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Effect of a Sand Substrate on the Growth and Condition of *Apalone mutica* Hatchlings

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Abstract.—Smooth Softshell Turtles, *Apalone mutica,* prefer medium to large rivers with clean sand where they spend substantial amounts of time buried in the bottom sand substrate; however, the habitat requirements of hatchling *A. mutica* are poorly known. We designed a laboratory experiment to determine whether the presence of an underwater sand substrate affects growth and condition of hatchling *A. mutica.* We found that hatchlings maintained on a sand substrate grew faster and differed in body appearance compared to hatchlings maintained without a sand substrate. A readily accessible sandy substrate in which to bury may be a significant habitat component for *A. mutica* hatchlings. In addition, investigators maintaining *A. mutica,* and likely other softshell species in the laboratory, should consider the species' substrate requirements in their research design.

A soft substrate that permits burying is an important underwater habitat component for North American softshell turtles (Webb, 1962; Ernst et al., 1994). For example, Smooth Softshells, *Apalone mutica*, prefer medium to large rivers with clean sand where they spend substantial amounts of time buried in the sand in shallow water (Webb, 1962; Fitch and Plummer, 1975; Plummer and Shirer, 1975). In the laboratory, the importance of a suitable burying substrate to softshells is demonstrated by its effect on growth in hatchling *Trionyx sinensis* (Choo and Chou, 1984), activity in *Trionyx triunguis* (Krause et al., 1999), and activity, thermal preference, and levels of aggression in hatchling *A. mutica* (Nebeker and Bury, 2001).

Softshell turtles and their eggs have been the subjects of various laboratory research projects that have addressed an assortment of ecological, physiological, and evolutionary questions. For example, the effects of environmental variables during egg incubation have been studied (e.g., Packard et al., 1979; Leshem et al., 1991; Mullins and Janzen, 2006), as well as physiological aspects of older juveniles and adults (Smith et al., 1981; Khosatzky, 1981; Robertson and Smith, 1982). Choo and Chou (1984) and Nebeker and Bury (2001) represent the few studies that have addressed the immediate posthatching biology of softshells. Indeed, the ecology of hatchlings is the poorest known life-history stage for turtles (Kuchling, 1999; Moll and Moll, 2004). Recently, we studied facets of the posthatching growth and development of *A. mutica* that required maintenance of hatchlings in the laboratory over a period of several weeks (Lee et al., 2007). Individuals were maintained in small plastic containers with approximately 1.5 cm of water but no substrate in which to bury. Toward the end of the study, we observed that hatchlings seemed to be less robust than freshly caught field hatchlings. Because field hatchlings normally spend substantial time buried in sand, we designed an experiment to determine whether the lack of a suitable burying substrate affected growth of *A. mutica*. If so, the information will benefit the species' conservation by identifying an important habitat requirement of hatchlings. Furthermore, the presence of a substrate effect in the New World *A. mutica* and the Old World *T. sinensis* (Choo and Chou, 1984) would suggest an importance of substrate for softshells in general. Incorporating this information into research design could improve laboratory techniques for future studies employing hatchling softshells.

Materials and Methods

Eggs of *A. mutica* were collected 26 May to 27 June 2005 from natural nests on sandbars of the White River near Georgetown, White County, Arkansas. Eggs were taken to the laboratory, weighed, and incubated according to established procedures (Plummer et al., 1994). Immediately after hatching, each of 50 hatchlings from 11 clutches was randomly assigned to one of two groups; each clutch was represented in both groups. Hatchlings in both groups emerged from eggs of similar initial mass (8.48

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± 0.212 g, $N = 25$; 8.53 ± 0.216 g, $N = 25$; $t = 0.188$, $P = 0.852$). Immediately after hatching, each hatchling was assigned to one of two treatment groups and placed in a 8 × 15 × 15 cm plastic container with approximately 1.5 cm of water and either with a 1.5-cm clean sand substrate (SAND group) or without a sand substrate (NOSAND group). Water was changed, and turtles were fed commercial catfish food (Cargill Aquafeed®) ad libitum three times a week. Photoperiod was 12 : 12 (L : D), and room temperature was held constant at 25°C. We measured size (body mass, carapace width, plastron length) of each hatchling every two weeks for a period of nine weeks.

