The Overwintering Strategy of Hatchling Painted Turtles, or How to Survive in the Cold without Freezing

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Painted turtles (*Chrysemys picta*) are common residents of shallow lakes and marshes across much of North America east of the Rocky Mountains—so common, in fact, that the species has become a “model organism” for studies on the ecology and evolution of chelonians (Wilbur and Morin 1988). The natural history of painted turtles differs from that of most other species in an important respect, however. Whereas neonates of other freshwater turtles usually emerge from their subterranean nest in late summer or autumn and move to a nearby marsh, lake, or stream to spend their first winter, hatchling painted turtles typically remain inside their shallow (8–14 cm) nest throughout their first winter and do not emerge above the ground until the following spring (Ernst et al. 1994). This behavior commonly causes neonatal painted turtles in northern regions—from Nebraska (Packard 1997, Packard et al. 1997a), northern Illinois (Weisrock and Janzen 1999), and New Jersey (DePari 1996) northward to the limit of distribution in southern Canada (Storey et al. 1988)—to be exposed during winter to ice and cold, with temperatures in some nests dipping below –10°C (Figure 1). Many of these hatchlings withstand such extremes and emerge from the nest when the soil finally thaws in the spring (Table 1).

Tolerance for cold

How do neonatal painted turtles survive in the cold? Early research on this subject indicated that hatchlings spend part or all of the winter in a frozen state, with up to 50% of their body water converted to ice in the extracellular space (Storey et al. 1988, Churchill and Storey 1992). The initial findings were persuasive (Storey 1990, Storey and Storey 1992): We are among those researchers who once subscribed to the notion that turtles survive winters in the field by exploiting a tolerance for freezing (Packard and Packard 1990). Work performed more recently, however, indicates that the correlation between overwintering by hatchlings in their nest and the indisputable ability of the animals to withstand freezing (Rubinsky et al. 1994) was spurious. Three sets of findings have led to the current view that tolerance for freezing is not a general means by which hatchling painted turtles withstand exposure to low temperatures in the field.

NEONATAL TURTLES, LIKE MANY COLD-TOLERANT INSECTS, EXPLOIT A CAPACITY FOR SUPERCOOLING TO WITHSTAND EXPOSURE TO SUBZERO TEMPERATURES

First, a problem that was apparent with the aforementioned theory almost from the outset concerns the limited capacity demonstrated by neonatal painted turtles in the laboratory to recover from freezing (Churchill and Storey 1992). Hatchlings frozen at high subzero temperatures clearly can withstand exposure to –2°C for a week or more (Churchill and Storey 1992, Packard et al. 1999b). Viability declines rapidly, however, when frozen animals are exposed to –3°C (Packard et al. 1999b), and even a brief exposure to –4°C is lethal (Churchill and Storey 1992, Costanzo et al. 1995, Packard et al. 1999b). Yet temperatures in natural nests commonly fall below –4°C (Storey et al. 1988, DePari 1996, Packard 1997, Packard et al. 1997a, Weisrock and Janzen 1999), often for extended periods, and many turtles survive (Table 1). The presumption is that animals that survived exposure in natural nests to temperatures below –4°C could not have been frozen.

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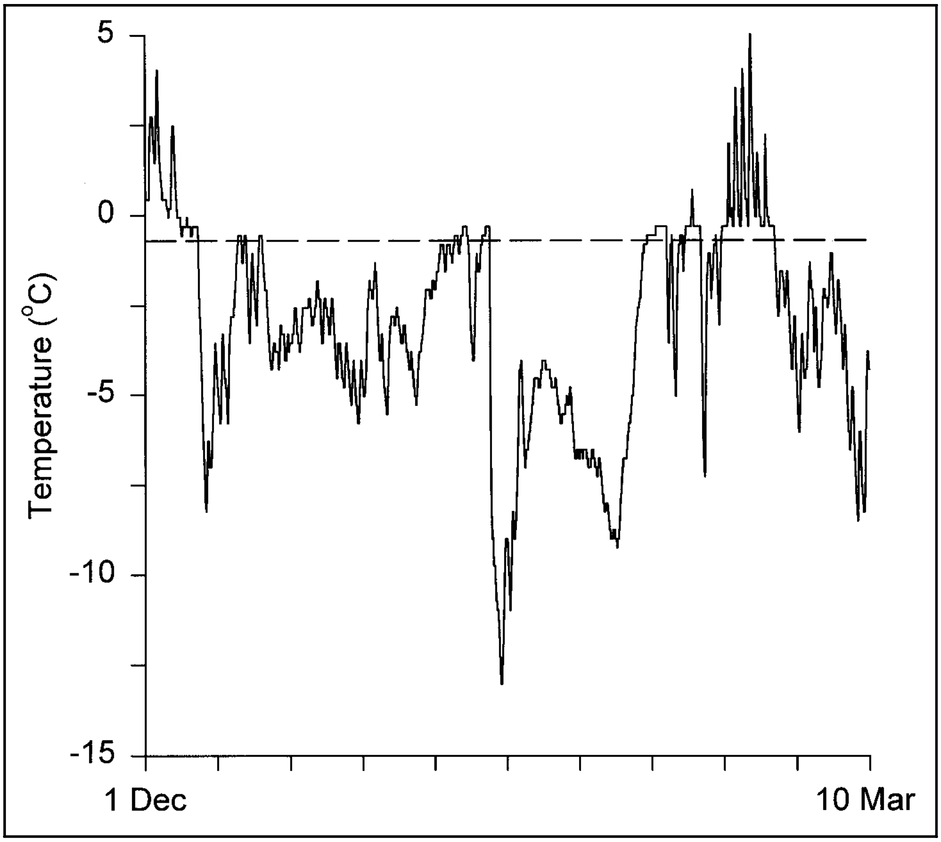

