Behavior, Hibernacula, and Thermal Relations of Softshell Turtles (*Trionyx spiniferus*) Overwintering in a Small Stream

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Abstract. – We used radiotelemetry to study the behavior, hibernacula, and thermal relations of free-ranging spiny softshell turtles (*Trionyx spiniferus*) overwintering in a small stream in Arkansas, USA. The softshells hibernated singly by burying in stream bottom sediments (various mixtures of mud, sand, and gravel) at sites within their summer home ranges. They did not hibernate in the deepest pools available, nor in readily available muskrat and beaver burrows. Water depths at hibernacula were deep enough ($\bar{x} = 46$ cm) to prevent buried turtles from reaching the water surface by neck extension to breathe air as commonly occurs during the active season. Burial depths in sediment were shallow enough ($\bar{x} < 5$ cm) to permit turtles to reach the water column for water breathing. Shifts in hibernacula occasionally occurred when brief warm periods stimulated activity. Body temperatures (T_b) of hibernating turtles ($\bar{x} = 7.3 \pm 3.1°C$; range, 1.5–16.2°C) closely tracked ambient environmental temperatures (T_a) and deviations ($T_b - T_a$) were randomly distributed around 0. Differences between T_b and T_a likely resulted from lags in reaching equilibrium body temperatures when water temperatures varied rapidly.

Key Words. – Reptilia; Testudines; Trionychidae; *Trionyx spiniferus*; turtle; ecology; hibernation; overwintering; thermoregulation; radiotelemetry; Arkansas; USA

Despite anecdotal observations by Mitsukuri (1905), Newman (1906), Cahn (1937), Plummer and Shirer (1975), and Plummer (1977) that softshell turtles (*Trionyx* spp.) hibernate by burying in the substrate on the bottom of ponds or streams, little is known regarding the ecology and physiology of overwintering softshells (Ultsch, 1989; Graham and Graham, 1997). Here we employ radiotelemetry to examine the behavior and hibernacula of free-ranging spiny softshells (*T. spiniferus*; Fig. 1) overwintering in a small stream. In addition, the question as to whether aquatically hibernating freshwater turtles are capable of maintaining body temperatures above that of ambient has been adequately tested only on small emydids (e.g., *Chrysemys picta*; Ernst, 1972; Peterson, 1987). Because *T. spiniferus* is approximately 10x more massive than *C. picta*, and is capable of altering heat-exchange rates in water to a greater extent than any other known reptile of similar size (Smith et al., 1981), we also analyze simultaneously measured body and ambient temperatures to test the hypothesis of possible thermoregulation by hibernating softshells.

The numerous terms used to describe the behavior and physiology of ectothermic and endothermic animals seeking refuge in seasonally cold environments have been confused. We follow Gregory (1982) and Ultsch (1989) in using the term overwintering as a general term to describe what animals actually do when passing the winter (e.g., remaining active, hibernation, or migration) whereas the term hibernation indicates seeking a refuge (hibernaculum) in which to overwinter.

The generic allocation of trionychid turtles has been disputed and unsettled since Meylan (1987) subdivided the genus *Trionyx*. Until the issue is settled, rather than adopting the name *Apalone* for American softshells as recommended by Meylan (1987), we follow Webb (1990) and Ernst et al. (1994) in retaining the generic name *Trionyx*.

MATERIALS AND METHODS

Gin Creek is a small, ca. 6 km long, partially spring-fed tributary of the Little Red River near Searcy, White County, Arkansas. Because the spring enters the creek at ca. 3.5 km from the creek mouth, the upper 2.5 km of the creek is often stagnant or mostly dry during the summer whereas the lower 3.5 km flows more or less throughout the year. Most of the length of Gin Creek passes through urban areas and thus receives a large amount of runoff from storm sewers, pavement, and other nonporous surfaces. Water levels can rapidly rise by 1 to 3 m after heavy rains, but also rapidly fall afterwards. Substrate in the upper 4.8 km of the creek is mostly hard clay whereas the lower 1.2 km is shale bedrock. Frequent scouring of the creekbed results in unconsolidated sediments being limited to deeper pools, shallow slower waters of inside bends, and small backwater areas created by snags.

