

this theory comes from the shape of the peripheral bones where the suture should have been.

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## Characteristics of Hibernacula Use by Spotted Turtles, *Clemmys guttata*, in Ohio

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Adaptations to cold stress of reptiles living in regions subject to seasonally cold environments may be either physiological or behavioral (Hutchison, 1979). Due to their ectothermic metabolism, these reptiles generally adapt to long-term or seasonal cooling rather than resist it physiologically. Supercooling can be found in all reptiles found in temperate climates, at least over limited periods of time (Lowe et al., 1971; Storey and Storey, 1992). Freeze tolerance has been found in box turtles (*Terrapene carolina*), eastern garter snakes (*Thamnophis sirtalis sirtalis*) and hatchling painted turtles (*Chrysemys picta*), with mechanisms allowing up to half of all body water to freeze without causing injury

(Costanzo et al., 1988; Costanzo and Claussen, 1990; Churchill and Storey, 1992). Actual physiological mechanisms that allow this freeze tolerance in reptiles are poorly understood, but include increases in tissue glucose, dehydration of some organs and sequestering of ice crystals outside the organs, temporary cessation of cardiac function when high body-ice is present, and cellular mechanisms to stabilize surface proteins (Storey and Storey, 1992; Costanzo et al., 1993).

Behavioral adaptations to seasonally cold temperatures by over-wintering ectotherms include the use of hibernacula (Storey and Storey, 1992). These habitats, which include specific repeatedly used retreats and general thermally protected areas, provide relatively stable thermal environments that minimize the exposure to sub-freezing temperatures. For semiaquatic species, such as the Emydidae, these locations are often under water, below the depth of ice formation in mud (Ultsch, 1989; Crawford, 1991). Dormant turtles in these hypoxic environments have additional physiological adaptations allowing increased tolerances for anoxia (Lutz, 1992).

General characteristics of winter dormancy sites and associated behaviors have been noted for many turtles such as painted turtles (*C. picta*) and snapping turtles (*Chelydra serpentina*) (e.g., Peterson, 1987; Meeks and Ultsch, 1990; Crawford, 1991; Brown and Brooks, 1994). Furthermore, Ultsch (1989) reviewed general ecological parameters of hibernacula sites in freshwater turtles. Detailed, repeated observations of the same hibernaculum are rare and hibernation specifics are lacking for most species.

Spotted turtles (*Clemmys guttata*) remain active in waters as cold as 3°C, even beneath ice (Ernst, 1982) and are reported at the far northern range of cold tolerance for freshwater turtles (Ultsch, 1989). Spotted turtles can be found from Maine, USA, west to Ontario, Canada, south to northeastern Illinois, east to northeastern Virginia, and south again along the coastal plain into Georgia and Florida (Ernst et al., 1994). In Ohio they are found with few exceptions only in the previously-glaciated northern parts of the state, having been abundant in the first half of this century in swamps, bogs, ditches, and ponds (Conant, 1951). Spotted turtle hibernacula are typically aquatic beneath a layer of ice and mud (Ward et al., 1976; Ernst, 1976; Ernst, 1982; Ultsch, 1989). Sometimes summer sites used for aestivation are continuously occupied through the following spring (Ernst, 1976; Lovich, 1988).

The objective of our study was to examine long-term hibernacula use by spotted turtles in a fen in Ohio and to determine the characteristics of winter dormancy sites used repeatedly over several winters.

*Study Site.* — Prairie Road Fen (PRF) is the largest of 40 remaining fens in Ohio. It is located in Moorefield township in northern Clark County along Buck Creek (39°59'N, 83°42'W). There are approximately 5 ha of fen habitat which have been protected in a 39.29 ha preserve owned by the Army Corps of Engineers and managed by the Division of Natural Areas and Preserves (DNAP), Ohio Department of

Natural Resources (ODNR), since 1981. This fen is characterized by several artisan springs and small streams with mean annual water temperature of 15°C. A 10 cm organic muck layer covers a 1–2 m layer of unconsolidated marl which lies over a layer of compacted clay, with less than 1 ha upland in the fen itself. Stuckey and Denny (1981) further described the flora of Ohio fens, including PRF.