Figure 1. A small data logger was used to measure the temperature inside a nest that contained eight hatchling painted turtles, all of which were alive at the time they were placed into the chamber in late October. Measurements are shown for 100 days, beginning 1 December 1995. Seven of the turtles were alive when the nest was reopened in March 1996. The dashed line identifies the equilibrium freezing point for body fluids of neonatal animals. From Packard et al. 1997a.

Second, hatchlings of several other chelonians also express some limited tolerance for freezing at high subzero temperatures, although the neonates of these other species typically do not encounter life-threatening conditions of ice and cold during winter. For example, hatchling Blanding's turtles (*Emydoidea blandingi*), map turtles (*Graptemys geographica*), and snapping turtles (*Chelydra serpentina*) usually avoid exposure to ice and cold by taking refuge in the unfrozen depths of a marsh, lake, or stream (Ernst et al. 1994), and neonatal box turtles (*Terrapene ornata*) dig deep into the soil and stay below the frost line (Costanzo et al. 1995). Similarly, hatchling slider turtles (*Trachemys scripta*) are seldom exposed to ice and cold because of the species' southerly distribution (Packard et al. 1997c, Tucker and Packard 1998). All these animals withstand "shallow freezing" (Costanzo et al. 1995, Packard et al. 1999b), so a limited tolerance for freezing may be a feature common to hatchling turtles generally. Such tolerance probably reflects nothing more than a widespread ability for cells in these animals to withstand the loss of small quantities of water to ice forming in the extracellular space and for these same cells to deal with the anaerobic state that accompanies a shutdown of the circulation (Storey and Storey 1992). Tolerance for freezing probably has little (if any) significance with regard to adaptation to cold (Packard et al. 1993, 1997c, 2000, Tucker and Packard 1998).

Third, freezing of hatchling painted turtles under ecologically relevant conditions in the laboratory usually results in their death, even in those cases in which the temperature of the frozen animals never goes below –4°C (Packard et al. 1997b, 1999a). On the rare occasion when a hatchling has survived such freezing at a high subzero temperature, the turtle began to freeze late in its exposure to cold and did not remain frozen for more than a few hours (Packard et al. 1999a). Longer exposure probably would have resulted in death of the animal (Packard et al. 1999b). An exception to this generalization may occur when the temperature of frozen animals never goes below –2°C (Willard et al. 2000), but the ecological relevance of such a situation then becomes an issue (Table 1; Packard et al. 1999a).

Why does water freeze?

The most likely explanation for the survival of neonatal painted turtles exposed to the rigors of winter at high latitudes is that they remain unfrozen, and they do so without the benefit of an antifreeze. The key to understanding how this feat might be accomplished is in understanding why it is that aqueous solutions freeze in the first place.

For water to change its phase from liquid to solid (or crystalline state), two conditions must be satisfied simultaneously (Franks 1985). Both are necessary, and neither is sufficient by itself. First, the temperature of the solution must go below the equilibrium freezing point (which is defined as the temperature at which liquid water and solid water can coexist indefinitely), so that ice is the favored, thermodynamically stable phase (Sweeney and Beuchat 1993). And second, a suitable organizing site must be present, that is, a site where molecules of liquid can condense to form incipient crystals of ice. If a suitable organizing site is not present, water will not freeze no matter how low the temperature goes.

Three kinds of organizing sites, each characterizing a distinct kind of freezing, are commonly recognized. First, water molecules may aggregate spontaneously to form suitable organizing sites, thereby leading to what is known as homogeneous nucleation (Franks 1985). Such an aggregation of

Table 1. Number of hatchling painted turtles surviving the winter of 1995–1996 in nests located on the Valentine National Wildlife Refuge in north-central Nebraska.

