

EFFECT OF WATER SALINITY AND FOOD SALT CONTENT ON GROWTH AND SODIUM EFFLUX OF HATCHLING DIAMONDBACK TERRAPINS (MALACLEMYS)¹

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At hatching and for many months afterward (until they reach about 50 g), diamondback terrapins cannot grow in salinities above about two-thirds seawater. Yet salinities near the nests are above this level. Very small terrapins reared in 100% seawater and offered one drink of fresh water every 2 wk can achieve limited growth. In 25% seawater at 28 C, growth of hatchling terrapins is stimulated in comparison with animals in fresh water or 50% seawater. Hatchlings grown in 0.25 Molal glycerol solutions, osmotically similar to 25% seawater, have the same growth rates as those in fresh water. Hatchling terrapins injected with NaCl have an elevated rate of sodium efflux (about 65 $\mu\text{mol}/100\text{ g wet mass}\cdot\text{h}$), indicating that the lachrymal salt glands are functional. At hatching, body water content (77.0%) is much higher than that of adults (64.5%), and body sodium concentration ($\mu\text{mol}/\text{g dry mass}$) is slightly higher. However, sodium concentration in units of $\mu\text{mol}/\text{g wet mass}$ is only 71% of adult levels. When feeding commences, body sodium concentration (as $\mu\text{mol}/\text{g wet mass}$) rises to adult levels. Further increases occur in salinities up to 50% seawater. There is an inverse relation between body size and water efflux in 100% seawater, and this difference appears to be one cause of the lesser tolerance of smaller terrapins to saline water. In contrast, sodium influx in hatchlings is only slightly elevated. The rate of sodium efflux in fed terrapins is directly correlated with feeding rate. However, the main source of sodium uptake at higher salinities appears to be incidental swallowing of water during ingestion of food—not the salt content of the food itself.

INTRODUCTION

Although certain aspects of osmoregulation in the terrapin are fairly well understood (Dunson 1984), no attempt has previously been made to study hatchling growth and sodium and water balance. In other estuarine or coastal reptiles, it is not unusual for small animals to be less tolerant of saline waters than large ones, although the mechanism of such a difference in tolerance is poorly understood (Dunson 1981, 1982, unpublished data; Mazzotti and Dunson 1984; Dunson and Seidel 1986). The objective of this study was to determine whether hatchling terrapins could grow and maintain water and electrolyte balance in

seawater. Salinity tolerance was found to be lower in hatchling terrapins than in adults, so a detailed study of the routes of uptake and loss of sodium and water was made in hatchlings. Attention was particularly devoted to measurements in saline water of growth, water efflux, sodium influx and efflux, body water and sodium contents, food sodium content, and the effects of periodic drinking of fresh water.

MATERIAL AND METHODS

EXPERIMENTAL ANIMALS

Hatchling terrapins were obtained by incubating eggs taken from nests or from females after induction of oviposition by injections of oxytocin (Ewert and Legler 1978). Eggs were placed in covered plastic dishes on paper towels moistened with tap water and incubated at 28–30 C. Clutches (24) were obtained from the area near Chincoteague, Virginia, between June 11 and 30 in 1982–1984. A single clutch was obtained from a female captured in February, 1983, in Florida Bay; she laid eggs in captivity on July 19. Incubation times

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were about 53–60 days. In between exposures to the experimental saline treatments, hatchlings were kept in fresh water. They were placed in a tank tilted at one end so that a choice between land and water could be made. At the dry end of the tank, there was an infrared spotlight and a black light-blue ultraviolet fluorescent lamp. The photoperiod was 12L:12D. Terrapins were fed chopped fathead minnows (*Pimephales promelas*) every 2 to 4 days, with occasional supplements of squid. Other meats such as clam (*Mercentaria*) were eagerly taken when offered. Under these conditions the terrapins quickly became quite tame, would readily accept food from the hand, and obviously associated the presence of humans with food. This is an important attribute of these animals since it was critical that they feed readily despite confinement in small dishes and manipulation during measurement of body effluxes.

