Environmental Physiology of Terrestrial Hibernation in Hatchling Turtles

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Abstract. Hatchling turtles hibernate either under water or on land. In northern regions, those hibernating within the frost zone must cope with desiccating conditions and extreme cold. Terrestrial hibernators exhibit several morphological, physiological, and behavioral adaptations to conserve water and to tolerate or avoid freezing. Here we review the environments inhabited by hatchling turtles and the strategies they use to overcome the challenges of life in the cold.

Hibernation Habitats and Winter Microenvironment

In late spring and early summer, female turtles deposit their eggs in shallow nests, excavated in suitably friable soils. The embryos complete development in 60 to 90 days and hatching occurs in late summer. Before the advent of winter, the hatchlings of many aquatic species emerge from their nests and, like adults, hibernate in thermally buffered aquatic habitats (Ultsch, 1989). Terrestrial hibernation, however, may enable hatchlings to reduce their risk of predation at a time when resources necessary for rapid growth are in decline (Wilbur, 1975). Field and laboratory studies have provided support for the model of Gibbons and Nelson (1978), which predicts that species that emerge in the fall forgo the benefits of terrestrial hibernation because they are unable to survive harsh winter conditions in the nest (Obbard and Brooks, 1981; Packard et al., 1993; 2000; Costanzo et al., 1995, 2000b, 2001b; Sims et al., 2001). Nevertheless, the offspring of a few northern turtles hibernate on land, either inside the natal nest or in the soil column below (Costanzo et al., 1995). Much of the research on the subject of hibernation in hatchling turtles has focused on species that

hibernate inside the natal nest, since these species presumably encounter the most challenging of environmental conditions (but see D. Jackson's chapter in this volume). Variation in the hydraulic and thermal regimes among nests during embryonic development has long been known to influence the gender (Vogt and Bull, 1984) and size (Packard and Packard, 1988) of the resulting hatchlings. However, recent study (Costanzo et al., 2004) has shown that microenvironmental conditions within hibernacula at the same locale also vary considerably. Thus, nest-site selection by mother turtles may be an important determinant of winter survival (Tucker and Pauktis, 1999). Hatchlings that overwinter within the nest chamber require adaptations to limit water loss and, in some cases, to tolerate exposure to subfreezing temperatures (Costanzo et al., 2001b).

**Water Conservation**

Desiccation may adversely impact terrestrial hibernators because precipitation commonly falls as snow and because the water potential of the frozen soil matrix is low (Costanzo et al., 2001b). In addition, hatchlings are particularly prone to evaporative water loss because their surface area is large relative to their body mass (Mautz, 1982; Nagy et al., 1997). Morphological and physiological mechanisms of water conservation therefore may be critical to winter survival.

Low ambient temperatures and a reduction in metabolic rate may serve to reduce water loss in hibernating turtles (Gregory, 1982). Most terrestrial hibernators are highly resistant to dehydration, whereas species that typically hibernate in aquatic habitats lose body water via evaporation from body surfaces relatively easily when held in conditions of low relative humidity (Costanzo et al., 2001b). Although hatchlings apparently are unable to absorb moisture from their surroundings (Costanzo et al., 2001b), desiccation-resistant species conserve water by retaining urine (Costanzo et al., 2000b) and may offset any water loss (and perhaps even gain water) through oxidative metabolism of lipids and glycogenolysis (Wilson et al., 2001; Costanzo et al., 2004). Nevertheless, terrestrially hibernating hatchlings may dehydrate significantly during exceptionally dry winters (Costanzo et al., 2004). The relative importance of desiccation as a winter mortality factor, however, has not been determined.

