

Rates of Water Loss and Estimates of Survival Time under Varying Humidity in Juvenile Snapping Turtles (*Cheelydra serpentina*)

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Juvenile snapping turtles may be highly susceptible to dehydration during overland movement because of their aquatic nature and large surface area to volume ratio. The present study examined the influence of body size and relative humidity on rates of evaporative water loss and estimates of survival time. Larger hatchlings had higher rates of evaporative water loss but longer survival times resulting from their greater absolute water contents. Rates of evaporative water loss decreased, and estimated survival times increased with increasing relative humidity. The findings suggest that evaporative water loss may be an important factor influencing survivorship and behavior during overland movements of juvenile snapping turtles.

SNAPPING turtles (*Cheelydra serpentina*) of various age classes are known to make extensive overland migrations as part of their reproductive behavior (reviewed in Ernst et al., 1994) as well as in response to detrimental environmental conditions such as drought or food shortage (Gibbons et al., 1983; Parker, 1984; Yeomans, 1995). However, snapping turtles, like other highly aquatic turtles, may be particularly prone to dehydration during these overland forays as a result of relatively high rates of evaporative water loss (Schmidt-Nielsen and Bentley, 1966; Ernst, 1968; Chessman, 1984). High rates of water loss may be especially problematic for neonatal and juvenile snapping turtles by virtue of their relatively high surface area to volume ratio. Thus, the body water content of the animal and the rate of evaporative water loss together may markedly influence the ability of the juvenile snapping turtles to complete prolonged overland migrations. Although the effects of variation in body size and water content on the desiccation tolerance of neonates have been preliminarily examined in one study (Finkler, 1999), the influence of environmental factors (e.g., relative humidity, temperature, convection) on evaporative water loss and desiccation tolerance has yet to be examined.

The present study examined the influence of relative humidity and body size on rates of evaporative water loss and desiccation tolerance of yearling snapping turtles. The amount of time the turtle can survive without rehydration should increase with increasing relative humidity because of reduced rates of water loss. Moreover, larger juveniles, with their larger absolute water contents and relatively smaller surface areas to volume ratios, should be able to survive longer out of water than can smaller juveniles.

MATERIALS AND METHODS

The juvenile snapping turtles used in this study were reared from eggs from four clutches collected in Washtenaw County, Michigan, on 1 June 1998. The eggs ranged from 10.157–13.602 g in mass (mean = 11.560 g). Eggs from each clutch had been half buried in moistened vermiculite (0.5 ml H₂O/g dry vermiculite, with a water potential of about –325 kPa) and incubated in a pair of Hova Bator 2362N incubators (GQF, Inc.) at 25 C. Eggs hatched over a four-day period between 17 and 20 August 1998. Hatchlings were housed in plastic shoeboxes filled with water to approximately 5 cm in depth. Animals were induced to hibernate, beginning on 15 November 1988, by lowering their temperature from 25 C to 5 C over 30 days with a change of 0.7–0.8 C daily. They remained at 5 C until 15 February when the temperature was raised 0.7–0.8 C per day until 15 March. Otherwise, animals were maintained at 25.0 C on a 12:12h L:D photoperiod and fed commercial catfish feed ad libitum three times per week.

Testing of the animals was conducted between 16 May and 17 June 1999. At the time of testing (approximately nine months posthatching), turtles varied considerably in size, ranging from 27.4–40.6 mm in carapace length and 6.019–15.999 g in initial body mass. Differences in size appeared to result primarily from differences in feeding, because preliminary analyses found neither measure of body size at nine months to be significantly influenced by either initial egg mass or clutch.

To determine the water and solid contents of fully saturated individuals prior to dehydration, 12 randomly selected animals were sacrificed and their carcasses dried to a constant mass at 60 C. Another 10 animals were used for deter-

