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given here seem to confirm Shine's suspicion that diets of arboreal and terrestrial snakes are similar. One can speculate on the reasons causing this apparent similarity, but our data are not yet sufficiently precise, and further ecological studies on this subject would be of great interest. As concluding remark, it would be strongly recommendable that ecologists avoid general deductions on food habits of a predator simply on the basis of its habitat: examples of arboreal snakes not preying on birds (e.g., see Shine, 1987) and terrestrial snakes occasionally eating birds (Luiselli and Anibaldi, 1991; Luiselli and Agrimi, 1991; Luiselli and Rugiero, 1991; Agrimi and Luiselli, 1992) should be kept in mind.

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Freeze tolerance is a remarkable adaptation that enhances the winter survival of some vertebrate ectotherms. Although freeze tolerance in certain anurans was reported over a decade ago, this capacity was demonstrated in reptiles only recently. Adult garter snakes (Thamnophis sirtalis) can survive the freezing of at least 36% of their body water and remain frozen for at least 48 h without injury (Costanzo et al., 1988). Hatchlings of the aquatic painted turtle (Chrysemys picta), which overwinter terrestrially within natal nests, tolerate 24 h of freezing at −4 °C (Storey et al., 1988). Although adult C. picta retain some freeze tolerance (Johnson, 1990), they most likely escape sub-zero temperatures by hibernating underwater (Ultsch, 1989).

Freeze tolerance occurs in box turtles (Terrapene spp.) that inhabit regions characterized by severely low winter temperatures (Costanzo and Clausen, 1990; Costanzo et al., 1990; Doroff and Keith, 1990). The eastern box turtle (T. carolina), in particular, hibernates in shallow burrows excavated in loose soil, where it may be exposed to subfreezing temperatures (e.g., Clausen et al., 1991). Consequently, this species is highly freeze tolerant, able to survive temperatures < −3.6 °C for at least several days and the freezing of > 58% of its body water (Costanzo and Clausen, 1990).

The physiological mechanisms conferring freeze tolerance in reptiles are poorly understood. In con-
Glucose concentrations in organs from cold-conditioned box turtles \( (T. \text{carolina}) \) sampled unfrozen or after freezing 68-88 h to a cloacal temperature of \(-3.0 \, ^\circ\text{C}\). Means (shown \( \pm 1 \text{SEM} \); \( N = 4 \) /treatment) were statistically compared using Student’s \( t \)-tests for independent samples.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Unfrozen</th>
<th>Frozen</th>
<th>Change (%)</th>
<th>( t )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>22.1 ± 4.8</td>
<td>63.4 ± 14.0</td>
<td>+166.9</td>
<td>2.8</td>
<td>0.016</td>
</tr>
<tr>
<td>Heart</td>
<td>2.1 ± 0.7</td>
<td>7.2 ± 0.7</td>
<td>+242.9</td>
<td>5.0</td>
<td>0.001</td>
</tr>
<tr>
<td>Muscle</td>
<td>4.8 ± 1.2</td>
<td>12.1 ± 1.8</td>
<td>+152.1</td>
<td>3.4</td>
<td>0.007</td>
</tr>
<tr>
<td>Eye</td>
<td>1.0 ± 0.4</td>
<td>6.0 ± 1.7</td>
<td>+500.0</td>
<td>3.0</td>
<td>0.013</td>
</tr>
<tr>
<td>Brain</td>
<td>4.7 ± 1.0</td>
<td>16.7 ± 1.4</td>
<td>+255.3</td>
<td>6.9</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Glucose concentrations were significantly higher in frozen relative to unfrozen tissues (Table 1), suggesting that in box turtles this compound may have a cryoprotective function. By far the highest concentrations occurred in the liver. Perhaps in \( T. \text{carolina} \), as in freeze tolerant anurans (Storey, 1990), the liver is the primary site of glucose production. The magnitude of the increase in tissue glucose (2.5 to 6.0-fold) was notably less in \( T. \text{carolina} \) than is typical of freeze tolerant anurans (e.g., up to 50-fold; Storey, 1990); however, because our turtles were tested following hibernation, their glucose production capacity might be considerably less than that possible during autumn and winter. Nevertheless, cold-conditioned \( T. \text{carolina} \) are highly freeze tolerant even in spring (Costanzo and Claussen, 1990); thus it appears that adequate cryoprotection is afforded by even modest glucose concentrations. This may be particularly true of turtles inhabiting mild climates. Interestingly, the glucose levels of our \( T. \text{carolina} \) strikingly resemble those measured in wood frogs collected from the same habitats in south-central Ohio (Costanzo et al., 1992).

Although \( T. \text{carolina} \) clearly mobilized glucose in our freezing tests, glucose may not be the sole (nor even primary) cryoprotectant agent used by this species. Accordingly, hatching painted turtles and garter snakes tolerate freezing but accumulate little or no glucose (Storey et al., 1988; Costanzo et al., 1988). Perhaps, then, in contrast to the case with freeze tolerant anurans, glucose is of minor importance in reptilian freeze tolerance. In considering alternative cryoprotectant systems, Storey (1990) speculated that hatching painted turtles maintain a sizable pool of free amino acids that colligatively regulates cell volume during freezing. Additionally, Costanzo and Claussen (1990) suggested that the winter increase in osmotically active blood constituents, including uric acid, which increases 3- to 5-fold (Hutton and Goodnight, 1957), might enhance freeze tolerance in \( T. \text{carolina} \). Future research should not only investigate the efficacy of glucose in diminishing freezing damage, but also provide a careful screening for additional cryoprotectants.