Minimum temperature (°C)	Longest interval (hours) with temperature continuously below –4°C	Survivors
–3.0	0	8
–3.0	0	8
–4.0	0	8
–4.7	7	8
–5.5	61	8
–5.7	21	8
–6.5	178	8
–7.2	67	5
–7.9	168	8
–8.7	75	7
–9.7	99	5
–10.5	182	3
–12.0	195	0
–12.5	51	0
–12.7	279	7
–14.3	295	0
–20.3	395	0
–21.0	377	0

Eight live hatchlings were placed into each nest in late October; animals were recovered the following March (Packard et al. 1997a).

water molecules can form at any temperature below the equilibrium freezing point for the solution in question. However, in an animal with as little body water as a 5 g painted turtle, these aggregates seldom become large enough, or persist long enough, at temperatures above –20°C for the freezing process to proceed (Franks 1985). Temperatures in nests with overwintering painted turtles usually remain above –20°C (DePari 1996, Packard 1997, Packard et al. 1997a, Weisrock and Janzen 1999), so hatchlings apparently are not at great risk of freezing by homogeneous nucleation. Thus, we can exclude homogeneous nucleation from the current discussion.

Second, ice may begin to form when molecules of liquid condense on appropriately configured surfaces of certain impurities suspended in the solution (e.g., dust particles or microbes). This process, which is termed *heterogeneous nucleation* (Franks 1985), is the common cause of spontaneous freezing of aqueous solutions at temperatures above –20°C. This process is potentially of great importance to overwintering painted turtles. Each kind of impurity, or mote, has a limited range of temperatures over which it is capable of acting as an organizing site (Dorsey 1948). If the effective range of temperatures for the mote in question is below the equilibrium freezing point for the solution, the solution will become supercooled, that is, the solution will remain unfrozen at temperatures below the equilibrium freezing point but above the effective temperature for the mote. The liquid is said to be in a metastable state, but this state should not be taken to mean that the solution is unstable (Dorsey 1948).

Finally, preexisting crystals of ice may provide suitable organizing sites for liquid water to condense (Franks 1985), so seeding a solution with ice may cause water to freeze. Water in most soils freezes at temperatures near the equilibrium freezing point (Williams 1967), which usually varies between –0.1°C and –0.5°C, depending on particle size and water content of the medium (Bodman and Day 1943). The equilibrium freezing point for body fluids of hatchling painted turtles is approximately –0.7°C (Storey et al. 1991, Packard and Packard 1995, Costanzo et al. 2000b), so animals in the wild actually come into contact with ice in frozen soil before they themselves can freeze. When the temperature of the frozen soil goes below the equilibrium freezing point for body fluids, however, the turtles are immediately at risk of being caused to freeze by the crystals of ice contacting their body surfaces (Layne 1991, Costanzo et al. 1999). Animals that are caused to freeze in this way are said to have been inoculated.

Freezing by heterogeneous nucleation

Why don't neonatal painted turtles freeze by heterogeneous nucleation when the temperature in their nests goes below –0.7°C? The simple answer is that body fluids of the animals do not contain motes that are capable of eliciting freezing at temperatures commonly encountered in the field (Packard and Packard 1997, 1999, Costanzo et al. 1998, 2000a, Hartley et al. 2000). This point can be illustrated with a temperature profile for an animal that was acclimated to 2°C and then exposed to declining temperatures (Figure 2). The turtle was in a dry, ice-free environment, so it was not at risk of freezing by inoculation. Water in the turtle did not begin to freeze until the temperature had declined to a limit of supercooling at –18.2°C, as evidenced by the spike in temperature (the "exotherm") resulting from the release of latent heat of fusion as water changed phase from liquid to solid in the body of the hatchling (Claussen and Costanzo 1990). Such a temperature is considerably lower than those that turtles usually encounter in nests in the field (Storey et al. 1988, DePari 1996, Packard 1997, Packard et al. 1997a, Weisrock and Janzen 1999), so overwintering hatchlings are not at appreciable risk of freezing by heterogeneous nucleation.

Freezing by inoculation

Why don't turtles freeze by inoculation when they make contact with ice at temperatures below the equilibrium freezing point for their body fluids? The answer is that exposed skin on the head and the limbs of hatchling painted turtles resists the inward penetration of ice from surrounding soil (Packard et al. 1997b, 1999a). This point can be illustrated with a temperature profile for an animal that was placed in an artificial nest constructed in a jar of damp, loamy sand (Figure 3a). Water in the sand was caused to freeze at approximately –0.5°C