Growth tests were carried out in an incubator with each terrapin placed individually in a covered glass dish with just enough water to cover the carapace (100 ml for hatchlings). Turtles were continuously immersed during the course of a standardized 2-wk test period. Terrapins were first fed in the communal tank and then weighed 2 days later before placement in individual bowls. This day was then designated zero time, and seven feedings were made in the separate bowls at 2-day intervals (days 0, 2, 4, 6, 8, 10, 12). The final mass was determined on day 14, again on the second day after the previous feeding. Growth was estimated as the change (%/day) in initial wet mass. Measurement of ^{22}Na content was made on days 0, 4, 8 and 12 (see description of efflux below). Food consumption was estimated by counting the number of pieces of minnows that were eaten. Minnows were cut transversely into pieces small enough to be swallowed readily. The percentage of food consumption was calculated as the number of pieces eaten divided by the number offered. A standard amount of food that slightly exceeded the ad lib. consumption was offered during each experiment. In one series of tests the effect of salinity on growth of Virginia hatchlings fed minnows every 2 days was continued for about 30 days. Five different groups of five turtles each were uti-

lized. All five groups were tested first for 32 days in 25% seawater, then transferred to salinities of 0%, 25%, 50%, 75%, and 100% seawater for 33 days.

Water, sodium, and potassium contents were measured on freshly laid Virginia eggs (from one clutch), on recently emerged hatchlings that had not been in water (from a single clutch), and on terrapins grown at various salinities. Wet masses of each sample were taken with a Mettler electronic balance. Then water content was determined by drying in an oven at 100 C. Concentrated nitric acid was added to dissolve the tissue, then distilled water was used to dilute the sample. Sodium and potassium concentrations were determined in an air propane flame with a Varian Techtron model 1280 atomic absorption spectrophotometer (in Pennsylvania) or a Process and Instruments Corp. flame emission photometer with internal lithium standard (in Virginia). Water and cation contents of potential food items were similarly determined. Gastropods appear to be an important part of the natural diet, and it is likely that the shells differ considerably in composition from the body. Whole snails were analyzed and compared with crushed and dissected parts separated into body and shell portions. Fathead minnows were obtained from a commercial dealer in Pennsylvania. Squid were purchased frozen from a firm based in Hampton, Virginia. The remaining molluscs were collected near Chinco-teague Island, Virginia, in waters of about 30–32 ppt.

Artificial seawater (Instant Ocean) was used for all salinity exposures. Full strength (100% seawater) was defined as 35 ppt. All salinities are subsequently reported as a percentage of this value. Dilutions were checked with an American Optical refractometer, periodically calibrated by measurement of sodium concentrations.

WATER EFFLUX

The unidirectional loss of water (efflux) was measured in two groups of terrapins, hatchlings and yearlings. Six Virginia hatchlings taken randomly from a group of 60 were kept in fresh water for 2 wk without feeding, and then three were tested in fresh water and three in seawater. Each of the terrapins was injected with $^3\text{H}_2\text{O}$ (50 μCi)

intraperitoneally. They were placed immediately into individual 100-ml baths, the water was agitated, and a 200- μ l sample was taken after 5 min for liquid scintillation counting (Beckman model LS3801). This initial sample provided a measurement of any leakage from the injection site. The total amount of $^3\text{H}_2\text{O}$ injected was then determined by diluting an aliquot in a 1-liter bath and removing a 200- μ l sample after stirring. Samples from each turtle's bath were taken at 2 and 8 h and the content of $^3\text{H}_2\text{O}$ assayed. After subtraction of the initial leak value, efflux was linear up to 8 h, indicating that equilibration of the injected dose within the body occurred very rapidly. Effluxes were calculated with a two-compartment equation (Stokes and Dunson 1982).