*Methods.*—We studied spotted turtle winter ecology at PRF from March 1991 until March 1996. During the first three winters we regularly monitored hibernacula use, at least semi-monthly, and during the final winter recorded the temperature characteristics of known hibernacula. Only sporadic checks to confirm use of hibernacula were made during the fourth winter.

We located spotted turtles through trapping, intensive searches of fen habitat, and random encounters. We searched intensively along streams and on marl mud flats. As many as 15 volunteers at one time searched the rest of the preserve using both random and systematic searches. We examined every break in the vegetative mat or hole  $\geq 5$  cm in diameter throughout the fen and adjacent upland and shrub areas (7 ha) for hibernating turtles during months when turtles were known to occupy hibernacula. We examined each hole up to 1 m depth or until the compacted clay layer was reached. We also searched the substrate of streams and stream banks for hibernating turtles. When we located hibernating turtles in groups of 3 or more, we placed them in buckets of fen water at the same water temperature ( $\pm 2^\circ\text{C}$ ) as that from which they were removed to keep them inactive until they could be processed. This method minimized disturbance of turtles. After measuring, we returned turtles to their hibernacula.

Each turtle was marked by notching the marginal scutes (Cagle, 1939). Sex was determined by plastron shape, cloacal position, and eye color. In adult males, the plastron is concave; it is flat in females and juveniles. In adult males, the cloaca is located  $> 0.5$  cm distal to the edge of the plastron but barely clears the plastron on females. Males have brown eyes; those of females are orange. Immature turtles were not sexed, as eye color and plastron shape are distinctive only in adults (Ernst et al., 1994). We defined immature turtles as those with carapace length  $\leq 6.0$  cm, typically with 5 or fewer annular rings on each scute.

In September 1995 we placed 6 temperature data loggers at PRF (Onset Instruments, accurate to  $\pm 0.5^\circ\text{C}$ , placed in a waterproof sealed plastic tube). In order to examine thermal differences between a heavily used hibernaculum and unused locations, we set two data loggers each into two different hibernacula. One study hibernaculum was a large site used each winter of the study. The other was a hibernaculum formerly used by one turtle two winters before but unoccupied since. In each of these hibernacula we placed a data logger at 1 m depth, where the deepest turtles were found hibernating. We also placed a data logger at 30 cm depth, the level where open water reached mud in the hibernacula. We placed another data logger in a stream at 1

m depth, and one on the fen surface in an opaque tube just outside the largest hibernaculum. No effort was made to record air temperatures above the surface, and indeed this surface recorder was often below snow level. We programmed data loggers to record temperature every 3 hours. We recovered the data loggers 18 May 1996.

We used  $\chi^2$  analysis in order to test for differences in the patterns of hibernaculum use between years and sexes. Differences in the dates turtles were found initially occupying the hibernacula were tested using a Mann-Whitney rank sum test. Differences in temperatures between occupied and unoccupied hibernacula were examined using a rank sum analysis of variance.

*Results.*—We found 9 hibernacula occupied by 50% of all known spotted turtles at PRF (TLL, unpubl. data). All were within a 0.01 ha (85 m<sup>2</sup>) area, although we extensively searched elsewhere. Five hibernacula contained 1 turtle each, two contained 2 turtles each, one had 8 turtles, and one contained 34 turtles during peak use the first winter. Each of the seven hibernacula with 3 or fewer turtles were simple, vertical holes in the vegetative mat approximately 7 cm in diameter, with none  $> 70$  cm deep. None of these were used again in later years, although most of the hibernating turtles were found alive in later summers. Each was contained within the saturated, organic muck layer of the fen. The two larger hibernacula were more complex and measured 15 x 20 cm at the surface and were reused in each winter examined. Each contained some horizontal passage in addition to the primary vertical hole. Due to our inability to reach the end of the horizontal passage on one of the two larger hibernacula, complete data on repeated hibernaculum use only come from the largest hibernaculum.