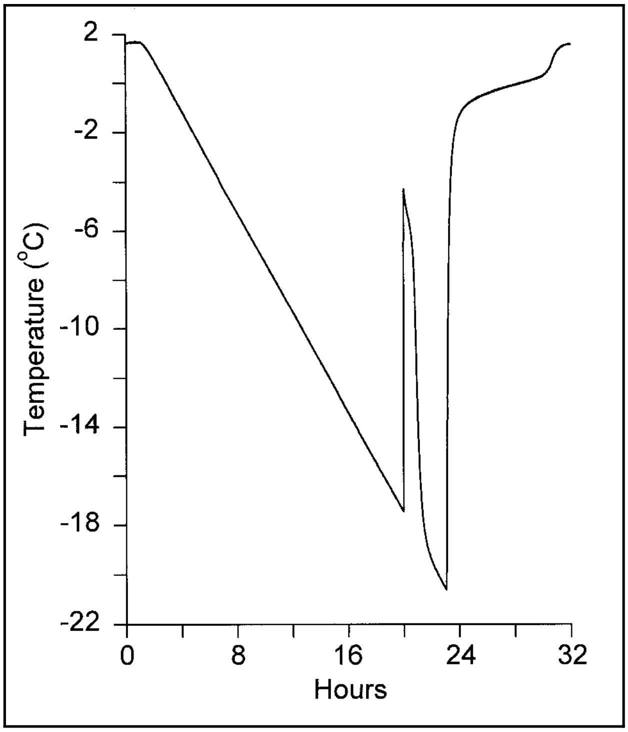

Figure 2. Temperature at the surface of the carapace of a hatchling painted turtle that was cleaned, dried, and then placed into an environment in which it would not make contact with crystals of ice. The release of latent heat of fusion as water in the body of the turtle changed from liquid to solid caused the spike in temperature at −18.2°C.

by seeding water in the soil with chips of ice. After the soil solution had frozen to an equilibrium, the temperature in the "nest" was reduced by 1°C per day until it reached –10.7°C. The temperature profile lacks the temperature spike that would result if water began to freeze in the animal itself, indicating that the turtle was unfrozen and supercooled at temperatures below the equilibrium freezing point for its body fluids. The animal was alive at the end of the experiment, despite being exposed to temperatures below –0.7°C for 11 days, being held at –10.7°C for 24 hours, and being in contact with ice the entire time.

The barrier to penetration of ice seems to reside in the outer (α-keratin) layer of the epidermis of skin from exposed surfaces on the head and the limbs (Willard et al. 2000). Those are the surfaces (other than the shell itself) that actually make contact with soil in the nest, because hibernating turtles tend to withdraw their extremities inside the shell, leaving exposed only part of their face, the distal surface of the forelimbs, and the planar surface of the hind feet. The basal part of the α-keratin layer of exposed epidermis contains a layer of lipid that is absent from the epidermis of hatchlings of several other species (Figure 4), all of which are readily penetrated by ice growing inward from frozen soil (Packard and Packard 1993a, Packard et al. 1993, 1997c, 2000, Willard et al. 2000). Moreover, damage to the epidermis results in an increase in susceptibility of neonatal painted turtles to inoculation, whereas other species (which already are highly susceptible to inoculative freezing) are little affected (Willard et al. 2000). Thus, the unique structure of the integument of neonatal painted turtles apparently is key to the success of their "strategy" for overwintering.

The cutaneous barrier to penetration of ice sometimes fails, and the animal freezes as a result. This point can be illustrated with a temperature profile for another animal that was placed into an artificial nest and then exposed to subzero temperatures (Figure 3b). In this example, a conspicuous spike appeared in the temperature profile at approximately –8.3°C. This temperature is well above the limit of supercooling, so the turtle apparently was caused to freeze by ice penetrating into a body compartment from the environment. The turtle was dead when it later was recovered from the jar of soil.

The integument of this second animal actually presented a formidable barrier to the penetration of ice for several days (Figure 3b). Such a delay in inoculation is the norm: Few animals begin to freeze at a temperature near the equilibrium freezing point for their body fluids (Packard and Packard 1993a, Packard et al. 1997b, 1999a). One explanation for such delayed inoculation is based on the fact that the vapor pressure of supercooled body fluids is higher than that of ice in frozen soil (Salt 1963). According to this hypothesis, water vapor tends to diffuse outward through breaks in the skin, at corners of the mouth, and so on. The vapor passing into air spaces in the soil condenses on the nearest crystal(s) of ice, thereby causing the crystal to grow toward the source of the vapor. When the crystal grows into and through the gap followed by vapor moving outward, the body fluids are inoculated, and the animal freezes and dies. The probability of inoculation increases with the duration of exposure to subzero temperatures, and it increases also as the temperature goes lower (Packard and Packard 1993a, Packard et al. 1997b). Moreover, the risk of inoculation is slightly higher for animals overwintering in sandy soil than for those in clayey soil (Packard and Packard 1997), and ice penetrates body compartments more frequently in wet soils than in dry ones (Costanzo et al. 1998, 2001). The noteworthy points, however, are that delayed inoculation is an unfortunate accident and that survival usually depends on an animal avoiding inoculation altogether (Packard et al. 1997b, 1999a).

Acquisition of cold tolerance

Despite the impressive ability of hatchling painted turtles to undergo supercooling during the coldest months of winter, the animals actually have only a modest capacity for supercooling in the first few weeks after hatching. Indeed, recently hatched turtles freeze spontaneously at temperatures near –6°C (Costanzo et al. 2000b, Packard et al. 2001), a limit of supercooling so high that few animals would survive winters at higher latitudes in North America if this value set their lower limit of tolerance (Table 1). However, the animals acquire a capacity for deeper supercooling as winter approaches, and