mination of water contents at "ecological death" (i.e., the point where the physiological performance of an animal is so reduced that it cannot escape detrimental environmental conditions that would lead to true physiological death). I chose loss of righting response as the endpoint for this test of physiological tolerance; although the general effectiveness of this endpoint in environmental tolerance studies has been challenged by some (Lutterschmidt and Hutchison, 1997), righting response is likely an ecologically relevant trait to juvenile turtles (Burger, 1976; Finkler and Claussen, 1997; Steyermark, 1999). The mechanical act of righting can normally be completed in a few seconds (Finkler and Claussen, 1997; Steyermark, 1999); thus a prolonged inability to right despite actively attempting to do so should serve as an effective indicator of severe physiological impairment. To test for loss of righting response, animals were placed in pairs into 5.5-liter still-air desiccators employing CaSO_4 as a desiccant. The animals were desiccated at 25 C until they began showing overt signs of dehydration stress (e.g., sunken eyes, reduced activity, etc.). Animals were then removed from the desiccators and placed upon their backs on a counter top. The observer then left the room and watched the animals through a window in the door. Upon righting themselves, the animals were returned to the desiccators, and the procedure was repeated after 2 h of additional desiccation. Animals that could not right themselves within a 30-min period of actively trying to do so were weighed, anesthetized, and sacrificed; and their carcasses were dried in a 60 C drying oven to a constant mass. Linear regressions of total water content and total solid content against carapace length in desiccated and nondesiccated animals were used to estimate these values both at saturation and at loss of righting response for individuals tested for rates of water loss.

Twenty-four turtles were used to assess rates of evaporative water loss (EWL) under four separate relative humidity levels. To avoid possible testing sequence effects, the animals were randomly assigned to six separate testing orders. After weighing the turtles to the nearest milligram, four animals were placed into a 5.5-liter still-air desiccator that suspended the juveniles over either 250 g of anhydrous CaSO_4 , 300 ml of supersaturated solutions of CaCl_2 or NaCl , or 300 ml of distilled water. At 25.0 C, these various substances generate approximate relative humidities of 23%, 32%, 75%, and 100%, respectively (Winston and Bates, 1960). Because evaporation from the animal may cause the actual relative humidity of the chamber to be higher,

relative humidity ($\pm 5\%$) inside the desiccators was recorded with Hobo H8 data loggers (Onset Computer Corp.) positioned at the center of the plate among the turtles. The data loggers gave consistent readings with each other and with a Fisher Traceable Digital Hygrometer ($\pm 1.5\%$ RH) when tested over supersaturated CaCl_2 and NaCl solutions. The turtles were desiccated for a 24-h period at 25.0 C, after which the animals were reweighed and returned to their housing for a minimum of two days between testing intervals. EWL (g/h) was determined by dividing the difference in mass between the two weighings by the duration of the desiccation period. This method is based on the assumption that changes in mass were primarily the result of evaporative water loss, although other activities such as defecation and metabolic consumption of stored energy undoubtedly accounted for some of the observed mass loss as well (most notably at high relative humidity). In addition, an estimate of survival time (EST) for a given animal under a given relative humidity condition was calculated by dividing the difference between the turtle's estimated water contents at the start of the desiccation period and at loss of righting ability (g) by the turtle's EWL (g/h) at that humidity level.

The influence of relative humidity on EWL and on EST was evaluated with repeated-measures analyses of covariance (ANCOVAs) conducted using the MIXED procedure on SAS 6.12 (Statistical Analysis Systems Institute, Inc., Cary, NC, 1996, unpubl.). In these analyses, experimental run was a repeated effect, clutch and testing order were random effects, and mean humidity during experimental run and carapace length were covariates. The MIXED procedure correctly estimates covariance parameters for random effects but does not assess significance levels for this type of effect (Packard et al., 1999; Tucker and Paukstis, 1999). As such, differences among clutches and testing order are not evaluated in the result summaries below. Carapace length was chosen as the covariate representing overall body size because this variable remained effectively constant over the course of the experiment, whereas body mass fluctuated in the short term because of food consumption, defecation, and a number of other factors. Data for EST were log-transformed to normalize the data prior to analysis. Linear regressions of EWL versus carapace length within humidity treatment were formulated using PROC REG on SAS 6.12 to illustrate the influence of relative humidity on the association between body size and rate of mass loss.