**Cold Hardiness: Freeze Tolerance and Supercooling**

**Freeze Tolerance**

Extreme cold is another challenge confronting some terrestrial hibernators, as hibernaculum temperatures may occasionally fall below the equilibrium freeze-
ing point of their body fluids, ca. −0.6°C (Costanzo et al., 1995; DePari, 1996; Packard et al., 1997; Nagle et al., 2000). One strategy to cope with extreme cold is freeze tolerance (i.e., survival of somatic freezing). Some turtles are well adapted to survive somatic freezing and can tolerate the freezing of more than half of their body water (Churchill and Storey, 1992a). To date, freeze tolerance has been demonstrated in hatchling *Chrysemys picta* (Storey et al., 1988), *Emydoidea blandingii* (Packard et al., 1999), *Malaclemys terrapin* (Baker et al., manuscript in preparation), *Terrapene ornata* (Costanzo et al., 1995), and *Trachemys scripta* (Churchill and Storey, 1992b), as well as adult *C. picta* (Claussen and Kim, 1993), *Terrapene ornata* (Costanzo et al., 1995) and *Terrapene carolina* (Costanzo and Claussen, 1990). These findings support Ultsch's (1989) hypothesis that freeze tolerance may be common among the Emydidae, a family of mostly North American pond turtles, although recent work suggests that one emydid, the northern map turtle (*Graptemys geographica*), appears to be freeze intolerant (Baker et al., 2003). Freeze tolerance has been examined in taxa within other families (i.e., Kinosternidae, Trionychidae, Chelydridae; Costanzo et al., 1995), but only hatchling *Chelydra serpentina* (Chelydridae) tolerate even modest freezing (Packard et al., 1993; Costanzo et al., 1995). Collectively, these findings cast doubt on the tenuous assertion that freeze tolerance is a common attribute among hatchlings of all species of turtles (Packard and Packard, 2001).

Turtles capable of surviving periods of somatic freezing must be specially adapted to cope with osmotic and ionotropic perturbations, ischemic anoxia, and metabolite end-product accumulation, which can contribute to freezing-related injury (Storey et al., 1988). The rapid accumulation of lactate in the brains of *C. picta* and *T. scripta* suggests that freezing induces acute anoxic stress (Hemmings and Storey, 2000) and therefore anoxia tolerance may be fundamental to freeze tolerance (Greenway and Storey 1999). There are limitations to this strategy since hatchlings of freeze-tolerant species tolerate somatic freezing only so long as their body temperature remains above ca. −4°C (Storey et al., 1988; Costanzo et al., 1995; Packard et al., 1999).

**Freeze Avoidance by Supercooling**

Another survival strategy used by hatchling turtles is freeze avoidance by sustaining a state of supercooling. Lacking freeze tolerance, hatchlings that routinely encounter subzero temperatures must rely on freeze avoidance. In order to supercool extensively, hatchlings must eliminate endogenous and ingested
ice-nucleating agents (INAs) that would otherwise seed the freezing of body fluids. Purging of INAs occurs during acclimatization to winter conditions (Costanzo et al., 2003) and is critical to the seasonal development of cold hardiness (Costanzo et al., 2000b). Generally, hatchlings can supercool extensively (from −8º to −20º C), owing in part to their small size (Costanzo et al., 2001b), if they manage to remain free of INAs.

A survival strategy based exclusively on freeze avoidance also has limitations. Since supercooled fluids are metastable, ice nucleation in turtle tissues can occur spontaneously or may be triggered by contact with ice and INAs in the environment (Costanzo et al., 2000a). It has long been presumed that ectotherms can recover from supercooling to low temperatures without injury (e.g., Spellerberg, 1972; Costanzo and Lee, 1995). However, recent studies (Packard and Packard, 1999; Hartley et al., 2000; Costanzo et al., 2001a) have shown that prolonged supercooling or chilling to very low temperatures causes physiological stress or even mortality. Owing to diminished tissue perfusion, survival in extreme cold probably requires a capacity for sustained anaerobic respiration as well as a tolerance for the decrease in pH associated with lactate accumulation (Hartley et al., 2000; Costanzo et al., 2001a; Baker et al., 2003). Anoxia-intolerant species may be constrained in their use of supercooling as a winter survival strategy, but this hypothesis has not been tested empirically.

