individual reaching 22 g body weight in 13 months of captivity (Fig. 1) and another reaching 35 g in 15 months (Fig. 2).

Many of the adults of this captive group, especially the males, were very colorful. Green and red, with shades of maroons, purples and browns, were not uncommon. Hatchling color was more subdued, but was unique to each specimen.

The successful captive breeding of *Homopus areolatus* in an indoor environment reported here resulted from a focused approach to the species' husbandry, diet, microclimate, behavior, and reproductive parameters. With the survival status of many tortoises in the wild facing increasing threats, this captive breeding approach may be considered part of the overall conservation strategy for selected species.

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Thermal Limits of Incubation in Embryos of Softshell Turtles (*Apalone mutica*)

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Unlike most turtles, softshells (Trionychidae) possess rigid-shelled eggs in which embryonic development is relatively unaffected by substrate moisture (Packard et al., 1979, 1981; Packard, 1991; Gettinger et al., 1984) except for mortality resulting from excessive water loss (Leshem and Dmi'el, 1986). Also unlike most turtles, the sex of softshell embryos is independent of incubation temperature (Ewert and Nelson, 1991). However, incubation temperature may have other important consequences. For example, in smooth softshells (*Apalone mutica*), incubation temperature directly affects the physiology and morphology of developing eggs and embryos as well as the behavior, locomotor performance, and survivorship of hatchlings and possibly also their fitness (Ewert, 1979; Janzen, 1993). Smooth softshells normally lay their eggs in shallow nests in clean, unvegetated sand substrates on exposed sandbars (Fitch and Plummer, 1975; Plummer, 1976; Ewert, 1979). Such nests should experience extremes in temperatures due to their solar exposure and lack of moderating substrate moisture (Packard and Packard, 1988). In this paper, we evaluate the effects of constant incubation temperature on eggs and hatchlings of *A. mutica* in the laboratory and describe temperature variation in natural nest sites in the field.

Materials and Methods. — Eggs of *A. mutica* were collected from natural nests on sandbars in the White River near Georgetown, White County, Arkansas on 5 days from 2-9 June 1992, a time at the beginning of the approximate 45-day nesting season at this locality (Fig. 1; Plummer, unpub.). Eggs were individually marked in the field and transported to the laboratory where they were weighed and aged accord-

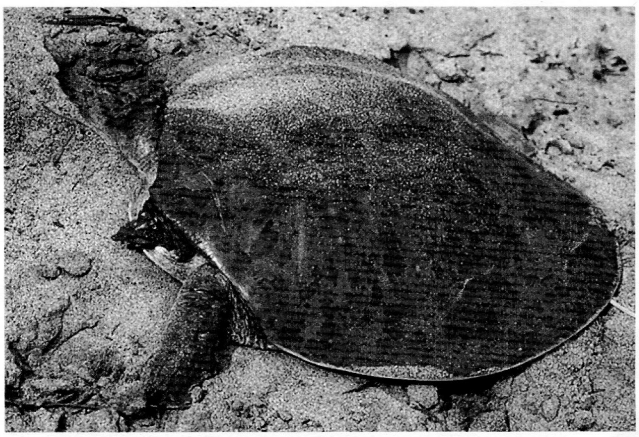

Figure 1. Female *Apalone mutica* nesting on study site sandbar.

ing to the diameter of the chalk spot (Ewert, 1979, 1985). Eggs in clutches which had been deposited on the day of collection had spots ranging from 0-5 mm and were assigned an age of 1 day (n = 70). Nests judged to be 2 days old, based on the condition of nesting tracks and sand disturbance, contained eggs with spots ranging from 6-10 mm (n = 72). Older eggs had larger spots and were arbitrarily assigned ages of 3 days (11-15 mm; n = 60), and 4 days (16-20 mm; n = 28). Eggs were individually buried in moist (5% water by weight) sand in covered plastic boxes and incubated in Hovabator incubators (G.Q.F. Manufacturing Co., Savannah, Georgia) at one of seven constant temperatures: 21, 24, 27, 30, 33, 36, and 39°C. Sand temperatures in each incubator varied < ±1°C as measured by indwelling thermister probes. Percent moisture of the sand was maintained by adding water weekly to boxes according to the weight lost by evaporation. Eggs from each clutch were distributed throughout treatments to minimize potential parental effects. Upon hatching, we measured hatchling weight (HW) and plastron length (PL). For eggs that did not hatch, we opened each and determined the extent of development.