Five yearling Florida Bay terrapins ranging from 55 to 109 g (mean 84 g) that had been exposed to various salinities earlier were kept in fresh water for the previous 3 wk. The protocol for flux measurement was similar to that for the hatchlings, except that the injected $^3\text{H}_2\text{O}$ was about 1 $\mu\text{Ci/g}$ wet mass, and the bath was 800 ml seawater. The baths were sampled at 0, 2.5, and 5 h. After subtraction of the zero time "leak" value, the rate of increase of $^3\text{H}_2\text{O}$ in the bath was linear, as before.

SODIUM INFLUX

The rate of uptake of ^{24}Na from 100% seawater was measured with three hatchlings in October. They had previously been kept in fresh water in a communal tank. Each terrapin was placed individually in 10 ml seawater isotopically labeled with $^{24}\text{NaCl}$ (about 0.5 $\mu\text{Ci/ml}$). At 1, 2, 8, and 12 h after placement in seawater, the terrapins were removed and rinsed for 2 min. They were then placed for 2 min in a beaker (while held motionless with a foam pad) on top of a NaI crystal attached to a photomultiplier and a multichannel analyzer (Canberra series 30). Uptake of ^{24}Na was most rapid during the first hour and then declined and remained at a steady rate for the next 11 h. The radioisotope accumulated by 1 h was thus assumed to include considerable amounts of surface absorption and not to represent the true uptake into the extracellular fluid. The influx was calculated between 1 and 12 h using a standard

two-compartment equation (Stokes and Dunson 1982). Note that this represents the sodium influx of unfed terrapins and probably reflects primarily the diffusional permeability of the integument. A much larger influx is presumed to occur during feeding because of incidental swallowing of seawater (estimated from high efflux values).

SODIUM EFFLUX

A test for the presence of a functional salt gland in hatchlings was made by intraperitoneal injection of NaCl loads (2 mmol/100 g wet mass) into three unfed terrapins (9.8–10.2 g) in November. Two days previously they had been similarly injected with $^{22}\text{NaCl}$ (0.2 μCi). The rate of loss of ^{22}Na was followed as above for 1 day in 100% seawater, and the efflux was calculated (Stokes and Dunson 1982). It has been suggested that Na efflux above a value of about 5 $\mu\text{mol}/100\text{ g wet mass} \cdot \text{h}$ represents salt gland secretion (Robinson and Dunson 1976).

Numerous measurements were made of the rates of Na efflux of terrapins exposed to various salinities, offered periodic drinks, and fed food differing in salt content. All of these animals were being tested in the standard 2-wk growth procedure described above. They were previously injected with $^{22}\text{NaCl}$ (0.2 μCi), and their gamma radiation was assayed at 4-day intervals on days 0, 4, 8, and 12. An exponential curve fit was made on an HP-85 computer ($\text{cpm} = ae^{-bt}$, where cpm = radioactivity in the terrapin at time = t). Efflux was then calculated in the usual fashion for the 12-day interval. Effluxes were remarkably constant as long as the feeding rate was maintained. Assuming that the terrapins were in a steady state, these effluxes should represent excretion of sodium that came in via the integument (diffusional), the food, and drinking (incidental to feeding and otherwise).

RESULTS AND DISCUSSION

NEST DEPOSITION SITES

Hatchling terrapins are small (5–8 g) and may have very limited powers of dispersal. Therefore the placement of the nests by adult females indicates what salinity conditions the young must be exposed to at hatching and for some time thereafter. In

the study area near Chincoteague Island, there are no rivers. Salinities along this area of the eastern shore of Virginia remain near oceanic levels (28–33 ppt) throughout the year. Nesting sites of terrapins are commonly found in any area of higher elevation near tidal marshlands, regardless of the distance from the mainland and areas of potential freshwater runoff. Four main nesting areas were monitored between 1982 and 1984. One was on the mainland at the head of a tidal channel dredged for a boat basin, another was an elevated road bed next to a dredged channel on Wallops Island, a third was a high natural beach on the south side of Chincoteague Point Island (adjacent to Chincoteague Channel), and the fourth was a natural strand of sand on the northern end of Morris Island (between Assateague and Chincoteague Islands). None of these sites was close to sources of brackish water. Their common feature was the presence of sandy soil elevated about the high tide line. At the Morris Island site, some spring tides would probably inundate the area and cover the nests. Many nests on Chincoteague Point Island appeared also to be close to the high tide line. There are no data available on the tolerance of the eggs to submergence in seawater.