Based on mark-recapture and radiotelemetry monitoring, activity of softshell turtles in this small population is restricted to a central ca. 2.5 km of Gin Creek (Plummer et al., 1997). Within this area, the creek ranges from ca. 3 to 7 m in width and has alternating shallow riffles and deeper pools. The substrate is highly dissected hard clay. At normal summer water levels, depth averages ca. 35 cm and is highly variable over short distances, ranging from < 10 cm to ca.
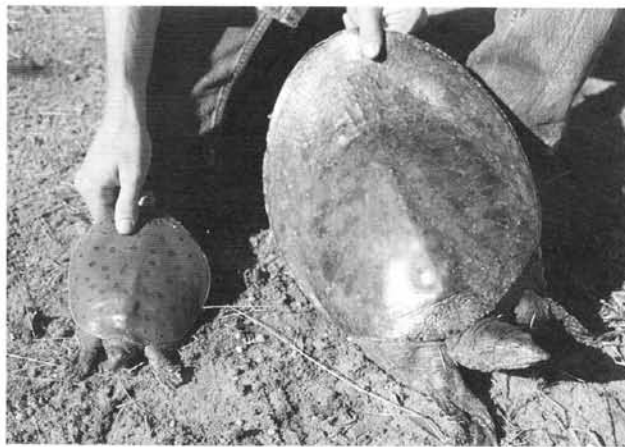
Figure 1. An adult male (smaller) and female (larger) *Trionyx spiniferus* from Gin Creek, White County, Arkansas.

100 cm in the deeper pools. A beaver (*Castor canadensis*) dam created the largest pool (ca. 100 m long) in the creek. The dam was destroyed by humans in each of the two winters of the study but each time was quickly rebuilt by the beavers. In this pool, and at several other smaller natural pools, underwater burrows dug into the bank by both beaver and muskrat (*Ondatra zibethicus*) created possible refugia for turtles. In addition to softshells, other turtles in Gin Creek include common snappers (*Chelydra serpentina*), common musk turtles (*Sternotherus odoratus*), eastern mud turtles (*Kinosternon subrubrum*), cooters (*Pseudemys concinna*), and sliders (*Trachemys scripta*).

We marked locations along the creek with plastic flagging and, in 1995–96, installed a maximum-minimum thermometer on the bottom of the stream at a depth of ca. 30 cm. At our study site (latitude ca. 35°N), winters are relatively mild; annual precipitation is ca. 130 cm. During the study, ice cover on the creek was infrequent, patchy, usually less than 2 cm thick (maximum 6 cm), and never lasted more than a week.

To obtain core body temperatures of adult female softshells, temperature-sensitive radiotransmitters (Model CHP-2P, Telonics, Inc., Mesa, AZ) were surgically implanted into the body cavity of two turtles in 1994–95 and four turtles in 1995–96 (including one turtle also implanted in 1994–95). Transmitters (ca. 6 x 12 x 30 mm, 6–7 g) were inserted into the body cavity through a small incision made in the body wall anterior to the hind legs near the bridge. A small hole was drilled in the lateral portion of the mid-carapace through which protruded a 20 cm external antenna (24 gauge teflon-coated stranded copper wire). The incision was closed with nylon sutures. Transmitter mass averaged less than 0.4% of turtle mass. To obtain ambient water temperatures concurrent with body temperatures, temperature-sensitive transmitters were attached with stainless steel wire to the posterior carapace of selected softshells bearing implanted transmitters (one turtle in 1994–95 and three turtles in 1995–96). All temperature-sensitive transmitters each year were simultaneously calibrated in a circulating water bath to insure measurement congruence. Also, in 1995–96, non-temperature-sensitive transmitters (Model SM-1, AVM Instrument Co., Ltd., Livermore, CA) were attached to the carapace of two softshells without implants.

From late October through November, the softshells became less active when water temperatures decreased, and vice versa. In December of both years, with few exceptions, turtles became dormant at specific underwater hibernacula for the balance of the winter. Thus, beginning in December, we visited the study area from 1 to 7 days a week until late February to mid-March when turtles again became active. For each hibernaculum, we recorded location, water depth, and burial depth. We also collected substrate samples for later qualitative analysis in the laboratory, including substrate composition and relative organic content. For turtles which shifted hibernacula during brief warm periods in the winter, we recorded the same data for each new hibernaculum. At each visit, we recorded the location of each turtle, the pulse rate for each temperature-sensitive transmitter, the current water temperature, and the maximum and minimum water temperatures reached since the prior visit. We also

Table 1. Characteristics of hibernacula selected by *Trionyx spiniferus* along Gin Creek, Arkansas — site number, turtle number, weight, sex, date selected, location along creek, water depth, burial depth, primary and secondary composition of substrate, and relative organic content of substrate. Mean water depth was 46.4 ± 16.7 cm and mean burial depth was 4.8 ± 2.6 cm.