TABLE 1. RESULTS OF MIXED-MODEL REPEATED-MEASURES ANCOVA ON POTENTIAL FACTORS INFLUENCING RATE OF EVAPORATIVE WATER LOSS (EWL) AND ESTIMATED SURVIVAL TIME (EST) DURING DESICCATION.

| Parameter | Source | df | F | P |
|-------------|-------------------------------------|-------|--------|---------|
| EWL (g/h) | Experimental run | 3, 83 | 0.44 | 0.71 |
| | Relative humidity | 1, 83 | 0.06 | 0.81 |
| | Carapace length | 1, 83 | 12.33 | 0.0007 |
| | Relative humidity × Carapace length | 1, 83 | 7.49 | 0.007 |
| Log EST (h) | Experimental run | 3, 84 | 2.01 | 0.11 |
| | Relative humidity | 1, 84 | 304.34 | <0.0001 |
| | Carapace length | 1, 84 | 87.01 | <0.0001 |

RESULTS

Total water content of the turtles was correlated with carapace length both at full saturation {water content (g) = 0.661[carapace length (mm)] - 12.42, $r^2 = 0.993$, $P < 0.0001$ } and at loss of righting ability {water content (g) = 0.177[carapace length (mm)] - 0.372, $r^2 = 0.502$, $P = 0.022$ }. The slopes of these two linear regressions differ significantly (ANCOVA: $F_{1,18} = 58.49$, $P < 0.0001$), indicating that larger turtles can tolerate greater water loss before loss of righting ability than can smaller animals. There was no difference in total dry body mass between animals examined at full saturation and those examined at loss of righting ability (ANCOVA: $F_{1,19} = 0.01$, $P = 0.94$).

Evaporative water loss was significantly influenced by an interaction between humidity level and carapace length (Table 1). Rate of mass loss was significantly correlated with carapace length in the two lower humidity levels (mean RH = 54.3%: $y = 0.0025x - 0.0135$, $r^2 = 0.319$, $P = 0.004$; mean RH = 66.3%: $y = 0.0014x +$

0.0129, $r^2 = 0.259$, $P = 0.011$) but not in the two higher humidity levels (mean RH = 91.8%; $y = 0.0005x + 0.0150$, $r^2 = 0.043$, $P = 0.33$; mean RH = 99.2%, $y = 0.0073 + 0.0003x$, $r^2 = 0.027$, $P = 0.44$). EWL decreased with increasing relative humidity (Fig. 1).

Estimated survival time increased exponentially with increasing relative humidity (Table 1, Fig. 2), with a change of nearly an order of magnitude over the range of relative humidity examined in this study. Body size also significantly influenced EST (Table 1) with larger animals having greater estimated survival times.

DISCUSSION

Although largely aquatic, juvenile snapping turtles occasionally spend considerable amounts of time on land, most notably during the nest to water migration following emergence from the nest (Ernst et al., 1994; Congdon et al., 1999). In addition, events such as drought may force juveniles to emigrate from smaller bodies

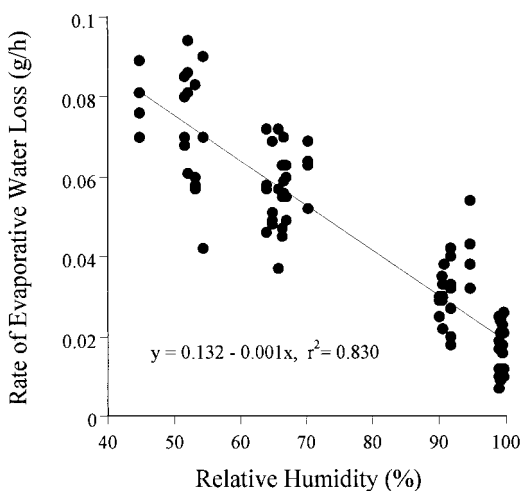


Fig. 1. Relationship between EWL (g/h) and relative humidity (%) in juvenile snapping turtles.

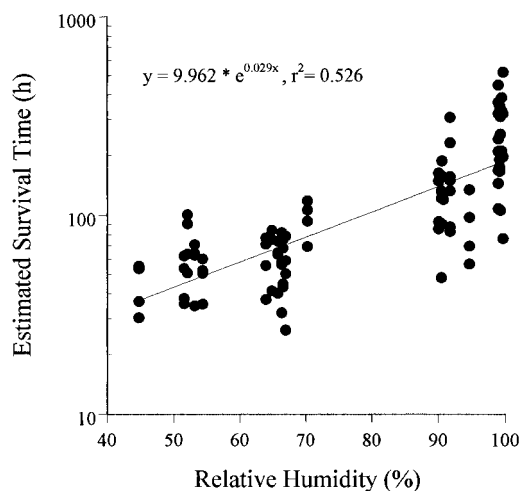


Fig. 2. Relationship between EST (h) and relative humidity (%) in juvenile snapping turtles.