Very little is known about nesting in the Florida Bay terrapins, but it appears that they nest on the sand berms that occur along the edges of many islands. P. Patti (unpublished observations) dug up one nest in such a location at Trout Cove Island, and the presence of old eggshells has indicated the use of similar locations on other islands. It is unlikely that the hatchlings could survive in the open bay, so they may seek shelter and food in the interior lagoons of the islands. The presence of large numbers of adults in these flooded interior sites indicates that they are suitable habitat for adult terrapins (Wood 1981). Presumably the young, as in most turtle species, are extremely cryptic and elude detection.

SALINITY AND GROWTH

Data on nest placement indicate that hatchling terrapins in Florida and Virginia are exposed to 100% seawater. Therefore it was quite surprising to find that they can not grow in 100% seawater in the laboratory (fig. 1).

In contrast to the inhibition of growth at high salinities, there was a striking acceleration of growth in progeny of one Florida Bay female terrapin in 25% seawater (fig. 1). Twelve Virginia hatchling terrapins each tested at 28 C also showed a significant stimulation (*t*-test, $P = .001$) of growth in 25% seawater ($3.3\% \pm 1.1\%/day$) in comparison with fresh water ($1.4\% \pm 0.4\%/day$). Another group of six hatchlings reared in 0.25 molal glycerol (osmotically equivalent to 25% seawater) had the same rate of growth ($1.3\% \pm 0.7\%/day$) as the freshwater group (*t*-test, $P = .36$). Note also how closely these growth rates correspond to those obtained for Florida Bay hatchlings (fig. 1). Thus it appears that (a) there is a growth optimum near 25% SW for hatchlings from Virginia or Florida Bay, and (b) hatchlings require a certain level of salt intake for optimum growth, and they do not obtain adequate amounts in fresh water. Of course these hatchlings were fed freshwater fish rather than their natural food of marine invertebrates. However, data presented below will establish that much greater amounts of salt are gained from ingestion of saline water incidental to feeding than are taken in directly via the food itself.

HATCHLING WATER AND ION CONTENT

Immediately after hatching, terrapins have a higher water, sodium, and potassium concentration than do whole, freshly laid eggs (table 1). Their body composition is very similar to that of the freshwater painted turtle at hatching. In comparison with adult male terrapins, new hatchlings have a much higher water content, a similar sodium concentration (expressed as $\mu\text{mol/g}$ dry mass), but a lower sodium concentration (expressed as $\mu\text{mol/g}$ wet mass). Once feeding starts, body sodium concentration of hatchlings rises considerably (see table 2, figures for 0% seawater) so that (expressed per gram dry matter) it considerably exceeds that of the adults. However, it appears that sodium concentrations expressed in $\mu\text{mol/g}$ wet mass are quite comparable in adults and hatchlings. This suggests that extracellular fluid volumes are relatively larger in hatchlings, but that fluid sodium concentrations are similar to those of adults. Many other vertebrates (e.g., trout; Shearer 1984) also possess a higher water