Inoculation Resistance
Supercooling is a viable strategy of winter survival only if hatchling turtles are able to remain unfrozen during subzero chilling episodes (Lee and Costanzo, 1998). This is a particularly significant challenge confronting terrestrially hibernating turtles because the soil surrounding them teems with various INAs, such as soil particles, dust, and ice-nucleating microorganisms, which can inhibit supercooling (Costanzo et al., 2000a, 2001c, 2004; Baker et al., 2003). In addition, contact with ice in the winter microenvironment can readily trigger the freezing of supercooled turtles (Packard and Packard, 1993a; Costanzo et al., 1998).

Several studies (Costanzo et al., 1998, 2001c, 2004; Baker et al., 2003) have shown a strong association between soil characteristics and susceptibility to inoculative freezing (Fig. 1). Soil moisture is an important factor because it influences the probability that a hatchling will come into contact with ice in the soil matrix. For the same reason, susceptibility to inoculative freezing is influenced by characteristics of the soil such as particle size, porosity, and adsorbive capacity (Costanzo et al., 1998, 2001c). Intuitively, experimental tests of a species'
Fig. 1. Effect of soil characteristics on inoculation resistance, as indicated by the temperature of crystallization ($T_c$) of hatchling painted turtles (Chrysemys picta) cooled in a matrix of frozen soil. Response indicates the effect of variable moisture content in native loamy sand and the addition of 10% (w/w) clay or peat to native soil (inset; mean values identified by different letters were statistically distinguishable, $P < 0.05$). Adapted from Costanzo et al., 1998.
capacity for inoculation resistance should use native substrata and ecologically appropriate moisture levels; not doing so (e.g., Packard and Packard 1993a, 1993b, 1995) has led to some erroneous conclusions.

Capacity to resist inoculation by external INAs does not vary appreciably among populations of the same species (Costanzo et al., 2001c), but it is considerably greater in species that hibernate terrestrially than in aquatic hibernators (Costanzo et al., 2001b). Taxonomic variation in this trait may reflect differences in the amount of skin exposed to the environment (Costanzo et al., 2001b) as well as differences in the ultrastructure of the integument (Willard et al., 2000). For example, superior inoculation resistance in hatchlings of the painted turtle (*Chrysemys picta*) has been attributed to the presence of large deposits of unsaturated lipids in the dermal and epidermal layers of skin that is chronically exposed to the environment (Willard et al., 2000). Hatchling *G. geographica* also resist inoculation at low temperatures (Baker et al., 2003), but whether this

![Figure 2](image_url)

*Fig. 2. Relationship between susceptibility to ice inoculation, as indicated by the temperature of crystallization (T), of hatchling turtles (n = 4–9) cooled in a matrix of frozen soil, and the rate of evaporative water loss (EWL; n = 3–10). Means are shown ± 1 SEM. Adapted from Costanzo et al., 2001b.*
species also possesses an abundance of integumental lipids remains to be determined. Generally, species adept at resisting inoculative freezing also tend to have low rates of evaporative water loss, perhaps because a similar morphological attribute governs both properties (Fig. 2). This association attests that the integument plays a major role in adaptation to terrestrial hibernation (Costanzo et al., 2001b).

Conclusions

Despite the length and severity of winter in temperate North America, this region is populated by a diverse assemblage of semiaquatic and aquatic turtles. The hatchlings of a few species overwinter terrestrially and may experience desiccating and subfreezing temperatures. The survival of these turtles on both local and regional scales may be influenced primarily by nest temperature, precipitation, and the physical and hydric characteristics of nest soil that influence freezing risk (Costanzo et al., 2001c, 2004). At least one species, the painted turtle (C. picta), reportedly exhibits both freeze tolerance and a well-developed capacity for supercooling and may utilize either strategy, depending on physiological and environmental conditions.

References