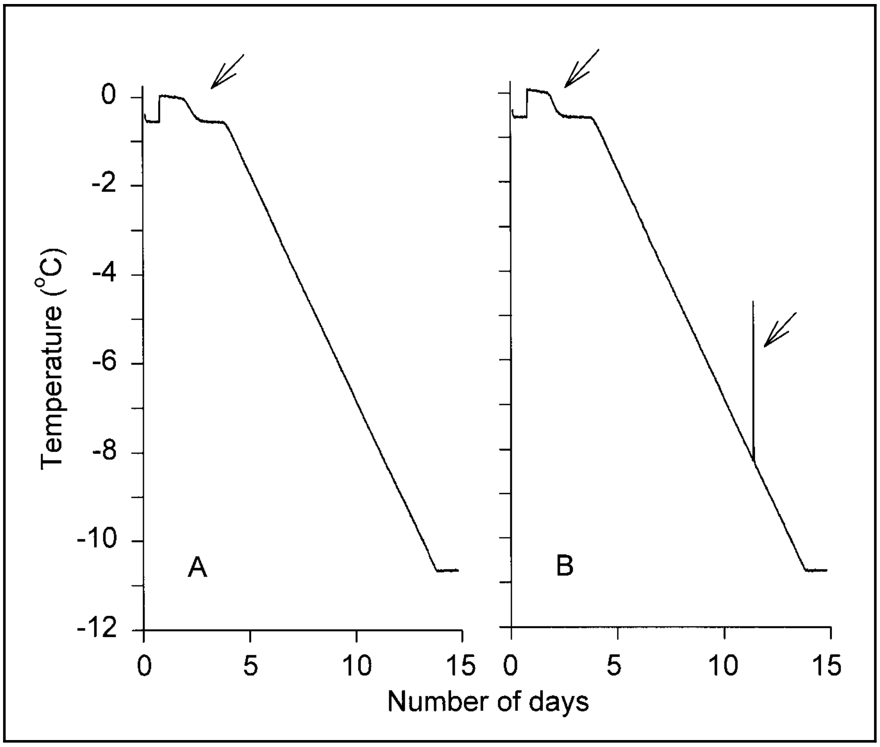

Figure 3. Temperatures at the surface of the carapace of hatchling painted turtles that were confined inside artificial nests formed in a moist, loamy sand. Water in the soil was caused to freeze at approximately –0.5°C by placing chips of ice on the surface (arrows at upper left of each panel). After the temperature had stabilized, the temperature in the nests was reduced by 1°C per day to minima near –10.7 °C. (a) The turtle survived for 11 days at temperatures below the equilibrium freezing point for its body fluids. Note the absence of a freezing exotherm (temperature spike) other than that for water in the soil. (b) This turtle did not survive exposure to low temperatures. The arrow at right identifies a freezing exotherm for the hatchling. Modified from Packard et al. 1997b.

the new limits for supercooling are lower than temperatures that are commonly encountered in the field (Figure 5).

The likely cause for the high limit of supercooling in newly hatched turtles is the presence in their gut of potent nucleators. Turtles in the process of hatching typically ingest quantities of soil and eggshell (Packard et al. 2001), perhaps to provide the animals with a reserve of calcium (from the calcareous layer of the eggshell) to support skeletal growth in the neonatal period. Soil contains an abundance of nucleating agents (Costanzo et al. 1998, 2000a, 2001) that presumably initiate freezing of water in the gut at relatively high subzero temperatures. When contents of the gut begin to freeze, the ice propagates across the wall of the gut and seeds the formation of ice in extracellular fluids of the turtle. Needless to say, the limit of supercooling varies among animals hatching on different substrates because of differences in the kinds of nucleators that are ingested by turtles in the process of hatching (Figure 5).

Over the course of the autumn (or perhaps as a result of exposure to progressively declining temperatures), the turtles purge their gut of its contents, including the soil that was ingested at the time of hatching (Packard et al. 2001). This purging of the gut seemingly removes the nucleators and enhances the ability of turtles to supercool