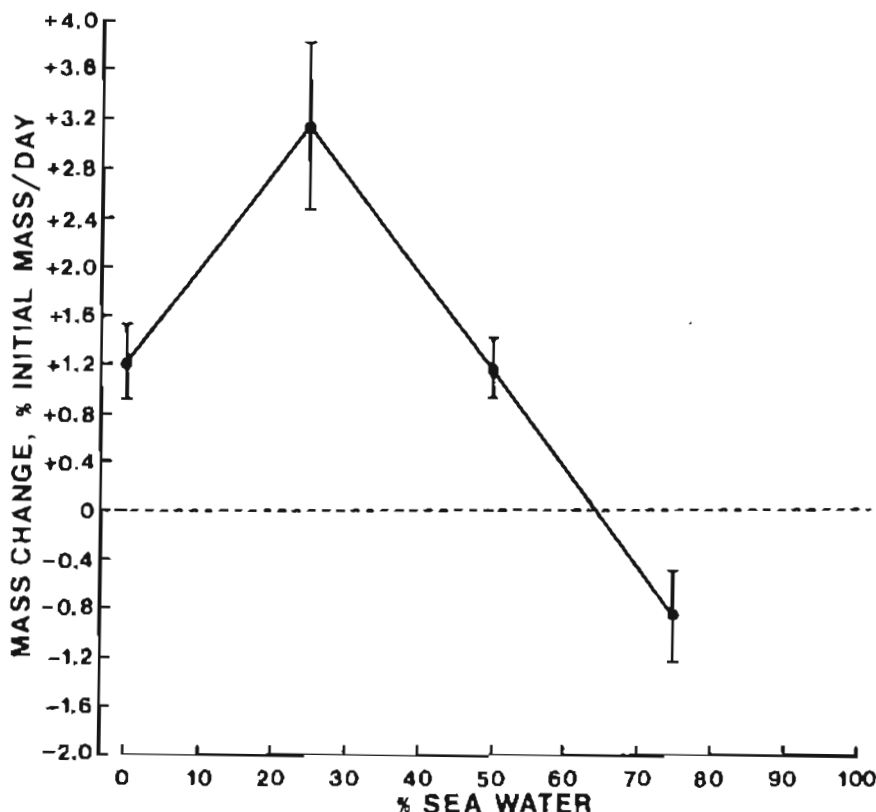


FIG. 1.—Growth rates of six hatchling terrapins from Florida Bay fed fathead minnows on alternate days at different salinities (28 C). Turtles were immersed in water and had no access to other salinities for drinking water. Initial masses for the 0%, 25%, 50%, and 75% seawater tests were 7.5 ± 0.2 , 8.5 ± 0.5 , 12.6 ± 1.2 , and 12.3 ± 1.4 g, respectively. Means \pm SD.

content, and thereby sodium content/gram dry mass, at smaller sizes.

EFFECT OF SALINITY ON BODY CONTENT

Five groups of Virginia hatchling terrapins grown at 23 C at five different salinities

were subsequently killed and analyzed for water and ion content (table 2). All groups were first tested in 25% seawater for 32 days. Their initial masses were not significantly different, nor were their rates of mass gain in 25% seawater (F -test, $P > .05$). However,

TABLE 1
WATER AND ION CONTENT OF FRESHLY LAID EGGS AND TURTLES IMMEDIATELY AFTER HATCHING^a

Species	Sample	Wet Mass, g	Water, % Wet Mass	Na (μ mol/g dry mass)	K (μ mol/g dry mass)	Na (μ mol/g wet mass)
<i>Malaclemys terrapin</i>	Fresh eggs	$8.3 \pm .4$ (3)	$71.1 \pm .2$	234 ± 7	109 ± 3	68
<i>M. terrapin</i>	Fresh hatchlings	$7.8 \pm .5$ (5)	$77.0 \pm .6$	333 ± 25	179 ± 12	77
<i>Chrysemys picta</i>	Fresh hatchlings	$4.6 \pm .2$ (4)	76.1 ± 1.6	327 ± 19	158 ± 12	78
<i>M. terrapin</i> ^b	Adults in seawater	224 (12)	64.5	307	...	109

^a Means \pm SD; n in parentheses.

^b From Robinson and Dunson (1976).