Our dissections of frozen \( T. \text{carolina} \) revealed many ice crystals that had accumulated dorsal and ventral to the viscera in both thoracic and abdominal cavities. The heart, eye, and brain (but not the liver or muscle)
Based on their studies with wood frogs, Lee et al. (1990; 1992) hypothesized that organ dehydration significantly during freezing (Table 2), beneficially reduces the quantity of ice forming within tissues and thereby limits mechanical damage. Furthermore, the withdrawal of water concentrates cryoprotectant within tissues.

The level of dehydration occurring in some tissues of T. carolina was generally lower than that typical of wood frogs (Costanzo et al., 1992). Also, both the liver and muscle of our turtles remained hydrated during freezing, whereas in wood frogs these organs dehydrate markedly. These findings imply that the organ dehydration response is relatively less developed in T. carolina. However, the removal of even a modest amount of water may significantly enhance organ tolerance to freezing stress in this species. Further, the considerable dehydration of both the eye and brain suggests that this process may be of particular importance in the cryoprotection of the nervous system.

Cardiovascular performance figures importantly in the physiological adaptations promoting anuran freeze tolerance (Layne et al., 1989; Lee et al., 1990). Therefore, we made continuous electrocardiogram recordings on a Grass polygraph during the freezing and thawing of three turtles, each fitted with a cloacal thermocouple and platinum subdermal electrodes on both forelimbs and one hind limb (ground). Body temperature (Tb) and heart rate (HR) were recorded at 1-h intervals during freezing to a cloacal temperature of -3 C and subsequent thawing at 5 C.

Prefreeze cooling was characterized by parallel decreases in Tb and HR. Coincident with the onset of freezing, Tb and HR stabilized at -0.5 C (the approximate melting point of plasma) and 2.0-2.5 beats min-1, respectively, owing to the release of the latent heat of crystallization. Subsequently, both parameters gradually decreased as freezing progressed (Fig. 1). Cardiac electrical activity ultimately ceased 47 ± 3.9 h after freezing commenced (Tb = -2.1 ± 0.2 C), was absent for the next 25 ± 5 h, and spontaneously resumed (Tb = -1.4 ± 0.2 C) during thawing, about 20 h before the ice had completely melted (Fig. 1). Generally, HR tracked changes in Tb during freezing and thawing.

In wood frogs cardiac electrical activity ceases only after many hours of freezing (Layne et al., 1989) and resumes early in the thaw (Layne and First, 1991). This sequence also occurred with our T. carolina. Maintenance of cardiovascular function during freezing in both species seems critical because glucose, which is mobilized only after freezing commences, must be distributed to tissues via the vasculature. In our box turtles cardiac activity ceased only after Tb had fallen to -2.1 C, a temperature associated with >40% body ice content (Costanzo and Claussen, 1990). How the heart of freeze tolerant vertebrates continues to function despite progressively increasing mechanical and osmotic stress is presently unknown. Owing to its relatively large body size, T. carolina is a useful model for investigating cardiovascular responses to freezing.

In summary, the freezing responses of T. carolina, like those of freeze tolerant anurans, included: (1) an increase in tissue glucose; (2) a dehydration of some organs and concomitant sequestration of ice in perivisceral spaces; and (3) a persistence of cardiac function during freezing, followed by its cessation at a high body ice content and an early resumption during thawing. Despite these similarities, the magnitude of both the glucose mobilization and dehydration responses was relatively less in T. carolina. Whether freeze tolerance in box turtles and other reptiles depends on additional, as yet unidentified physiological responses remains to be determined.

### Table 2. Water contents of organs from cold-conditioned box turtles (Terrapene carolina) sampled unfrozen or after freezing 68-88 h to a cloacal temperature of -3.0 C. Means (shown ± 1 SEM; N = 4/treatment) were statistically compared using square root-arcsine transformed values and Student’s t-tests for independent samples.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Water content (% of fresh mass)</th>
<th>Change (%)</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unfrozen</td>
<td>Frozen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>70.3 ± 3.2</td>
<td>70.6 ± 3.4</td>
<td>+0.4</td>
<td>0.1</td>
</tr>
<tr>
<td>Heart</td>
<td>78.7 ± 0.6</td>
<td>76.2 ± 0.5</td>
<td>-3.2</td>
<td>3.2</td>
</tr>
<tr>
<td>Muscle</td>
<td>77.9 ± 1.1</td>
<td>77.3 ± 1.1</td>
<td>-0.8</td>
<td>0.4</td>
</tr>
<tr>
<td>Eye</td>
<td>87.7 ± 0.1</td>
<td>83.9 ± 1.9</td>
<td>-4.3</td>
<td>2.0</td>
</tr>
<tr>
<td>Brain</td>
<td>83.3 ± 0.8</td>
<td>72.1 ± 3.9</td>
<td>-13.4</td>
<td>2.8</td>
</tr>
</tbody>
</table>

Fig. 1. Profile of cloacal temperature and heart rate of a 424 g box turtle (Terrapene carolina) during freezing to -3 C and subsequent thawing during exposure to 5 C. The onset of freezing was clearly marked by exothermy at time zero; thawing was induced at 62 h and, as judged from the cessation of endothermy, completed by 96 h.
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