Site no.	Turtle no.	Weight (g)	Sex	Date y/m/d	Location (m)	Water Depth (cm)	Burial Depth (cm)	Substrate Composition	Relative Organic Content
1	4	2900	F	941201	1480	35	5	mud/sand	mod
2	4	2900	F	950115	1410	30	3	mud/gravel	low
3	16	2100	F	941201	1740	40	5	sand/mud	mod
4	16	2100	F	950118	1430	30	3	gravel/mud	low
5	16	2100	F	951221	1460	35	2	mud/gravel	low
6	45	4100	F	951202	2836	25	5	mud	high
7	45	4100	F	960208	2843	75	5	mud	high
8	46	600	M	951221	1736	55	10	sand/mud	low
9	63	3400	F	951207	1689	70	7	mud/sand	mod
10	63	3400	F	960208	1782	70	3	gravel/mud	low
11	80	3300	F	951221	2896	35	3	mud	high
12	80	3300	F	960208	2848	55	10	mud	high
13	94	2100	F	951221	2117	55	2	mud/gravel	mod
14	94	2100	F	960208	2207	40	4	gravel/mud	low

recorded the occurrence of precipitation and qualitative conditions of water clarity.

Statistical analyses were conducted with SYSTAT (SYSTAT, 1992). Unless otherwise stated, results are given as means accompanied by ± one standard deviation.

RESULTS

Hibernating softshells buried themselves singly in the loose sediments of the stream bottom. There was no evidence of communal hibernation nor of movements to distant hibernacula. Long-term radiotelemetry monitoring indicated that each hibernaculum was located within the portion of the summer home range in which the turtle was last active (Plummer et al., 1997). Water depth and composition of sediment at hibernacula were highly variable (Table 1). Approximately 40% of hibernacula were in gravel-mud mixtures, 30% in mud-sand mixtures, and 30% in mud. In each case, water was deep enough (46.4 ± 16.7 cm, $n = 14$) to prevent a buried turtle from reaching the water surface by extending the neck to breathe air as commonly occurs during the active season. However, softshells did not hibernate in the deepest pools available. The depth of burial in sediment (4.8 ± 2.6 cm, $n = 14$) was shallow enough to permit each turtle to reach the water by extending its neck. No softshell in our sample hibernated in the readily available beaver or muskrat burrows. Most softshells switched hibernacula occasionally during a winter (mean = 1.8 hibernacula per turtle, range 1–2). Such changes occurred at water temperatures > 12°C and involved moving to a location within 120 ± 108 m ($n = 6$) of the previous location. Considering within-winter changes in hibernacula, the single 1995–96 hiber-

naculum of turtle no. 16 ranged 30–280 m from its two hibernacula in 1994–95. All turtles successfully emerged from hibernation in apparent good health and with little change in weight compared to late fall measurements (–0.7 ± 0.2% change).

Body temperatures (T_b) of hibernating softshells ($7.3 \pm 3.1°C$, range 1.5–16.2°C, $n = 215$) closely tracked ambient temperatures (T_a) (Fig. 2). Deviations of body temperature from ambient temperature were normally distributed around 0 (Kolmogorov-Smirnov = 0.08, $p > 0.05$, $n = 136$). Analysis of covariance revealed that the slopes of the regression of body temperature on ambient temperature for different turtles were significantly different from one another ($F_{3,128} = 3.05$, $p < 0.05$). Using Huey's (1982) method of analyzing the extent of thermoregulation, the slope of the regression line of body temperature on ambient temperature was significantly different from 1 in one turtle but not in three other turtles (Table 2), indicating thermoconformity.

One softshell (turtle no. 80) hibernated in mud substrate with a particularly high organic content (mostly decomposing leaves) and returned to the same exact hibernaculum on two different occasions after brief periods of activity. This fidelity suggested a particularly favorable hibernation environment. However, neither body nor ambient temperatures at this site were warmer than mainstream water temperatures measured concurrently (1-tailed paired t-test; for T_b, $t = -1.85$, $p > 0.10$, df = 16; for T_a, $t = -0.897$, $p > 0.75$, df = 16).