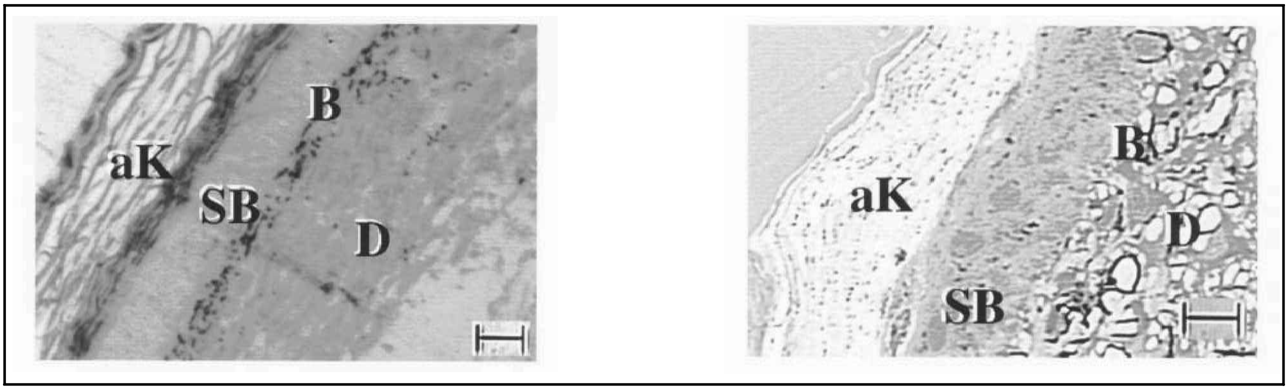

Figure 4. Micrographs of skin from the exposed outer surface of a forelimb of a hatchling painted turtle (left) and a hatchling snapping turtle (right). Neonatal snapping turtles are highly susceptible to inoculation when they contact crystals of ice (Packard and Packard 1993a). The outer surface of the skin is at the upper left of each panel. Preparations were stained with Sudan Black to demonstrate lipids. Scale bars are 10 µm. D indicates dermis; B, basal layer of the epidermis; SB, suprabasal layer of the epidermis; aK, α-keratin layer of the epidermis. Note the darkly stained region (lipid) in the α-keratin layer of skin from the painted turtle. The basket-weave appearance of the outer portion of the α-keratin layer is an artifact caused by the removal of additional lipid when the sample was prepared for microscopy. From Willard et al. 2000.

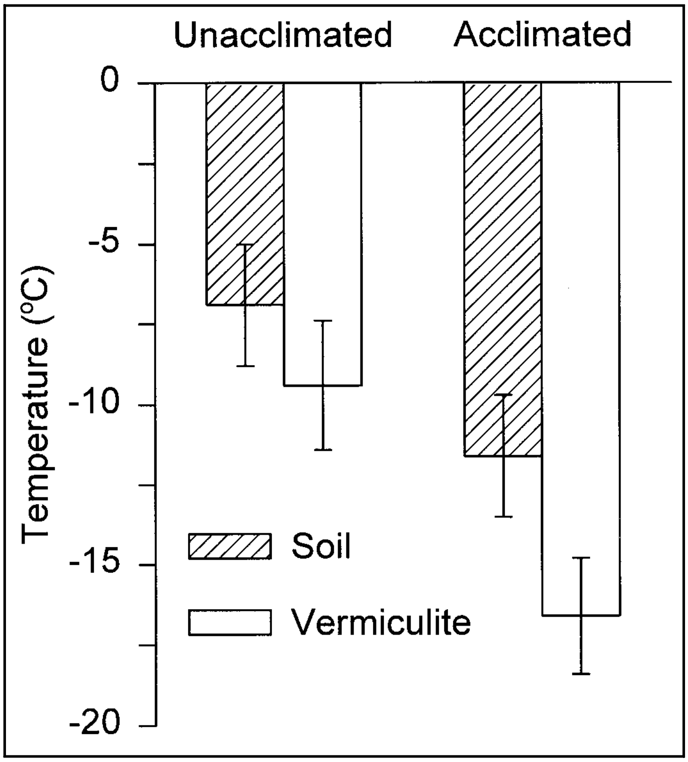

Figure 5. Least-squares means (± 2 SEM) for limits of supercooling for painted turtles hatching in the laboratory on moist substrates of vermiculite or native soil. Unacclimated turtles were studied in mid-September, whereas acclimated animals were studied in mid-November. From Packard et al. 2001.

(Figure 5), albeit some residual effect of soil type may linger (Figure 5; Costanzo et al. 2000b). A similar process of gut purging occurs in terrestrial arthropods that survive the cold of winter by supercooling (Salt 1966).

In addition, the integument of newly hatched painted turtles has only a limited capacity to resist the penetration of ice crystals into body compartments from frozen soil. Consequently, recent hatchlings are at high risk of inoculation whenever the temperature in frozen soil falls below the equilibrium freezing point of their body fluids (Costanzo et al. 2000b). Of course, the animals are unlikely ever to be exposed to ice and cold in the interval immediately after hatching (i.e., in August or September), but they clearly could not withstand the rigors of winter without an adjustment in the barrier properties of the skin. The barrier function is enhanced either by a simple process of maturation or by the turtles' acclimation to lower temperatures (Costanzo et al. 2000b). However, the actual change that occurs in the integument is unknown, but presumably entails a change in the kind or amount of lipid in the α-keratin layer of the epidermis (Figure 4).

Physiology of supercooled hatchlings

Although neonatal painted turtles need to remain unfrozen to survive the winter, remaining unfrozen is no guarantee that they will be alive when the ground thaws in the spring. Whereas unfrozen turtles generally recover from exposure to temperatures above –8°C, mortality increases rapidly as the temperature goes below this level (Packard and Packard 1999). The most likely explanation for this mortality lies in a complex interaction between temperature, circulatory function, and metabolism.

Declining temperature leads to the depression of many physiological functions in hatchling painted turtles. One of these functions is cardiac activity (Birchard and Packard 1997). Indeed, heart rate, which is nearly 1 beat per minute at 0°C, declines rapidly with declining temperature and apparently goes to zero at approximately –10°C (Figure 6). Once the heart has stopped beating, peripheral tissues cease to be perfused.