TABLE 2
EFFECT OF WATER SALINITY ON WATER AND ION CONTENT OF HATCHLING TERRAPINS AT THE CONCLUSION OF A 33-DAY PERIOD

SALINITY, % SEAWATER	INITIAL MASS, g	INITIAL CARAPACE LENGTH (CL), cm	% wet mass	FINAL H ₂ O CONTENT			FINAL IONS		
				ml/cmCL	Na (μmol/g dry mass)	K (μmol/g dry mass)	Na (μmol/g wet mass)		
0	8.0 ± 1.4	3.1 ± .3	77.4 ± 3.0	2.09 ± .35 ^a	494 ± 38 ^b	203 ± 19 ^a	103 ± 3 ^b		
25	8.3 ± 1.3	3.2 ± .2	81.0 ± 1.0	2.43 ± .28 ^b	592 ± 63	199 ± 30	112 ± 6		
50	8.9 ± 1.2	3.3 ± .2	81.6 ± 2.2	2.27 ± .30	704 ± 83 ^c	207 ± 14	139 ± 16		
75	9.6 ± 1.0	3.4 ± .2	79.5 ± 1.8	2.13 ± .09	658 ± 113	210 ± 15	139 ± 16		
100	10.0 ± 1.0	3.4 ± .2	78.7 ± 1.1	1.94 ± .10 ^{a,b}	688 ± 147 ^b	212 ± 16 ^a	146 ± 24 ^b		

NOTE.—At 23 C, *n* = 5 for each salinity. Food was fathead minnows. Means ± SD.

^a Not significantly different from each other, *t*-test.

^b Significantly different from each other, *t*-test.

^c One value of 1,032 deleted.

TABLE 3
EFFECT OF BODY SIZE ON WATER AND SODIUM FLUXES IN UNFED
DIAMONDBACK TERRAPINS (*Malaclemys terrapin*)

Body Mass, g	Test Salinity % Seawater	"Acclimation" Salinity % Seawater	Water Efflux (ml/100 g wet mass · h)	Sodium Influx (μ mol/100 g wet mass · h)	Sodium Efflux (μ mol/100 g wet mass · h)
224-270 (11) ^a	100	100	.16 \pm .05	10.0 \pm 6.3	6-58 ^b
84 \pm 21 (5)	100	0	1.0 \pm .1	...	4-74 ^c
7.5 \pm .6 (3)	100	0	1.02 \pm .12	17.4 \pm 4.9	65 \pm 28 ^d
7.0 \pm .5 (3)	0	0	2.74 \pm 1.19

NOTE.—Means \pm SD; *n* in parentheses.

^a From Robinson and Dunson (1976).

^b Highest value when NaCl injected, 3 mmol/100 g.

^c Highest value when fed clams.

^d Injected NaCl load, 2 mmol/100 g.

after 33 days at one of five salinities, some interesting effects were observed on body contents (table 2). Body water was analyzed in units of ml/cm carapace length to more clearly distinguish small changes (see Robinson and Dunson [1976] for further discussion). Water/unit length was highest in 25% seawater and significantly lower in 100% seawater (*t*-test, $P = .003$). Between 25% and 100% seawater, the relation between salinity (S in %) and body water per unit length (W in ml/cm) was as follows: $W = 2.599 - 0.00645S$; $r^2 = .46$; $P < .005$. Sodium concentration increased significantly with increasing salinity, although the primary effect occurred between salinities of 0 and 50% seawater (table 2). At 50% seawater and above, body sodium concentration was constant. Between 0% and 50% seawater, the relation between salinity (S in %) and body sodium concentration (Na in μ mol/g dry mass) was as follows: $Na = 491.8 + 4.179S$; $r^2 = .70$; $P < .01$. Body potassium concentrations remained remarkably constant at all salinities.