The softshells appeared to require warmer minimum body temperatures for activity than emydid turtles. Commonly we observed syntopic emydids (mostly *Trachemys*) either actively swimming or basking at water temperatures ranging from 4 to 13°C, whereas the lowest water temperature that we observed for active *Trionyx* was 12°C. These minimum temperatures were similar to those reported for *T. muticus* (13.5°C; Plummer, 1977) and *T. sinensis* (ca. 15°C; Chu, 1989, but see Khosatzky, 1981). In addition to, and in combination with, aerial basking on warm fall and winter days, *T. spiniferus* occasionally sought warmer water at the water's edge or in shallow backwater areas as does *T. muticus* (Plummer, 1977). For example, on 12 December 1995, we observed turtle no. 80 using a combination of aerial and aquatic basking at the water's edge. The anterior half of her body was on land and the posterior half in water. Her body temperature was 18.1°C, ambient shore water temperature was 14.9°C, and midstream water temperature was 13°C.
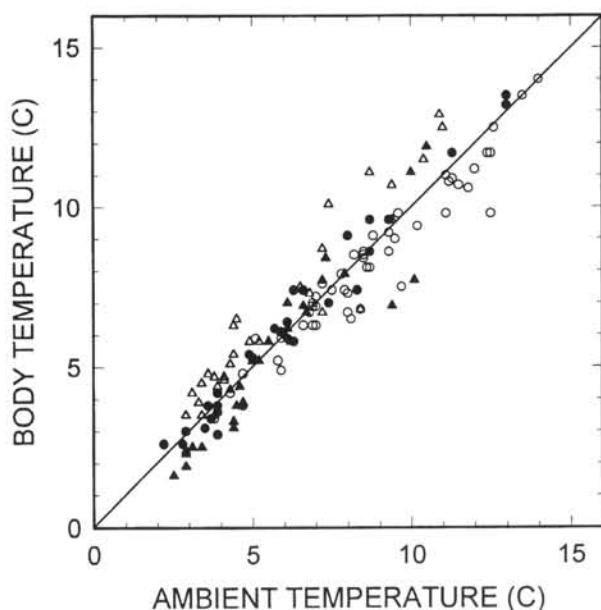

Figure 2. The relationship between body temperature (T_b) and ambient temperature (T_a) in four individual hibernating *Trionyx spiniferus*. The line of equality ($T_b = T_a$) is shown. Symbols indicate individual turtles as follows: open circles = no. 4; solid dots = no. 16; open triangles = no. 63; solid triangles = no. 80.

Table 2. Regression statistics of body temperature (T_b) on ambient temperature (T_a) in hibernating *Trionyx spiniferus*. The slopes are significantly different from 0 for all turtles ($p < 0.001$).

Turtle no.	n	Slope of T_b on T_a	r	Probability of $\beta = 1$
4	50	0.92	0.97	< 0.05
16	28	1.05	0.99	> 0.10
63	29	1.06	0.95	> 0.20
80	28	1.08	0.94	> 0.20

DISCUSSION

There is considerable variation among freshwater turtle species regarding the ecology and physiology of hibernation. Important variables seem to be location of hibernacula (aquatic vs. terrestrial), water depth, sediment burial, aggregation, migration, winter movements, and tolerance of various physiological stresses, especially anoxia (Gregory, 1982; Gatten, 1987; Ultsch, 1989). The free-ranging softshells in this study were typical of many hibernating freshwater turtles in that they buried singly in bottom sediments in shallow water and moved only occasionally during the winter.

Of the few studies which have simultaneously examined body and ambient environmental temperatures of hibernating freshwater turtles, most have demonstrated that equilibrium body temperatures conform to ambient temperatures (Ernst, 1982, 1986; Peterson, 1987; Ernst et al., 1989). Baldwin (1925), Edgren and Edgren (1955), and Ernst (1972) suggested that some freshwater turtles might be capable of metabolic heat production. Ernst (1972) reported that cloacal temperatures of hibernating *Chrysemys picta* were greater than ambient sediment and water temperatures, but his results could not be repeated with more vigorous testing by Peterson (1987).

In comparison to the small emydid *C. picta*, two attributes of *T. spiniferus* suggested that it might be capable of maintaining body temperatures above ambient temperatures when hibernating. For example, *T. spiniferus* has a higher capacity for retaining heat in water than other turtle species of similar size tested (Smith et al., 1981). It also is a much larger species, averaging 3000 g in our sample compared to a mean weight of only 266 g for female *Chrysemys* in Ernst's (1971) population. Khosatzky (1981) reported that, under certain conditions, *T. sinensis* was able to metabolically raise its body temperature above that of ambient water.