Tissues of the supercooled turtles continue to function at such low temperatures, although metabolic activity is exceedingly low compared with levels sustained at higher temperatures. If the delivery of oxygen to peripheral tissues is curtailed by a shutdown of circulation, then the requirements of those tissues for adenosine triphosphate (ATP) must be met by anaerobic respiration. This supposition is supported by results of a recent study in which hatchlings were supercooled and then exposed for up to 25 days to temperatures of 0°C, –4°C, or –8°C (Hartley et al. 2000).

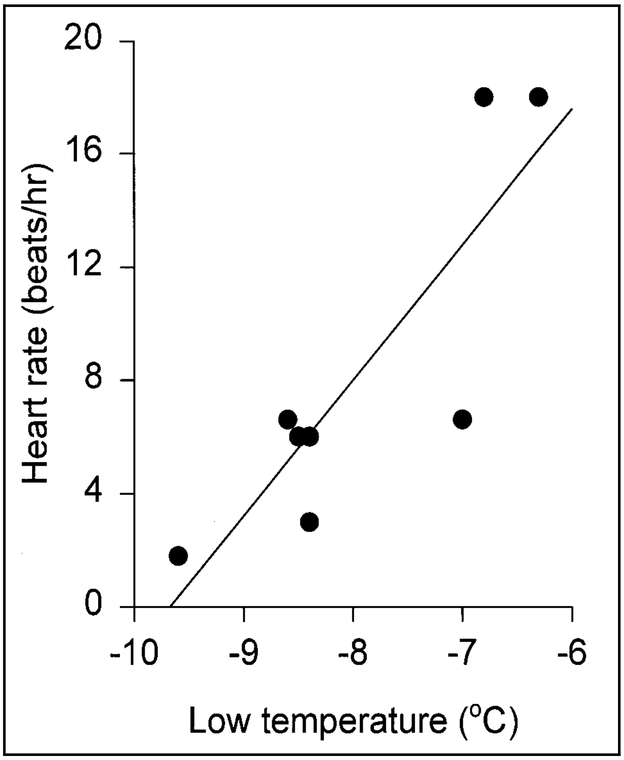

Figure 6. Heart rate of supercooled hatchlings of the painted turtle at different subzero temperatures. Extrapolating the line to lower temperatures indicates that cardiac activity would cease at –9.7 °C. Modified from Birchard and Packard 1997.

Turtles that were held at 0°C did not accumulate lactic acid, the end product of anaerobic glycolysis, over the course of 25 days (Figure 7). The low level of cardiac function in animals at that temperature apparently was sufficient to perfuse peripheral tissues with enough oxygenated blood for the tissues to regenerate ATP aerobically. At lower temperatures, however, peripheral tissues seemingly relied on anaerobic glycolysis for at least part of their requirement for ATP, because lactic acid accumulated in bodies of turtles during the course of their exposures (Figure 7). Lactate accumulated at a higher rate in animals held at –8°C than in those animals exposed to –4°C, indicating that the former group of turtles relied more on anaerobiosis to satisfy their requirement for ATP despite their lower overall metabolism.

Physiological consequences of anaerobiosis

The investigation of anaerobic metabolism in supercooled turtles provides insights into possible causes of death other than by freezing. For example, survival was high for hatchlings in reference groups, except in the case of animals exposed to –8°C for 25 days (Hartley et al. 2000). Lactate in bodies of turtles sampled after 25 days at –8°C was only slightly higher than was recorded for animals held at that temperature for 15 days (Figure 7). Our interpretation is that turtles held at –8°C survived for slightly more than 15 days but then died as the result of an acid–base imbalance caused by the accumulated lactic acid. This interpretation explains why turtles may die without freezing when they are exposed for extended periods to temperatures between –10°C and the limit of supercooling (Packard and Packard 1993b, 1999, Hartley et al. 2000).

Adult painted turtles, however, are noted for their ability to survive extended periods of submergence in cold, anoxic water by relying on anaerobic respiration and consequently accumulating lactic acid in their bodies (Jackson 2000). Why would hatchling painted turtles succumb to an acid–base imbalance that supposedly is elicited by an end product of anaerobic respiration when adult animals seemingly deal quite handily with the same general problem?

The answer may lie in the way adult animals avert an acid–base imbalance secondary to the production of lactic acid in anaerobic respiration. A substantial fraction of this acid is transported to the bony shell, where buffering is effected by as yet undetermined means (Jackson 2000). Additionally, calcium and magnesium carbonates are mobilized from the shell and then transported throughout the body to buffer additional lactic acid in the extracellular space (Jackson 2000). A patent circulation, therefore, is an essential component of the physiological mechanism used by adult animals to deal with the threat of lactic acidosis (Herbert and Jackson 1985). The stagnant anoxia experienced by deeply supercooled hatchlings, however, prevents them from moving lactate to the shell for buffering and from mobilizing strong ions from the shell for distribution to the extracellular compartment. This difference in circulatory function may provide an explanation for why adult animals held in anoxic water can tolerate accumulations of lactate that prove lethal to hatchlings in simulated hibernation (Hartley et al. 2000).