To monitor temperatures in natural nests, we selected typical nests constructed on a large sandbar (Fitch and Plummer, 1975; Plummer, 1976) known to contain the most *A. mutica* nests among all sandbars within a 17 km section of river for each of several years checked since 1977 (MVP, pers. obs.). We installed temperature probes at the top and bottom of each clutch and recorded temperatures either every 15 min with a CR10 Data Logger (Campbell Scientific, Inc., Logan, Utah) or every 24 min with HOBO-TEMP™ temperature loggers (Onset Computer Corp., Pocasset, Massachusetts). Locations of nests were marked with plastic flagging at a certain distance and compass bearing from each nest. Mammalian nest predators eventually ate every clutch that we attempted to monitor. Therefore, to obtain nest temperatures in mid and late incubation, we resorted to recording temperatures of backfilled depredated nests, leaving the probes intact at the originally installed levels. Temperatures recorded in a given depredated nest were indistinguishable from those recorded in the same nest immediately before predation and also were indistinguishable from those recorded on the same day in intact nests in close proximity. Monitored nests were constructed in microsites typical of those known to produce hatchlings in previous years (Plummer, pers. obs.). Temperatures in depredated nests were recorded from the date of oviposition to the predicted date of hatching based on laboratory results.

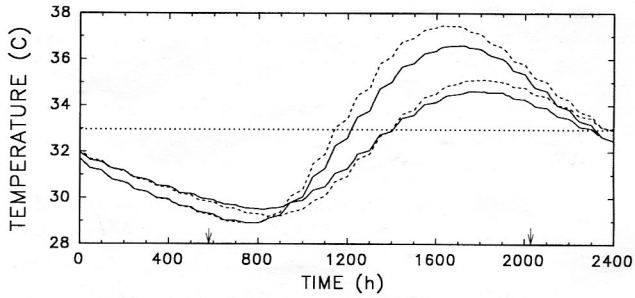

Figure 2. Hourly variation in temperatures on 18 June at the top and bottom of two typical and intact *A. mutica* nests (differentiated by solid and dashed lines). One nest was constructed on 3 June and depredated on 30 June; the other was constructed on 14 June and depredated on 19 June. Arrows indicate local times of sunrise and sunset. Dotted line indicates 33°C, the maximum temperature at which development occurred in the laboratory.

Statistical analyses were performed with SYSTAT (1992). To analyze the effects of treatment on hatching success, we transformed the proportion (p) of eggs hatching to arcsine $\sqrt{p}$. To analyze the effects of treatment on hatchling size, we used analysis of covariance with initial egg mass as a covariate. Unless stated otherwise, means are presented with their standard errors.

Results. — All clutches were fertile as each contained eggs which hatched. Initial egg weight did not differ among

Table 1. Egg and hatchling characteristics of *Apalone mutica* incubated at different temperatures. Incubation is the number of days from oviposition to pipping. Weights (HW) and plastrons (PL) of hatchlings were measured five days after hatching. For eggs which did not hatch, "no devel." indicates eggs not developing past primitive streak, "died early" indicates eggs containing dead embryos with total lengths <10 mm, and "died late" indicates eggs containing dead embryos with carapace lengths >20 mm.

	Treatment (°C)						
	21	24	27	30	33	36	39
No. clutches	20	21	21	21	20	20	19
No. eggs	33	33	33	33	33	32	33
Mean egg wgt (g)	7.2	7.6	7.5	7.6	7.5	7.4	7.1
SD egg wgt	0.83	0.97	1.00	1.14	0.98	1.01	0.82
No. eggs pip	0	2	25	24	20	0	0
No. eggs hatch	0	0	25	24	20	0	0
Percent pip	0	6.1	75.8	72.7	60.6	0	0
Percent hatch	0	0	75.8	72.7	60.6	0	0
No. no devel.	33	10	8	7	11	17	33
No. died early	0	0	0	0	0	9	0
No. died late	0	21	0	2	2	6	0
Mean incubation	—	120	75	58	50	—	—
SD incubation	—	2.8	2.3	1.8	1.8	—	—
Mean PL (mm)	—	—	27.3	27.8	27.7	—	—
SD PL	—	—	1.38	1.66	1.38	—	—
Mean HW (g)	—	—	5.3	5.5	5.5	—	—
SD HW	—	—	0.70	0.82	0.61	—	—