When hatchlings were 4–5 weeks old, we observed that turtles in the SAND group, which appeared similar to field-caught hatchlings, seemed to have a different appearance and feel than turtles in the NOSAND group. To test our subjective observations, we assessed the condition of hatchlings at an age of approximately 6–8 weeks in a double-blind classification study. The two participants were experienced in animal observation but had no prior knowledge of the purpose of the study or the treatments involved. The classifier was given a list of four classification criteria and allowed 15 min to observe all turtles, in identical, numbered containers in 2 cm of water, in a randomly arranged array to get a sense of the variation in the classification traits before classification began. After the 15-min initial observation period, the classifier was repositioned where he could not see the turtles, and the presenter, without verbal communication, presented each turtle in its container randomly to the classifier. The classifier examined each turtle individually and assigned it to one of two categories for each of four relative classification criteria based on appearance and feel of the carapace. The classification criteria and categories were (1) prominence of ribs on carapace: prominent/not prominent; (2) feel of carapace: slick/not slick; (3) color of carapace: dark/light; and (4) sloughed skin on carapace: present/absent. The classifier was instructed to ignore any perceived size, weight, or behavior differences among turtles.

Microorganisms were identified on swabs taken from the carapaces of two seven-week-old turtles in each treatment group. At the termination of the study, turtles from both treatment groups were transferred to two 15 × 40 × 105 cm plastic containers with a sand substrate. Turtles were maintained over winter on catfish food and crickets and were released at the site of egg collection in April 2006.

Turtle body size data were analyzed with repeated-measures MANOVA (SYSTAT Vers. 11; SYSTAT Software Inc., Richmond, CA). The primary independent variable was substrate; the independent variables clutch and egg mass were included to remove possible maternal effects. Dependent variables were body mass (g), plastron length (mm), and carapace width (mm). We used the Pillai Trace multivariate test statistic because it is robust to a wide variety of violations of MANOVA assumptions (Wilkinson et al., 1996). Classification data were analyzed with Chi-square tests of independence. Means are presented ± SE.

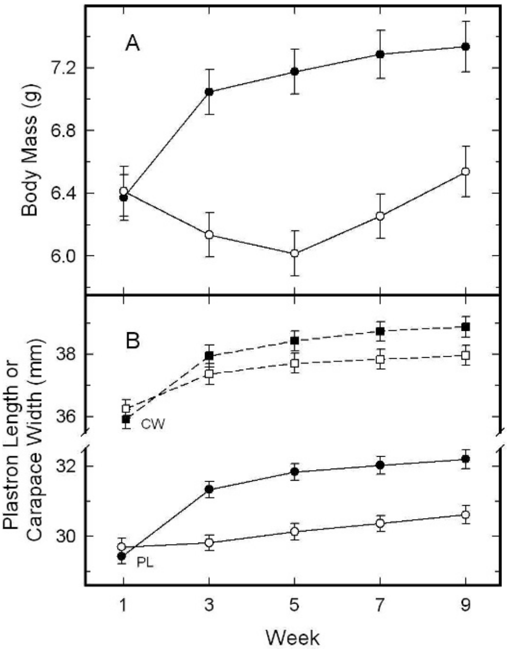

FIG. 1. (A) The relationship of body mass ($\bar{x}$ ± SE) and time after hatching for *Apalone mutica* hatchlings. Solid symbols are hatchlings maintained on sand; open symbols are hatchlings maintained without sand. (B) The relationship of plastron length ($\bar{x}$ ± SE; circles, solid line) and carapace width (squares, dashed line) with time after hatching for *A. mutica* hatchlings. Symbols as in A. $N = 25$ for each treatment group.

RESULTS

We predicted that SAND hatchlings would increase in body size faster than NOSAND hatchlings. MANOVA demonstrated that substrate treatment had an overall significant effect on body size measurements (Pillai Trace = 0.637, $P < 0.001$). Consistent with our expectations, SAND hatchlings had significantly different body size trajectories than NOSAND hatchlings (Fig. 1), as evidenced by significant week by treatment interactions (Table 1). As the trajectories diverged,

TABLE 1. Repeated-measures MANOVA for univariate tests within subjects in hatchling *Apalone mutica*.

Source	Body mass (g)			Plastron length (mm)			Carapace width (mm)		
	Pillai Trace	*F*	*P*	Pillai Trace	*F*	*P*	Pillai Trace	*F*	*P*
Week	0.284	4.254	0.005	0.329	5.267	0.002	0.328	5.242	0.002
Week × Treatment	0.617	17.347	<0.001	0.458	9.077	<0.001	0.286	4.310	0.005
Week × Egg weight	0.246	3.505	0.015	0.392	6.924	<0.001	0.265	3.881	0.009
Week × Clutch	0.066	0.755	0.561	0.083	0.974	0.432	0.108	1.303	0.284

separate ANOVAs revealed significant differences in body mass and plastron length beginning in week 3 when SAND hatchlings weighed 14.8% more and were 11.8% larger in plastron length than NOSAND turtles (Table 2). Carapace width became significantly different by week 7 when SAND hatchlings were 1.6% larger than NOSAND turtles. These differences were maintained or increased throughout the experiment except for the body mass difference in week 9, which decreased to 12.2% (Fig. 1).