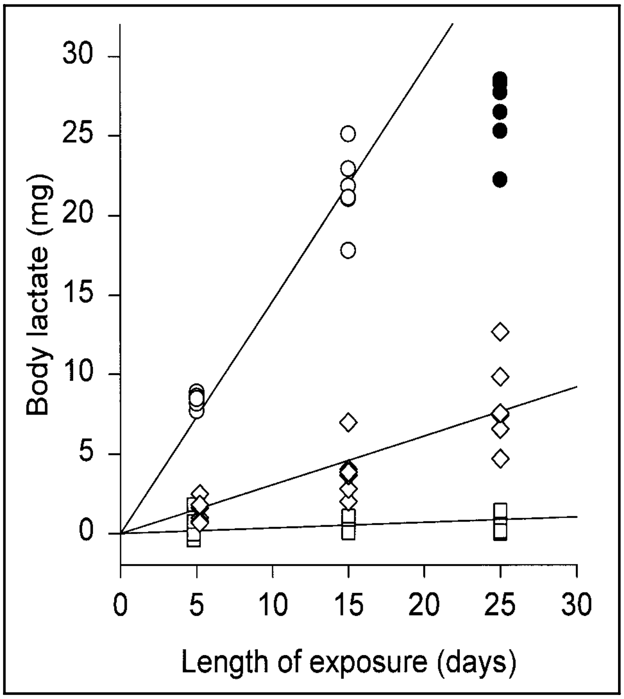

Figure 7. Lactate accumulated in bodies of unfrozen hatchling painted turtles held at 0°C (❑), –4°C (·), or –8°C (○). Animals held for 25 days at –8°C (●) probably were dead at the point in time when they were sampled, so data for these turtles were not used in computing the regression line. From Hartley et al. 2000.

Unanswered questions

Several important questions about the strategy for overwintering remain unanswered, but the most intriguing, perhaps, is why neonates remain inside the nest over the winter and thereby expose themselves to life-threatening ice and cold. Hatchlings of other species, many of which co-occur with painted turtles, emerge from nests before the arrival of cold weather and take refuge in the protective, unfrozen depths of marshes, lakes, and streams (Ernst et al. 1994). A common explanation to account for the unusual behavior of painted turtles is that hatchlings remaining inside the natal nest reduce their exposure to predators at a time of year (late summer and autumn) when resources in freshwater environments are declining in quality and abundance (Gibbons and Nelson 1978). Presumably, the hatchlings delay their entry into aquatic habitats until the spring, when resources are increasing in quality and abundance, the potential for rapid growth is maximized, and the benefits to turtles outweigh the risks of predation.

Despite the wide acceptance of this explanation, it may not be correct. A preliminary experiment we performed in collaboration with P. A. Sims (now at the University of Wisconsin at Madison) indicated that the natural-history strategy expressed by neonatal painted turtles results from an evolutionary constraint imposed by their physiology. Neonatal turtles in this experiment were placed into artificial hibernacula in jars of damp soil or reconstituted tap water and then held for 70 days at 4°C (i.e., at a temperature similar to that in winter in the unfrozen depths of a lake or pond). Survival was high for turtles hibernating in soil but extraordinarily low for those overwintering in water, which raises the possibility that neonates are unable to deal with the physiological stresses of living in water during the first winter. The stresses may be related to the animals' limited capacity for osmoregulation in cool water (Dunson 1985, 1986), or they may reflect the limited capacity of small turtles to absorb oxygen across the integument (Ultsch and Jackson 1982). Regardless of the cause for the physiological stress, the success of the species at higher latitudes in North America may depend critically on the ability of neonates to survive the winter in frozen soil. Other species, like the slider turtle, express the same natural-history strategy for overwintering by neonates, but young sliders are unable to resist the penetration of ice into body compartments from the environment (Packard et al. 1997c, Willard et al. 2000). Consequently, sliders seem to be limited in their distribution to regions where the soil does not freeze during winter to the depth of hibernating animals (Packard et al. 1997c, Tucker and Packard 1998).

Summary

Hatchling painted turtles typically spend their first winter inside the shallow, subterranean nest where they completed incubation the preceding summer. This behavior often exposes animals in northerly populations in midwinter to life-threatening conditions of ice and cold. The hatchlings apparently withstand exposure to such conditions by avoiding freezing, but they do so without the benefit of an antifreeze. In the interval between hatching and the onset of winter weather, turtles acquire capacities to resist the penetration of ice into body compartments from surrounding soil and to undergo moderate supercooling. However, cardiac function is diminished in hatchlings at subzero temperatures, thereby compromising the delivery of oxygen to peripheral tissues and eliciting increased reliance by those tissues on anaerobic metabolism for the provision of ATP. The resulting heightened production of lactic acid may disrupt the acid–base balance and lead to death even in animals that remain unfrozen. Although a natural-history strategy that involves prolonged exposure to potentially lethal conditions of ice and cold has been difficult to explain, it may stem from the limited capacity of neonatal painted turtles to hibernate successfully in water.

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