A total of 43 different adult (16 males and 27 females) and 4 juvenile turtles used the largest hibernaculum over the three year period. There was no sex-biased use of the hibernaculum in any of the three years ( $\chi^2 = 2.30$ , 2 d.f.,  $p = 0.3165$ ,  $n = 73$ ), nor were the number of years of hibernaculum use sex-biased ( $\chi^2 = 2.37$ , 2 d.f.,  $p = .3059$ ,  $n = 41$ ). Twenty-three turtles used the hibernaculum in only one of three years, 10 in two years, and 8 all three years. Similarly there was no difference in the probability of males vs. females returning to use the same hibernaculum ( $\chi^2 = 0.65$ , 1 d.f.,  $p = 0.4207$ ,  $n = 58$ ). The probability of a turtle returning a following year was 0.5000 (male 0.4348, female 0.5429). Most turtles absent during a winter were later relocated in the summer and do not represent mortality.

In 1991–92 there were 34 turtles in the large hibernaculum, 29 in 1992–93, and 14 in 1993–94. Seventeen of the 34 turtles in the hibernaculum in 1991–92 returned in 1992–93, and 11 in 1993–94. All but one of these 11 used the hibernaculum in each year. Twelve of the 14 in the hibernaculum in 1993–94 were also found there in 1992–93, including the 10 found in the hibernaculum all 3 years.

At each visit to the hibernaculum during the winter, one or more turtles were observed moving in the water column below the ice but above the mud layer. Furthermore, through-

out most of the winters, the layer of ice covering the hibernaculum had a hole of up to 2 cm diameter with open water penetrating through the ice layer. Occasionally turtles moved up to the hole, but seldom broke the water surface.

Although males arrived at the hibernaculum a mean 21 days earlier than females (14 and 25 November, respectively), the difference was not significant ( $p = .7143$ ,  $n = 39$ ) for any year nor cumulatively. One reason for this was the wide range of arrival dates for each sex. Turtles arrived as early as 25 September, and as late as 1 February if ice had not yet sealed the hibernaculum. Two additional female turtles arrived after the ice melted in the spring, and are not included in these analyses. Turtle departure correlated positively with the warming of water in the hibernaculum, usually a few days after the ice melted from the hole.

We recorded no temperature differences between the occupied hibernaculum and two other unoccupied holes. The temperature at 1 m depth was significantly warmer (by 1.5°C,  $p < 0.0001$ ) than the sub-ice temperature at 30 cm depth. The temperature at the bottom of the hibernaculum reached a winter low of 2.73°C from 14 to 19 February. The other study site at 1 m depth reached a low of 2.21°C during the same period. The upper temperature at the hibernaculum reached a low of 0.43°C during the same time period, which was a week after a surface low of -5.78°C recorded beneath snow cover. Turtles were observed to move in the water column above the mud, < 10 cm from the ice where water temperatures were 0°C.

The hibernaculum first iced over on 24 November 1995. Bottom temperatures first dropped below 4°C on 16 November, within a week of the other study sites. The last ice melted above the hibernaculum on 13 March. Temperatures rose in the hibernaculum slowly (< 0.05°C total) until 24 March, then rose 3° in 7 days. Sunny days on 14, 18, and 24 March drove daytime surface temperatures above 10°C, and turtles were observed basking on 24 March 1996. By the end of March the entire hibernaculum reached 4°C and had a constant temperature throughout. No turtles remained in the hibernaculum, although several would later return, but not submerge deeply.

Structurally, the largest hibernaculum consisted of two holes connected by a horizontal passage at 30 cm depth. The smaller hole was used only during the first winter, but the passage and the second hole were occupied all three winters. Solitary hibernacula were structurally similar in terms of depth to mud and impenetrable clay layer. The large hibernaculum with 8 turtles had a 50 cm high open air cavity above the waterline, but turtles over-wintered below the water-table at depths similar to turtles in other hibernacula.