Huey (1982) suggested that the extent of thermoregulation in an ectotherm may be estimated by regressing equilibrium body temperature on ambient temperature. A slope of 1 indicates thermoconformity whereas slopes greater or less than 1 indicate various amounts of physiological or behavioral thermoregulation (Huey, 1982). The null hypothesis of $\beta = 1$ was rejected for only one of the four softshells (no. 4; Table 2) suggesting that this turtle was actively thermoregulating. However, we reject this interpretation because the value of the slope (< 1) suggests active cooling, which is physically untenable.

A likely explanation for the deviations from thermoconformity was time lag between body temperature equilibration and the relatively rapid and frequent changes in water temperature with air temperature and solar radiation (Edgren and Edgren, 1955; Peterson, 1987). Daily variation in water temperatures in this small body of water usually ranged several degrees, except on relatively cold, cloudy days when ice cover was present. One might expect lags to be greater in the cooling phase since *T. spiniferus* in water heats almost twice as fast as it cools (Smith et al., 1981).

However, opposing the physiological basis for sustaining warmer than ambient body temperatures is the high surface-to-volume geometry of softshells (Boyer, 1965; Smith et al., 1981). Characteristics of the two hibernacula occupied by turtle no. 4 in 1995–96 (Table 1; shallow water, shallow burial depth, gravel-mud substrate, little organic content) do not suggest a physical environment which would enhance the retention of body heat, but rather suggest an environment which might be susceptible to rapid fluctuations in temperature. Indeed, inspection of the data in which deviations between body temperature and ambient temperature were large in no. 4 revealed that most of the large deviations occurred during two rapid warming periods. Continuous monitoring of both T_b and T_a would be necessary to verify that time lag was responsible for deviations between T_b and T_a.

At low temperatures spiny softshells are proficient water breathers and, unlike many turtles, are intolerant of anoxia such as occurs in mud only a few mm below the water-mud interface (Ultsch, 1985, 1989; Penney, 1987). Thus, softshells hibernating in mud may be dependent on using the water column for buccopharyngeal respiration during hibernation. Because Gin Creek is fed by a very shallow, fast-flowing spring run, oxygen concentrations probably were relatively high in the water column. The shallow burial depth that we observed is consistent with water respiration. On one occasion at water temperature of 12°C we observed a buried female extend her head and neck into the water column and pump her buccopharyngeal region for approximately one minute (Graham and Guimond, 1995). Similar observations were made on *T. spiniferus* by J. Lynch (*pers. comm.*) in early April 1994 in a sandpit off the North Platte River in Nebraska. Lynch observed each of six turtles buried in the sand under water to extend its head and neck into the water column (water temperature < 10°C) and expand its buccopharyngeal region.

The shallow burial depth that we observed also suggests that hibernating softshells do not behaviorally thermoregulate by exploiting a thermal gradient in sediment as perhaps does *Chrysemys*, which may bury at depths of up to 45 cm (Ernst, 1972; Peterson, 1987). It appears that buried hibernating softshells differ from buried softshells in the summer active season only in the slightly greater water depth of the former; burial depth appears to be the same in both seasons (average ca. 5 cm, maximum 10 cm; Plummer et al., 1997). The shallow burial depth may also have contributed to the temperature differences observed between the implanted and attached transmitters on an individual turtle. For example, the attached transmitter on a shallowly buried softshell (no. 16) often protruded into the water column rendering it more susceptible to rapid fluctuations in water temperature compared to the implanted transmitter which probably more closely reflected the substrate temperature.

Because the particular characteristics of overwintering may vary geographically in reptiles (Gregory, 1982; Sexton et al., 1992), similar studies in colder climates and different environments (e.g., lentic habitats) would be useful to clarify

the range in hibernacula and behavior that overwintering *T. spiniferus* normally display. For example, Graham and Graham (1997) recently reported that in Vermont, *T. spiniferus* inhabiting Lake Champlain migrated > 3 km up the Lamoille River where they overwintered in an apparent communal hibernaculum located in deep (6–7 m) water in a small depression of the river bottom. Turtles dispersed downstream in the spring when water temperatures reached 12°C.

Acknowledgments

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