Presence of sloughed skin and slick carapaces characterized NOSAND turtles, whereas absence of sloughed skin and nonslick carapaces characterized SAND turtles (Table 3). Of 17 turtles that were classified slick, 15 (88.2%) had sloughed skin. Of 33 turtles that were classified not slick, only five (15.2%) had sloughed skin. Microorganisms identified on carapaces from both treatment groups included the bacteria *Pseudomonas*, *Escherichia coli*, and *Salmonella*. At the termination of the experiment, 13 NOSAND turtles had carapacial growths of a water mold (Oomycota), which were absent on SAND turtles.

DISCUSSION

The presence of a soft substrate in which turtles may bury themselves is known to be an important habitat component for adult softshell turtles. We have demonstrated that the presence of an underwater sand substrate affects short-term growth and condition of *A. mutica* hatchlings in the laboratory. Divergence in growth between SAND and NOSAND turtles occurred soon after hatching and continued until the experiment was terminated at nine weeks. Further, the condition of SAND turtles diverged from the NOSAND turtles and subjectively appeared more similar to field-captured hatchlings. This similarity suggests that burying physically abrades the skin and shell of sloughed skin and prevents accumulation of a substrate that supports microbial growth. In addition to our results on hatchlings, a sand substrate is known to affect activity, thermal preference, and levels of aggression in *A. mutica* hatchlings (Nebeker and Bury, 2001).

For adult *A. mutica*, sand is of biological significance in ways other than permitting burying. For example, adult males forage (Plummer and Farrar, 1981) and spend most of their time (Plummer and Shirer, 1975) along sandbars, and adult females nest only in the clean sand (free of silt and organic matter) of sandbars large enough to elevate nests well above water level (Plummer, 1976; Doody, 1995). Thus, it appears that both juvenile and adult *A. mutica* are dependent on the presence of sand. This dependent association should be considered in the conservation biology of *A. mutica* populations. In addition, investigators using *A. mutica*, and likely other softshell species, in the laboratory should consider the species' substrate requirements in their research design.

Hatchling *A. mutica* conceivably could receive ecological benefits from burying such as escape from strong currents and predation or achieving

TABLE 2. Separate ANOVAs for effects of substrate treatment on body size traits within each trial in hatchling *Apalone mutica*.

Variable		Week 1	Week 3	Week 5	Week 7	Week 9
Body mass (g)	*F*	0.036	20.415	32.978	24.409	12.451
	P	0.851	<0.001	<0.001	<0.001	0.001
Plastron length (mm)	*F*	0.656	22.099	26.395	23.801	18.487
	P	0.422	<0.001	<0.001	<0.001	<0.001
Carapace width (mm)	*F*	0.634	1.434	2.519	4.149	4.064
	P	0.430	0.237	0.119	0.047	0.049

TABLE 3. Number of turtles for each classification character by treatment in hatchling *Apalone mutica*. Chi-square values and probabilities for independence of classifications and treatments are shown.

Character	State	Number of hatchlings SAND	NOSAND	X^2	P
Ribs	prominent	11	11	0.000	1.000
	not prominent	14	14		
Sloughed skin	present	0	20	33.333	<0.001
	absent	25	5		
Carapace color	dark	13	15	0.325	0.569
	light	12	10		
Carapace feel	slick	0	17	27.758	<0.001
	not slick	25	8		

favorable thermal conditions and energy conservation; however, the mechanism of how burying affects hatchling growth is unknown. Future studies should address this question, and we suggest several possible lines of investigation. (1) Because softshells maintained without sand may be more active than those maintained with sand (Choo and Chou, 1984; Krause et al., 1999; Nebeker and Bury, 2001), the increased energy required to fuel activity may decrease the energy available for growth. (2) The increased rate of growth in NOSAND turtles after five weeks (Fig. 1) suggests that different aged turtles respond differently to lack of substrate. Studies are needed to determine the possible long-term effects of a lack of burying substrate for hatchlings. (3) Exposed turtles with no opportunity to bury themselves may experience increased stress. (4) Turtles maintained on sand potentially ingest sand which would artificially increase body mass and possibly affect digestive efficiency (Lopez-Calleja et al., 2000).

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