*Discussion.* — Few details of spotted turtle hibernation beyond phenology and cloacal temperatures are available. Some studies have suggested possible communal hibernation. Perillo (1997) reported a communal hibernaculum with four spotted turtles. Ernst (1967) reported an aggregation of 16 spotted turtles in early March which may have been their

hibernaculum site. Netting (1936) reported the migration of 4 spotted turtles from what he reported as upland wintering sites which might have been one hibernaculum. No other studies confirm upland wintering sites. Characteristics of spotted turtle hibernation at PRF follows well-documented patterns for other species of turtles and confirm overwintering aggregations of spotted turtles.

Hibernacula depths and physical characteristics were similar to those of other aquatic turtles, but decidedly different from terrestrial turtles such as box turtles. None of the hibernating turtles at PRF were apparently exposed to subfreezing temperatures over winter. However, their movements late in November and December could easily have exposed them to subfreezing temperatures, although presumably not for long enough to substantially lower body temperature. Brown and Brooks (1994) reported that 13 of 18 snapping turtles (*Chelydra serpentina*), for which they had multiple years of data, returned to the same general area to hibernate, including 10 that chose sites within 1 m of previous sites. One of these used the exact same site in 4 consecutive years. They found groups of up to six turtles within 25 m<sup>2</sup>. Meeks and Ultsch (1990) found similar fidelity and aggregations in snapping turtles. Ernst et al. (1989) found 12 bog turtles (*Clemmys mühlenbergii*) that used the same hibernation sites two years in a row. They also noted that most turtles hibernated alone in their study, although four groups of 2–5 individuals each were located. Perillo (1997) reported fidelity to general areas (the same vernal pool or the same bog) in spotted turtles.

As fully terrestrial ectotherms, box turtles (*Terrapene carolina carolina*) minimize impacts of winter extremes through hibernaculum selection, but still endure somatic freezing. Hibernacula for 10 box turtles in southwestern Ohio near our study site were located within 14 cm of the surface, and averaged 5 cm depth (Claussen et al., 1991). Hibernation duration averaged 142 days. A few of the turtles shifted to other sites late in the fall, but most remained in one hibernaculum all winter. Body temperatures dropped to as low as -0.3°C. No correlation between body temperature and entrance into or egress from hibernacula was noted (Claussen et al., 1991).

The largest PRF hibernaculum was probably in use as early as the winter of 1988–89. On 9 April 1989 a DNAP employee noted approximately a dozen turtles in the immediate vicinity of what was later identified as the large hibernaculum (J. McCormac, DNAP (ODNR), *pers. comm.*) The high site fidelity combined with large aggregations at PRF seems maladaptive considering vulnerability to predators. Some turtles traveled as much as 1.5 km from their summer range to return to the primary hibernaculum site, so there must be some significant advantage.

There is no evidence that such aggregations themselves serve any thermal function. We found no evidence of occupied hibernacula being warmer than unoccupied ones. Peterson (1987) examined the body temperatures of 8 hibernating painted turtles and found none had body temperatures differing from ambient mud temperature. Similarly, Ernst

(1977) found a dormant bog turtle buried in the mud in May whose body temperature was that of the surrounding mud and Ernst and Zappalorti (1989) found no difference between substrate temperature and body temperature of hibernating bog turtles. However, earlier work by Ernst (1972) indicated that hibernating painted turtles could maintain elevated body temperatures. We find it difficult to imagine any substantial benefit to this since the mud is already protected from freezing and further elevating temperatures would result in increased oxygen demand in an already anoxic environment.

Aggregations could confer reproductive advantages by putting otherwise solitary turtles in close proximity early in the spring for mating. Ernst (1970) noted courtship activities in a Pennsylvania population of spotted turtles that began as early as 24 March when water temperatures rose above 8.8°C. Ernst (1967) reported a large mating aggregation of 16 spotted turtles on 16 March and speculated the pool may have served as a hibernaculum the previous winter.

Emergence of spotted turtles with a proximate cause of increased water temperature of as little as 2°C is consistent with published data on turtle emergence (Lee, 1983; Ultsch, 1989; Ultsch and Grobman, 1990; Crawford, 1991), and may be one of the few consistent cues available to a turtle buried in the mud beneath the water column.