of water as they dry out (Gibbons et al., 1983). Juvenile snapping turtles tend to select shallow waters more frequently than do their adult counterparts (Congdon et al., 1992) and, thus, may be more likely to be driven from their resident bodies of water during dry spells than their older conspecifics (Bodie and Semlitsch, 2000). During overland movement, the ability of the turtle to tolerate dehydration is primarily dependent on the severity of the dehydrating conditions, the duration of exposure to those conditions, and the amount of water contained within the body of the animal.

The range of relative humidity used in this study is similar to the average relative humidity of southeast Michigan, and the temperature is representative of temperatures encountered in the region during the months of May through September (National Oceanic and Atmospheric Administration, 1985). Precipitation events of ≥ 0.25 mm occur on average once every three days at this location, although substantially longer intervals may also occur (National Oceanic and Atmospheric Administration, 1985). Temperature and relative humidity values used herein also fall within the range of average daily temperatures (20.7–32.1 C) and relative humidity (31.4–82.7%) recorded at ground level in areas traversed by hatchling turtles in an enclosure study conducted by Finkler et al. (2000). This suggests that turtles migrating overland could experience prolonged exposure to dehydrating conditions analogous to those experienced in this study, although direct measurement of relative humidity in microhabitats normally traversed by turtles in the field is needed to support this assertion.

The findings of the present study suggest that larger juveniles within a cohort may have greater chances of surviving prolonged overland migrations than do their smaller counterparts. Although larger turtles have greater rates of evaporative water loss, they can also sustain greater losses of water before succumbing to dehydration. Size-related effects on water budget may be an important factor influencing survival during overland movement, particularly in populations inhabiting relatively xeric regions of the species range where drier conditions would increase evaporative water loss and permanent bodies of water would be more sparsely distributed (Finkler, 1999; Packard, 1999).

The findings also suggest that available hydric conditions could have considerable influence on survivorship during overland migration. Juveniles can likely survive on land for much longer periods under humid conditions than under dry conditions. The data in this study sug-

gest that few juveniles of this size could survive more than four days at 25 C and a relative humidity of $\leq 70\%$, but most could survive more than four days at relative humidities of 90% or above (Fig. 2). Congdon et al. (1999) reported that hatchlings from southeastern Michigan may take as long as five days to migrate a linear 45 m distance. Although estimated survival times calculated in this study may underestimate true survival time, because at least some of the mass lost was likely solid mass lost through urination and defecation (note the mass loss at approximately 99% RH in Fig. 1), the data clearly demonstrate that desiccating conditions can have considerable influence on overall water budgets. Behavioral regulation of evaporative water loss, therefore, may be crucial for survival during prolonged overland migration under dry conditions. Small or dehydrated individuals may be able to increase their chances of successfully completing a prolonged overland migration through selection of microhabitats that minimize evaporative water loss and by minimization of activity (Finkler et al., 2000). The observation by Noble and Breslau (1938) that hatchling snapping turtles are attracted toward high humidity areas further indicates that such behavioral modification of water loss may be an important component of water budgeting during terrestrial movements.

The present study examined the influence of variation in only a single environmental attribute on rates of evaporative water loss in neonatal snapping turtles. The influence of numerous other factors such as temperature, convection, and animal activity (Foley and Spotila, 1974; Heatwole and Vernon, 1977), as well as responses during simultaneous variation in multiple environmental conditions, need to be characterized before the importance of evaporative water loss to the ecology of juvenile snapping turtles can be fully evaluated for a given population. Moreover, comparative data for other species of aquatic turtle are presently unavailable. Such comparisons may reveal further insights on the importance of water budgets in the ecology of these organisms during what arguably is the most vulnerable period of their life histories (Congdon et al., 1994).

ACKNOWLEDGMENTS

Hatchlings from this study were reared from eggs collected under Michigan DNR permit CO868. This study was conducted with the approval of the Indiana University Kokomo IA-CUC (Protocol 99-3). I thank D. Schultz, R. Schultz, and A. Schultz for their assistance in

collecting eggs. Previous drafts of this manuscript were improved with suggestions by D. Claussen and A. Steyermark.

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