EFFECTS OF BODY SIZE

Adult male terrapins have low rates of water efflux (about 0.2 ml/100 g · h) and sodium influx (10 μ mol/100 g · h). They have a variable rate of sodium efflux that may increase to about 60 μ mol/100 g · h after salt loading (Robinson and Dunson 1976; table 3). Smaller terrapins of two sizes differed in only one important respect; they had a fivefold greater water efflux in 100% seawater (table 3). Water efflux of hatchlings

in fresh water was even higher. It is important to note that all of these measurements were made on unfed animals. The water efflux may differ dramatically when the terrapins are feeding. It is also possible that the salinity of prior exposure may have had some effect on the water efflux. Since the two smallest groups had been in fresh water previously, an acclimation effect may have caused the difference observed between young and adults. Note, however, that the hatchling's salt glands were fully functional when NaCl was injected; sodium efflux was equal to that of salt-stimulated adults.

There is a striking increase in salinity tolerance within the first year of growth that these physiological measurements do not reveal. In October, 12-g Virginia hatchlings offered minnows as food lost 0.9% \pm 0.4% mass/day in 75% seawater. By March of the next year, the 31-g terrapins gained 1.2% \pm 0.4%/day in a 2-wk growth test at a salinity of 75% seawater. By April three terrapins of 42-50 g were even able to grow in 100% seawater (0.5%-0.7%/day). Yet two other, slightly smaller turtles (31 and 33 g) either lost mass or gained very little. Therefore, it appears that, when terrapins achieve a body mass of about 50 g, they are capable of growth in 100% seawater. However, it is not yet clear what changes in osmoregulation account for this increase in tolerance.

EFFECTS OF DRINKING RAINWATER

Dunson and Lazell (1982) and Mazzotti and Dunson (1984) have concluded that

TABLE 4

THE EFFECT OF PERIODIC CHANGES IN SALINITY AT FEEDING TIMES ON FEEDING RATE, GROWTH RATE, AND SODIUM EFFLUX OF HATCHLING DIAMONDBACK TERRAPINS (*Malaclemys terrapin*)

Conditions	Initial Wet Mass, g	Mass Change, % Initial/Day	Sodium Efflux ($\mu\text{mol}/100$ g wet mass · h)	Feeding, % Eaten
100% SW, (7FW)	8.1 \pm .4	+45 \pm .20	3.9 \pm 1.5	69 \pm 19
25% SW, (7FW)	7.8 \pm .7	+41 \pm .65	4.1 \pm 2.2	74 \pm 12
100% SW, (1FW)	8.4 \pm .4	+60 \pm .53	12.3 \pm 4.1	29 \pm 12
25% SW	7.8 \pm 1.3	+2.07 \pm .64	12.4 \pm 3.9	53 \pm 19
100% SW (1-14% SW)	8.8 \pm 1.1	...	17.9 \pm 16.9	20 \pm 10
50% SW	10.2 \pm 2.9	...	20.7 \pm 8.4	60 \pm 17

NOTE.—Fed fathead minnows at 28 C. Means \pm SD; $n = 6$ for each experiment. SW = seawater; FW = freshwater; number preceding salinity in parentheses indicates times this solution offered along with food.

black rats and small crocodiles in south Florida may drink from rainwater floating on the sea surface. The notion that terrapins also drink rainwater has been suggested, although the accounts were only anecdotal (Dunson 1970). A test of the possibility that terrapins drink brackish water was made by simulating periodic rainfall in the laboratory. Terrapins too small to grow in 100% seawater were offered fresh or brackish water only at the time of feeding minnows (about 2-4 h each time). In December, terrapins kept in 100% seawater (15 g) gained $1.0\% \pm 0.7\%$ /day when offered 11% seawater at all seven feedings during a 2-wk growth trial. In February (body mass 23 g) excellent growth occurred ($1.6\% \pm 0.3\%$ /day) when only three of the seven feedings were made in 11% seawater. A further reduction to feeding only once in 11% seawater still yielded growth of $1.1\% \pm 0.7\%$ /day in terrapins of 27 g.

