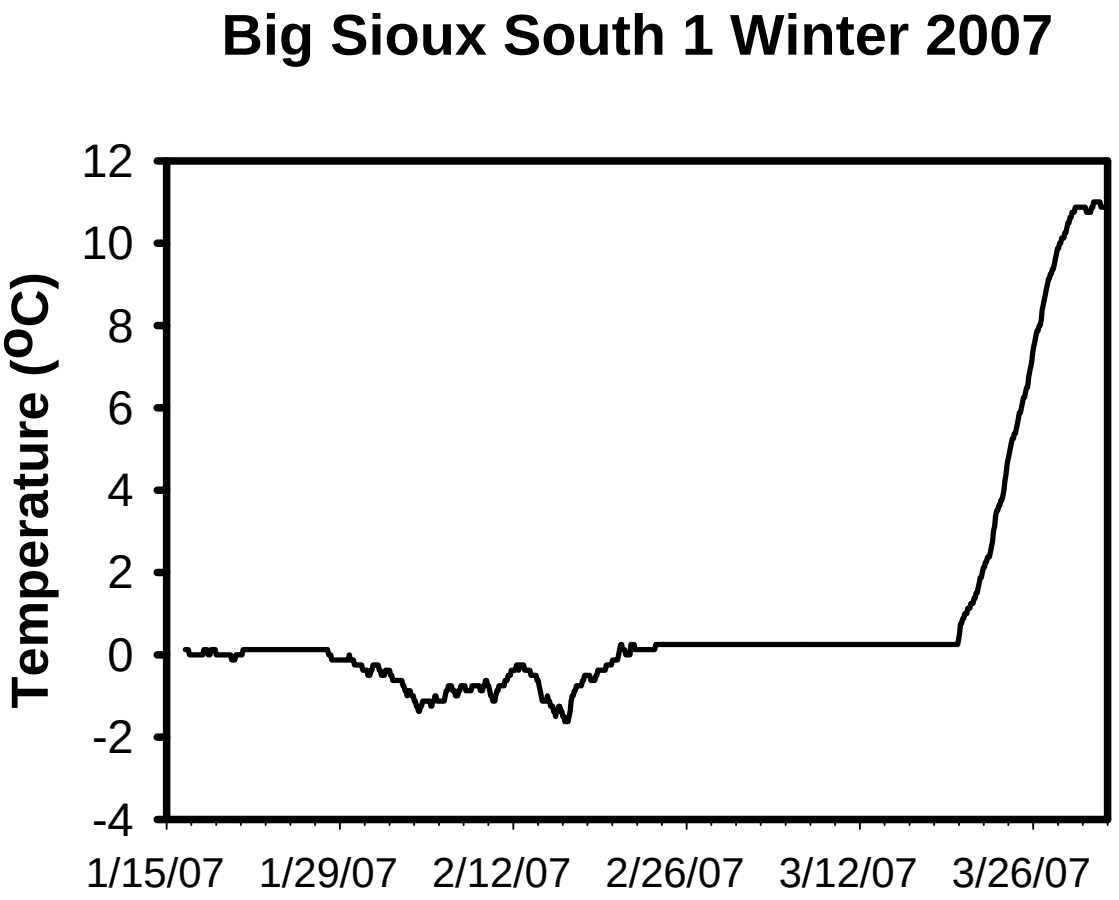

Figure 6: Hibernaculum temperature profile for a site on the Big Sioux River near Akron, Iowa, for winter 2006-2007. Temperatures fell below the body fluid freezing point for cricket frogs (-0.6°C) for two prolonged periods of 8 (3-11 February) and 3 (14-17 February) days, and reached temperatures as low as -1.65°C. The hibernaculum site was likely submerged under water for the flat portions of the trace in late January, and again for a period beginning around 26 February and continuing through the rest of the winter.