Three of the spotted turtles studied by Ward et al. (1976) were found in January under 1.3 cm of ice, although no mention is made of their activity levels. One unmarked turtle was noticed just below the surface of open water 13 cm deep in February, and one can infer it must have been active during the winter to get there. They also described hibernacula somewhat dissimilar to the ones we found; the pools turtles selected in which to hibernate were "remarkably consistent" and irregularly shaped with the longest dimension of 4.6 m, and 0.6 to 0.9 m deep. Turtles generally selected sites in 10–15 cm water near the edges of pools; that depth was consistent with spotted turtles at PRF.

Our spotted turtles were active throughout the winter, with a few moving at any given time. We could not determine which turtles were active, nor whether each turtle was active some time during the winter, nor how long turtles remained active.

Turtle movement in water under ice has been noted in other turtles (e.g. Carr, 1952; many records cited in Ultsch, 1989), as well as in spotted turtles (Ernst, 1982). Such movements probably expose the turtle to increased oxygen during the late winter before ice melt (Ultsch and Jackson, 1982). Tolerance to prolonged anoxia may limit the northern range of turtles (Ultsch et al., 1985). Turtle movements in the primary hibernaculum were associated with a 2 cm hole in the ice which was apparently maintained by turtles swimming up to where the water was likely richer in oxygen. No similar holes were seen above other hibernating turtles, so we assume this to be atypical, although it was observed in 3 of 5 winters.

Hibernaculum use for spotted turtles at PRF did not differ by sex nor over years, although fewer turtles used the

main hibernaculum the third year. Characteristics of solitary hibernacula were consistent with other published data on aquatic turtles except for the large aggregations in the same hole. These aggregations have significant implications in the conservation of spotted turtles, a species in decline over much of its range due to habitat loss, raccoon (*Procyon lotor*) predation, and over-collection (Conant, 1951; Lovich, 1987). Raccoon populations have increased 700% over the last decade in this portion of Ohio (ODNR estimates). Large turtle aggregations present obvious vulnerabilities to predators and collectors. If these aggregations are not restricted to PRF, locating them and protecting those sites would significantly facilitate long-term survival.

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## Observations of Reproductive Behavior of Male Green Turtles (*Chelonia mydas*) at a Nesting Beach in Cyprus

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Relatively little is known of the mating behavior of marine turtles, although many anecdotal accounts have been recorded (see Ehrhart, 1982). All of these observed matings have been of turtles at sea. Studies have suggested that mating in marine turtles occurs prior to the oviposition of a female's initial clutch of the season, when she can mate with several different males and sperm are stored for the fertilization of subsequent clutches of the season (Owens, 1980; Gist and Jones, 1989; Galbraith, 1993).

In this study we report incidental observations of green turtles (*Chelonia mydas*) mating at a nesting beach in Cyprus. Observations were made during June 1995, between 2 and 4 weeks after the onset of the nesting season, while conducting night surveys at Alagadi Beach, a major marine turtle nesting site in northern Cyprus (Broderick and Godley, 1996). Although continual night work has taken place at this study site over six successive nesting seasons, observations of this unusual mating behavior only took place in 1995.

We observed mating green turtles on four separate occasions. On one occasion the mating pair was in the sea, about 5 m from the shore, while on another, a mating pair appeared to have been washed onto the beach in stormy weather. Both pairs separated after approximately five minutes of observation. However, on two other occasions, during calm weather, the female crawled onto the beach with the male still attached in amplexus. In both cases, after the female ascended approximately 5-10 m up the beach, the male detached, appeared disoriented, and returned to the water. Both females went on to attempt nesting, one laying a clutch which hatched successfully. In the final case, the male was measured to have a curved carapace length (CCL) of 85 cm.

Similar published records of mating by marine turtles at or on nesting beaches do not appear to exist. The closest available record is some 1947 film footage of huge numbers of Kemp's ridley turtles (*Lepidochelys kempi*) nesting in an *arribada* at Rancho Nuevo, Mexico, which shows a nesting female on the beach with a male on top (P.C.H. Pritchard, pers. comm.).

Since these observations were made during the first 2 to 4 weeks of the nesting season it is possible that they occurred