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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Literature Cited

AGRIMI, U., AND L. LUISELLI. 1992. Feeding strategies of the viper *Vipera ursinii ursinii* (Reptilia, Viperidae) in the Apennines. Herpetol. J. 2:37–42.

ANGELICI, F. M., CAPULA, M., AND L. LUISELLI. 1993. Predation by Italian snakes upon birds: a preliminary synthesis. Atti 6th Congr. Hal. Ornitologia Torino, in press.

ARNOLD, E. N., AND J. A. BURTON. 1978. Reptiles and Amphibians of Britain and Europe. Collins, Glasgow. 244 pp.

BRUNO, S. 1990. Serpenti d'Italia e d' Europa. Editoriale Giorgio Mondadori, Milan. 224 pp.

CAPULA, M., AND L. LUISELLI. 1990. Contributo alla conoscenza della microteriofauna di un' area dell' Italia centrale (Monti della Tolfa, Lazio) mediante analisi del contenuto stomacale di *Vipera aspis* (Reptilia, Viperidae). Hystrix 2:101–107.

LUISELLI, L., AND U. AGRIMI. 1991. Composition and variation of the diet of *Vipera aspis francisciredi* in relation to age and reproductive stage. Amphibia-Reptilia 12:137–144.

———, AND C. ANIBALDI. 1991. The diet of the adder (*Vipera berus*) in two alpine environments. Amphibia-Reptilia 12:214–217.

———, AND L. RUGIERO. 1990. On habitat selection and phenology in six species of snakes in Canale Monterano (Tolfa mountains, Latium, Italy) including data on reproduction and feeding in *Vipera aspis francisciredi* (Squamata: Viperidae). Herpetozoa (Wien) 2(3/4):107–115.

———, AND ———. 1991. Food niche partitioning by water snakes (genus *Natrix*) at a freshwater environment in central Italy. J. Freshw. Ecology 6:439–444.

MONNEY, J. C. 1990. Régime alimentaire de *Vipera aspis* L. (Ophidia, Viperidae) dans les Préalpes fribourgeoises (ouest de la Suisse). Bull. Soc. Herpetol. France 53:40–49.

NAULLEAU, G. 1984. Les serpents de France. Rev. Francais. Aquar. Herpetol. 11:1–56.

———. 1987. Use of biotelemetry in the study of free-ranging snakes: example of *Elaphe longissima*. *In* J. J. Van Gelder, H. Strijbosch, and P. J. M. Bergers (eds.), Proc. Fourth Ord. Gen. Meet. Societas Europaea Herpetologica Nijmegen, pp. 289–292.

———. 1989. Etude biotélémétrique des déplacements et de la température chez la Coleuvre d'Esculape *Elaphe longissima* (Squamata, Colubridae) en zone forestière. Bull. Soc. Herpetol. France 52:45–53.

———, J. J. DUCAMP, AND A. MARIANI. 1989. Activity and thermoregulation studied by biotelemetry in *Elaphe longissima* in central west France. First World Congr. Herpetology, Canterbury (U.K.), 11–19 September 1989 (Abstract).

PIANKA, E. R. 1986. Ecology and Natural History of Desert Lizards. Princeton Univ. Press, Princeton, New Jersey. 208 pp.

SHINE, R. 1983. Arboreality in snakes: ecology of the Australian elapid genus *Hoplocephalus*. Copeia 1983: 198–205.

———. 1987. Food habits and reproductive biology of Australian snakes of the genus *Hemiaspis* (Elapidae). J. Herpetol. 21:71–74.

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Physiological Responses to Freezing in the Turtle *Terrapene carolina*

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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 subzero 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 Claussen, 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., Claussen 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 Claussen, 1990).

The physiological mechanisms conferring freeze tolerance in reptiles are poorly understood. In con-

TABLE 1. Glucose concentrations in 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 Student's *t*-tests for independent samples.

Tissue	Glucose concentration (mM/L)		Change (%)	t	*P*
	Unfrozen	Frozen			
Liver	22.1 ± 4.8	63.4 ± 14.0	+186.9	2.8	0.016
Heart	2.1 ± 0.7	7.2 ± 0.7	+242.9	5.0	0.001
Muscle	4.8 ± 1.2	12.1 ± 1.8	+152.1	3.4	0.007
Eye	1.0 ± 0.4	6.0 ± 1.7	+500.0	3.0	0.013
Brain	4.7 ± 1.0	16.7 ± 1.4	+255.3	6.9	0.001

trast, research on the wood frog (*Rana sylvatica*) has elucidated two primary adaptations that promote freeze tolerance. First, freezing initiates a rapid mobilization of glucose from liver glycogen which, following its distribution via the vasculature, concentrates in tissues (Storey, 1990). Glucose functions as a cryoprotectant by inhibiting freezing damage to cells (Costanzo and Lee, 1991) and tissues (Canty et al., 1986; Costanzo et al., 1991). Secondly, some water from visceral and peripheral organs is redistributed during freezing, ultimately accumulating as ice in coelomic and subdermal spaces. Organ dehydration reduces the amount of ice forming within tissues and associated freezing injury, particularly that caused by mechanical stress (Lee et al., 1990, 1992). A marked persistence of cardiovascular function during freezing promotes glucose distribution and organ dehydration (Layne et al., 1989; Costanzo et al., 1992). We undertook this investigation to determine whether similar adaptive responses to freezing also occur in eastern box turtles.