Table 2. Temperatures at the top and the bottom of clutches in typical intact natural nests early in incubation (18 June) and in typical depredated backfilled nests late in incubation (24 July).

	nest	level	mean	SD	min	max
18 June	1	upper	32.5	2.68	28.9	36.6
		lower	32.0	1.78	29.5	34.6
	2	upper	32.9	3.01	28.9	37.5
		lower	32.1	2.07	29.2	35.1
24 July	17	upper	36.0	3.34	31.4	41.4
		lower	35.5	2.39	32.1	39.3
	18	upper	35.8	3.33	31.4	40.9
		lower	35.4	2.31	32.1	38.8

treatments (ANOVA, $F_{6,227} = 1.80$, $P>0.05$). No eggs hatched from the 21, 24, 36, or 39°C treatments (Table 1). No observable gross embryonic development occurred in any egg in the 21 and 39°C treatments (Table 1). Of the developed eggs in the 24°C treatment, all embryos died in an advanced pre-hatching stage (Table 1). In the 36°C treatment, most eggs did not develop, and of those that did, most embryos died in early embryonic stages (Table 1).

Hatching occurred only in eggs in the 27, 30, and 33°C treatments (Table 1). Among these three treatments, hatching success was strongly, but not significantly, related to incubation temperature ($r = -0.95$, $P>0.20$, $n = 3$). Incubation time was strongly related to incubation temperature ($r = -0.97$, $P<0.001$, $n = 69$). Plastron length was significantly less in the 27°C than in the 30°C treatment ($F_{2,101} = 3.20$, $P<0.05$); hatchling weight was also less, but not significantly so ($F_{2,101} = 2.96$, $P=0.06$).

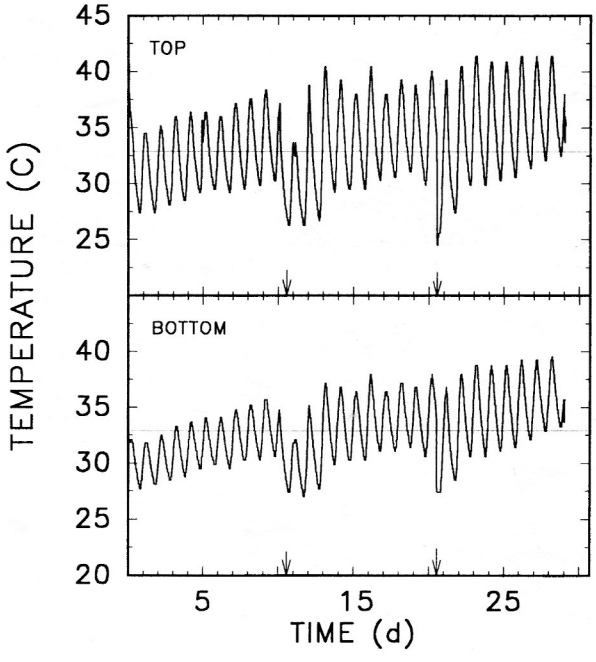

Figure 3. Daily variation in temperatures throughout July at the top and bottom of a typical *A. mutica* nest, constructed on 22 June, depredated on 24 June, and backfilled on 25 June. Arrows indicate beginning periods of precipitation. Dotted line indicates 33°C, the maximum temperature at which development occurred in the laboratory.

Mean depth to the top of the clutch in 25 natural nests was 12.5 cm (SD = 1.2). Nest temperatures varied in a diel cycle with temperatures at the bottom of the clutch lagging behind temperatures at the top in both the heating and cooling phases (Fig. 2). Compared to June, minimum and mean nest temperatures in July at both levels were approximately 2.5 - 3.5°C greater, and maximum nest temperatures were >4°C greater (Table 2; Fig. 3). During July, monthly mean upper nest temperatures were significantly different from mean lower nest temperatures (nest 17, upper = 33.5°C ± 0.09, lower = 32.9°C ± 0.07; $t = 6.08$, $P<0.05$; nest 18, upper = 33.3°C ± 0.09, lower = 32.7°C ± 0.07, $t = 4.78$, $P<0.05$), but the difference was only 0.6°C in each nest.

