REPTILES & AMPHIBIANS

Salinity Preferences of Diamondback Terrapin (*Malaclemys terrapin*) Headstarts: A Laboratory Study

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Abstract.—Salt marshes along the eastern coast of the USA are challenging environments due to their tidal nature, which causes constant fluctuations in the salinity levels of their brackish water. Diamondback Terrapins (*Malaclemys terrapin*) can inhabit these salt marshes due to their ability to tolerate highly saline water. The physiological mechanisms underlying this ability are well known in adults, but have not been thoroughly explored in hatchlings and juveniles, which cannot grow when exposed to high salinity. We examined salinity preferences of hatchlings and juveniles in the laboratory and concluded that hatchlings do not exhibit salinity preferences and develop this trait as juveniles.

Only a few vertebrates inhabit salt marshes despite their abundant nutrients (Greenberg et al. 2006), which may be due to the physiological challenge of exposure to salinity. Salinity is a critical environmental factor determining the presence of reptiles in coastal habitats (Dunson and Mazzotti 1989). Therefore, estuaries can present harsh conditions for these animals, mainly due to fluctuating salinities. Adult Diamondback Terrapins (*Malaclemys terrapin*) (hereafter "terrapins") employ behavioral and physiological mechanisms to cope with high salinity. They have salt glands that readily secrete excess sodium regardless of the acclimation concentration (Cowan 1981). Adults exposed to high salinities for extended periods avoid drinking high-salinity water by consuming freshwater from thin layers of rainfall water accumulation (Davenport and Macedo 1990). While hatchlings can also excrete sodium, their ability is less developed than that of adults.

Studies have shown that hatchling terrapins reared in the laboratory cannot grow in salinities above ~23 ppt, but their growth is stimulated in salinities of ~9 ppt (Dunson 1985). This could lead us to think that it would be adaptive for females to select nest sites based on local water salinities. However, field studies have suggested that other environmental factors, mainly nesting site characteristics, determine female nest location (Dunson 1985; Griffin et al. 2005). Holliday et al. (2009) exposed eight-month-old juvenile terrapins to the same four levels of salinity used in this study and found that varying salinity levels did not significantly alter metabolic rates, suggesting that salt excretion in these animals is not energetically costly, perhaps due to their ability to secrete salt through their nasal salt glands. However, despite this lack of effect on metabolic rate, the salinity-stressed turtles grew more slowly and were significantly smaller than turtles held at 10 ppt.

Studying neonatal physiological mechanisms that deal with environmental challenges is logistically challenging, so most of our understanding of these mechanisms is based on laboratory studies (Costanzo et al. 2008), which can provide insights into what happens during the following 3–4 years in the marsh, a period Baker et al. (2018) called the "missing years" since we have almost no knowledge about the lives of young terrapins in nature. The Wetlands Institute, located in Stone Harbor, New Jersey, initiated a headstart program in 1989 solely from eggs harvested from female terrapins killed on roads during the nesting season in June and July (Herlands et al. 1997). These eggs are incubated at female temperatures (>32 °C) and the hatchlings are distributed to several institutions that raise them until the following summer, when they are returned to The Wetlands Institute and released. We participate in this program and, in the past ten years, have conducted several physiological and behavioral studies with terrapins in our care. The laboratory choice experiment described herein was designed to ascertain whether terrapin hatchlings and juveniles exhibit salinity preferences. We hypothesized that hatchlings cannot discern salinity in the water and develop this capability and accompanying salinity preferences as juveniles.

Materials and Methods

Over the past several years, we brought batches of 10–30 hatchlings to our lab ~3 weeks post-hatching in late August

Reptiles & Amphibians ISSN 2332-4961
https://doi.org/10.17161/randa.v31i1.21078

or early September and kept them in our lab until the following June. While in our care, we examined the development of behavioral and basic physiological traits.

Ten three-week-old hatchlings obtained from The Wetlands Institute in late August 2013 were housed in aquaria filled with aged tap water and provided with small pebbles for climbing. We conducted two experiments, the first with newly hatched turtles from September to October 2013 (n = 10; body mass 6.3 ± 0.29 g) and the second with the same turtles as juveniles from February to April 2014 (n = 7; body mass 31.6 ± 1.63 g). Turtles were released at The Wetlands Institute in May 2014.

We tested four levels of salinity (0 ppt, 10 ppt, 20 ppt, and 30 ppt). Solutions were prepared by adding a specific amount of salt (Instant Ocean™) to 19,800 ml of water to achieve the desired concentrations. Salinity levels were verified by testing the density of the solutions using a Density Meter (Anton Paar DMA 5000 TM) and comparing them with published densities for each concentration.

We constructed an experimental chamber from the bottom of a Rubbermaid 120-L trash container with a lip 25 cm high, divided into compartments, and sealed with GE Silicone II Tub and Tile Sealant (Fig. 1). Turtles were placed one at a time in the chamber and allowed to acclimate for 60 minutes before their movements were recorded using surveillance cameras for 24 hours. Each experiment consisted of a test and control run using different salinity solutions. We analyzed our data using repeated-measures analysis of variance (RM-ANOVA) with treatment and compartment as fixed factors.

Figure 1. Experimental arena for juvenile Diamondback Terrapins (*Malaclemys terrapin*). Photograph by Itzick Vatnick.