Specimens of *T. carolina* (250–430 g) were collected from Scioto County, southern Ohio, during early spring 1990 and 1991. Turtles were cold-conditioned as described by Costanzo and Claussen (1990). Briefly, this involved housing them on damp moss and exposing them for several weeks to 5 C in a darkened room. Water was provided ad libitum, but food was withheld.

Four turtles were individually fitted with cloacal thermocouples, placed in an insulated jar, and cooled in an ethanol bath. Subsequent to the onset of freezing (which was clearly indicated by the abrupt cessation of cooling; cf. Costanzo and Claussen, 1990), the turtles required from 68–88 h to reach a cloacal temperature of −3.0 C ± 0.3 C (mean ± SEM). Frozen turtles were removed from the bath and immediately decapitated and dissected by severing the tissue between plastron and carapace. Their intact hearts, eyes, and brains, and portions (ca. 150 mg) of liver and skeletal (forelimb) muscle were excised, blotted, and weighed to 0.1 mg. Performed by a team of two investigators, all tissue harvesting and handling was completed within 2–3 min. Half of each sample (or one intact eye) was immediately prepared as described by Costanzo et al. (1992) for use in colorimetric glucose determinations (Sigma, no. 510), whereas the remainder was oven dried (65 C) to determine water content. For comparative purposes, additional data were obtained from four (unfrozen) specimens taken directly from their cages at 4 C. These turtles, unlike the ones used in freezing tests, were lightly anesthetized (10 mg/kg ketamine HCl) to facilitate their decapitation. However, judging from the drug's action and short induction time, it seems unlikely that this treatment would markedly alter the parameters under investigation.

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. 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. 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. 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. carolina* strikingly resemble those measured in wood frogs collected from the same habitats in south-central Ohio (Costanzo et al., 1992).

Although *T. carolina* clearly mobilized glucose in our freezing tests, glucose may not be the sole (nor even primary) cryoprotectant agent used by this species. Accordingly, hatchling 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 hatchling 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. 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. 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)

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.

Tissue	Water content (% of fresh mass)		Change (%)	t	*P*
	Unfrozen	Frozen			
Liver	70.3 ± 3.2	70.6 ± 3.4	+0.4	0.1	0.476
Heart	78.7 ± 0.6	76.2 ± 0.5	−3.2	3.2	0.010
Muscle	77.9 ± 1.1	77.3 ± 1.1	−0.8	0.4	0.359
Eye	87.7 ± 0.1	83.9 ± 1.9	−4.3	2.0	0.047
Brain	83.3 ± 0.8	72.1 ± 3.9	−13.4	2.8	0.016

dehydrated significantly during freezing (Table 2), indicating the probable origin of some of this water. Based on their studies with wood frogs, Lee et al. (1990; 1992) hypothesized that organ dehydration 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 (T_b) 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 T_b and HR. Coincident with the onset of freezing, T_b 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 (T_b = −2.1 ± 0.2 C), was absent for the next 25 ± 5 h, and spontaneously resumed (T_b = −1.4 ± 0.2 C) during thawing, about 20 h before the ice had completely melted (Fig. 1). Generally, HR tracked changes in T_b 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 T_b 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.

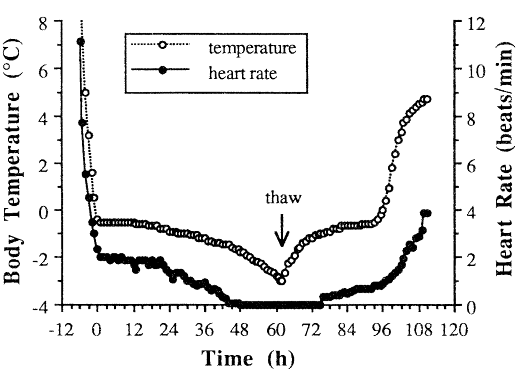

FIG. 1. Profile of cloacal temperature and heart rate of a 424 g box turtle (*Terrapene carolina*) during freezing to −3 C and subsequent thawing during exposure to 5 C. The onset of freezing was clearly marked by exothermy at time zero; thawing was induced at 62 h and, as judged from the cessation of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

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Literature Cited

CANTY, A., W. R. DRIEDZIC, AND K. B. STOREY. 1986. Freeze tolerance of isolated ventricle strips of the wood frog, *Rana sylvatica*. Cryo-Lett 7:81–86.