Discussion. — The shallow nests of *A. mutica* are constructed on the higher portions of unvegetated, unshaded sandbars (Fitch and Plummer, 1975; Plummer, 1976). Temperatures should be high in such exposed substrates lacking the moderating effects of moisture and organic content. Ewert (1979) suggested that requisite temperatures of incubating *Apalone* eggs are greater relative to many species of freshwater turtles with 25°C being an approximate lower threshold for normal development. Our data, and those of Janzen (1993) corroborate this high threshold, and also further refine it. At ≤24°C, eggs either did not develop (21°C) or if they did develop (24°C), failed to hatch. Hatchlings from eggs incubated at 25 and 26°C were smaller than those from higher temperatures (Janzen, 1993) and also exhibited locomotor and neuromuscular disorders and had greater mortality rates than hatchlings from eggs incubated at higher temperatures (Ewert, 1979; Janzen, 1993). Furthermore, hatchlings from eggs incubated at 27°C (present study) and at 28°C (Janzen, 1993) were smaller than those from eggs incubated at higher temperatures.

In nature, tolerance of hot, rather than cool, incubation temperatures may be a problem more commonly encountered by some turtle embryos. Eggs of *Malaclemys terrapin*, *Pelodiscus sinensis*, and *Emydura macquarii* may fail to develop in some shallow nests because of high temperatures (Burger, 1976; Choo and Chou, 1987; Thompson, 1988). In *A. mutica*, constant temperatures of ≥36°C were lethal. At 33°C, most eggs appeared to develop normally but there were a substantial number of early embryonic deaths compared to the 27 and 30°C treatments. Based on hatching success and hatchling size, our laboratory results and those of Janzen (1993) suggest that constant incubation temperatures of approximately 30°C may be optimal for normal development in *A. mutica*. However, our field data suggest that temperatures in intact natural nests (Fig. 2) may frequently exceed 30°C and rarely fall to 26°C, a lower level at which hatchling fitness may be reduced (Janzen, 1993). Additional data in backfilled depredated nests (Fig. 3) further emphasizes this incongruity. Unfortunately, we were unable to obtain temperatures to hatching from any one nest because of the high rate of nest predation and limited number of data loggers. Because it is unknown whether any of the nests that we monitored would have produced hatchlings,

our field data must be interpreted with caution. Perhaps thermal stress is a significant source of mortality for embryos in this population. Additional study in a population known to have a low rate of nest predation is needed to clarify this issue.

Reports of unusually high temperatures in successful sandbar nests of other turtles are known. Temperatures in a nest of *Podocnemis expansa* exceeded 36°C for >50% of the incubation period and were below 33°C for only <10% of the incubation period (Alho and Padua, 1982). Eggs of *Malaclemys terrapin* and *Carettochelys insculpta* tolerate brief periods of 40°C and above (Burger, 1976; Georges, 1992). Even isolated temperatures taken in *A. mutica* nests included values over 36°C in Texas (Ewert, 1979) and Kansas (Plummer, unpub.). Embryos of sandbar-nesting species also tend to develop relatively rapidly (Ewert, 1979, 1985) with rates being temperature sensitive throughout development in *A. mutica* (Ewert, 1985). Eggs of the softshell *Pelodiscus sinensis* may develop in as little as 28 days (Mitsukuri, 1895), the shortest incubation period reported for any turtle species (Ewert, 1979). Ewert (1979) suggested that high temperature tolerance and rapid development of eggs of sandbar nesting turtles may be an adaptation to a seasonally ephemeral and unpredictable resource (e.g., Roze, 1964; Plummer, 1976).

The problems in our field data notwithstanding, the dissimilarity between our laboratory and field results suggests that a better understanding of the limits of thermal tolerance in *A. mutica* embryos will require more information than yielded by constant temperature studies. For example, a knowledge of additional thermal variables such as the duration, frequency, and timing of exposure may be necessary (Packard and Packard, 1988). Although eggs in natural nests may tolerate brief periods of very high temperatures, extended periods may be lethal (Cunningham, 1939; Georges, 1992; present study). Additionally, older embryos may be more capable of tolerating higher temperatures than younger ones (Ewert, 1985; Packard and Packard, 1988). Our study augments that of Janzen (1993) in demonstrating the effects of incubation temperature on growth and survivorship in embryonic *A. mutica* and further underscores the importance of the thermal environment in the selection of nest sites by female turtles.

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