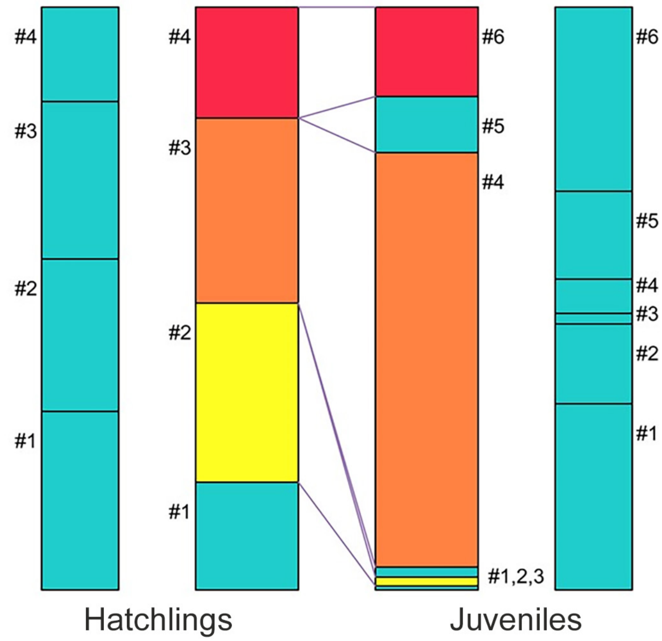

Figure 2. The portion of time hatchling and juvenile Diamondback Terrapins (*Malaclemys terrapin*) spent in each compartment. Numbers indicate compartment numbers and colors represent salinity (blue = 0 ppt, yellow = 10 ppt, orange = 20 ppt, red = 30 ppt). Outside columns are water controls and inside are salinity. See text for numbers and statistics.

Results

Fractions of time hatchlings spent in each of the compartments in the control (freshwater) treatment did not differ significantly ($F_{3,54}$ = 0.27, P = 0.8). Similarly, no significant differences were evident in the fractions of time spent in each of the compartments between the salinity treatment and the control ($F_{1,18}$ = 0.84, P = 0.4; Fig. 2).

Fractions of time spent by juveniles in each of the compartments during the control treatment did not differ significantly (Tukey's posthoc test: MSE = 0.99, df = 60). However, juveniles spent significantly different fractions of time in each compartment during the salinity treatment ($F_{5,60}$ = 4.31, P = 0.002; Fig. 2); specifically spending a significantly larger fraction of time in compartment 5 (62 ± 15%), which had a salinity of 20 ppt, the highest of all compartments (Dunnet's posthoc test: MSE = 0.99, df = 60, Fig. 2).

Discussion

Our study elicited the salinity preferences of terrapin headstarts in the laboratory at two stages of development, and is the first to demonstrate the ontogenetic development of this behavior. Our data indicate that terrapins did not exhibit a salinity preference immediately after hatching but developed a preference for a salinity of ~20 ppt as juveniles (~6 months after hatching). Ashley et al. (2021) exposed 4-week-old hatchlings to five salinity treatments (1, 5, 10, 20, or 35 ppt) for 75 days and supplied an ephemeral source of freshwater every three days. Hatchlings exposed to the higher concentrations of salt grew slower, had reduced appetites, and spent more time in the dish of freshwater, apparently drinking. Ashley et al. (2021) noted that hatchlings in the four groups of low salinity spent nearly all of their time in saline water, whereas hatchlings in the high-salinity treatments spent time in the freshwater dish even when it was dry, suggesting that hatchlings learned to seek freshwater if available and avoid exposure to high salinity.

Hatchlings in our study did not show a preference for low salinities, and this study could not discern whether this lack of preference early in life was due to an inability to discern salinity differences that develop later. Since salt exhibits profound effects on growth, surmising that the lack of preference is attributable to an inability to sense salinity rather than a preference is reasonable. Our protocol enabled us to observe preferences when given a choice rather than the effects of long exposure used by almost all studies examining the effects of salinity exposure. However, six-month-old juvenile terrapins clearly possess the ability to discern salinity and showed a distinct preference for a salinity of 20 ppt.

Allen and Littleford (1955) showed that feeding young hatchlings various food items showed that food preference is a developmental trait that develops early and continues to develop during the first few months of life. Therefore, hatchlings might similarly acquire an ability to discern environmental cues and develop preferences during their first year of life. The ability to discern salinity is crucial for juvenile terrapins since salinities fluctuate in their estuarine environment and can have a marked effect on the growth of juveniles (Dunson 1985; Holliday et al. 2009). However, hatchlings either overwinter in the nest or leave the nest and seem to overwinter in terrestrial refugia. Terrapins may be the only freshwater species that evolved this trait, and its evolutionary significance remains unclear (Muldoon and Burke 2012). Therefore, the ability to discern salinity may come into play only in the spring following hatching. At this age, juveniles can discern salinity and prefer a level of 20 ppt.

Maternal effects can influence offspring traits and performance (Rowe et al. 2017). Since we worked with headstarts we could not control for these effects. Also, our sample size is very small. However, our study assessed the same individuals at two stages of development, and our results are statistically robust. This study was one of the first conducted with headstarts from The Wetlands Institute and we have since conducted additional experiments examining other physiological functions (swimming, diving, terrestrial locomotion) as well as personality traits.

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