COSTANZO, J. P., D. L. CLAUSSEN, AND R. E. LEE. 1988. Natural freeze tolerance in a reptile. Cryo-Lett. 9:380–385.

———, AND ———. 1990. Natural freeze tolerance in the terrestrial turtle, *Terrapene carolina*. J. Exp. Zool. 254:228–232.

———, M. F. WRIGHT, AND R. E. LEE. 1990. Freeze tolerance and intolerance in hatchling turtles. Cryobiol. 27:678.

———, AND R. E. LEE. 1991. Freeze-thaw injury in erythrocytes of the freeze-tolerant wood frog, *Rana sylvatica*. Amer. J. Physiol. 261:R1346–1350.

———, ———, AND M. F. WRIGHT. 1991. Glucose loading prevents freezing injury in rapidly-cooled wood frogs. Amer. J. Physiol. 261:R1549–1553.

———, ———, AND ———. 1992. Cooling rate influences cryoprotectant distribution and organ dehydration in freezing wood frogs. J. Exp. Zool. 261:373–378.

CLAUSSEN, D. L., P. M. DANIEL, S. JIANG, AND N. A. ADAMS. 1991. Hibernation in the eastern box turtle, *Terrapene c. carolina*. J. Herpetol. 25:334–341.

DOROFF, A. M., AND L. B. KEITH. 1990. Demography and ecology of an ornate box turtle (*Terrapene ornata*) population in south-central Wisconsin. Copeia 1990:387–399.

HUTTON, K. E., AND C. J. GOODNIGHT. 1957. Variations in the blood chemistry of turtles under active and hibernating conditions. Physiol. Zool. 30:198–207.

JOHNSON, B. B. 1990. Freeze tolerance in the adult painted turtle, *Chrysemys picta*, and the effects of freezing on the cardiac cycle. Unpubl. MS thesis, Miami Univ., Oxford, Ohio. 57 pp.

LAYNE, J. R., R. E. LEE, AND T. L. HEIL. 1989. Freezing-induced changes in the heart rate of wood frogs (*Rana sylvatica*). Amer. J. Physiol. 257:R1046–R1049.

———, AND M. C. FIRST. 1991. Resumption of physiological functions in the wood frog (*Rana sylvatica*) after freezing. Amer. J. Physiol. 261:R134–R137.

LEE, R. E., JR., J. R. LAYNE, JR., J. P. COSTANZO, AND E. C. DAVIDSON. 1990. Systemic and organismal responses to freezing in vertebrates. Cryobiol. 27: 643–644.

———, J. P. COSTANZO, E. C. DAVIDSON, AND J. R. LAYNE. 1992. Dynamics of body water during freezing and thawing in a freeze-tolerant frog (*Rana sylvatica*). J. Therm. Biol. (in press).

STOREY, K. B., J. M. STOREY, S. P. J. BROOKS, T. A. CHURCHILL, AND R. J. BROOKS. 1988. Hatchling turtles survive freezing under winter hibernation. Proc. Natl. Acad. Sci. USA 85:8350–8354.

———. 1990. Life in a frozen state: adaptive strategies for natural freeze tolerance in amphibians and reptiles. Amer. J. Physiol. 258:R559–R568.

ULTSCH, G. R. 1989. Ecology and physiology of hibernation and overwintering among freshwater fishes, turtles, and snakes. Biol. Rev. 64:435–516.

Accepted 6 November 1992.

Literature Cited

Hibernation in the Eastern Box Turtle, Terrapene c. carolina
Dennis L. Claussen; Paul M. Daniel; Suping Jiang; Nadine A. Adams
Journal of Herpetology, Vol. 25, No. 3. (Sep., 1991), pp. 334-341.
Stable URL:
http://links.jstor.org/sici?sici=0022-1511%28199109%2925%3A3%3C334%3AHITEBT%3E2.0.CO%3B2-5

Demography and Ecology of an Ornate Box Turtle (Terrapene ornata) Population in South-Central Wisconsin
Angela M. Doroff; Lloyd B. Keith
Copeia, Vol. 1990, No. 2. (Jun. 27, 1990), pp. 387-399.
Stable URL:
http://links.jstor.org/sici?sici=0045-8511%2819900627%293%3A1990%3A2%3C387%3ADAEOAO%3E2.0.CO%3B2-C

Hatchling Turtles Survive Freezing during Winter Hibernation
Kenneth B. Storey; Janet M. Storey; Stephen P. J. Brooks; Thomas A. Churchill; Ronald J. Brooks
Proceedings of the National Academy of Sciences of the United States of America, Vol. 85, No. 21. (Nov. 1, 1988), pp. 8350-8354.
Stable URL:
http://links.jstor.org/sici?sici=0027-8424%2819881101%2985%3A21%3C8350%3AHTSFDW%3E2.0.CO%3B2-I