A further series of 2-wk growth tests was then set up to examine the value of periodic exposure to fresh or brackish water for the smallest hatchlings (table 4). These data are arranged in order of increasing sodium efflux; the size of the efflux should roughly correlate with the degree of salinity stress. If all seven feedings are made in fresh water, hatchling terrapins grow and undergo only a low rate of sodium efflux. This was the first indication of two most significant findings: (a) The smallest terrapins can grow in 100% seawater if fresh water is periodically available; and (b) The rate of sodium efflux

is related not to the amount of food eaten but to the salinity in which ingestion occurs. Therefore incidental swallowing of saline water during feeding may be the major route of sodium influx. In 25% seawater with seven freshwater "drinks," growth and efflux remained about the same. When terrapins in 100% seawater were fed only once in fresh water, growth again was the same, but sodium efflux increased substantially to about $12 \mu\text{mol}/100$ g · h, and feeding decreased. In 25% seawater with no "drinks," sodium efflux was the same, but growth increased dramatically to about 2%/day. In the final two tests, growth was not measured, but the relation between feeding rate and sodium efflux was more fully explored. It has been suggested above that the main source of sodium intake is via incidental ingestion of saline water during feeding. This was further confirmed by the observation that variation between individuals in feeding rate is directly correlated with the rate of sodium efflux. In fresh water, terrapins eating comparable amounts of food do not show such high and variable rates of sodium efflux. In 100% seawater with one "drink" of 14% seawater given in 2 wk, the rate of sodium efflux (E as $\mu\text{mol}/100$ g wet mass · h) was related to the amount of food eaten (F as % of offered fish eaten) in the following way: $E = -14.715 + 1.632 F$; $r^2 = .88$, $P < .005$. In 50% seawater with no "drinks" given, the relation was as follows: $E = 4.112 + 0.310 F$; $r^2 = .88$, $P < .01$.

TABLE 5
WATER AND ION CONTENT OF POSSIBLE TERRAPIN FOOD

Species	Sample	Water, % Wet Mass	Na ($\mu\text{mol/g}$ dry mass)	K ($\mu\text{mol/g}$ dry mass)
Fathead minnow, <i>Pimephales promelas</i> . . .	Whole fish	81.3 \pm .5 (4)	273 \pm 20	294 \pm 34
Squid	Mantle tissue	82.7	400	187
Mussel:	Body	73.4	717	309
<i>Mytilus edulis</i> ^a	Shell	148	6
Hard clam, <i>Mercenaria mercenaria</i>	Body	78.5 \pm .5 (3)	827 \pm 9	266 \pm 10
Mud snail, <i>Nassarius obsoletus</i>	Whole snail	27.3 \pm 1.7 (3)	374 \pm 18	23 \pm 4
<i>N. obsoletus</i>	Whole snail	30.8 \pm 3.1 (3)	378 \pm 8
<i>N. obsoletus</i>	Body	75.3 \pm 4.6 (3)	809 \pm 160
<i>N. obsoletus</i>	Shell	8.3 \pm 1.2 (3)	235 \pm 25
Periwinkle, <i>Littorina irrorata</i>	Whole snail	30.0 \pm 2.5 (3)	377 \pm 52
<i>L. irrorata</i>	Body	79.2 \pm 3.3	805 \pm 212
<i>L. irrorata</i>	Shell	6.9 \pm 2.2	149 \pm 27

NOTE.—Means \pm SD; *n* in parentheses.

^a Roesijadi and Creelius (1984).

Annual rainfalls at the study sites in Virginia and Florida are generally similar in total amount (104 cm vs. 124 cm, respectively) but very different in temporal distribution. Wallops Island, Virginia, has an extremely regular monthly distribution, with months of high (March) and low (October) precipitation differing by only 3.5 cm. In contrast, Tavernier, Florida (on Key Largo), has a pronounced wet and dry season, with a difference between the high and low months of 16.5 cm. Rainfall over Florida Bay peaks in June and October. The interior lagoon of one island harboring numerous terrapins varied typically between 51% and 86% seawater during repeated visits and once fell to 23% seawater after extremely heavy rains. These were average salinities of the entire lagoon; during rainfall hatchling terrapins could undoubtedly obtain virtually fresh water in direct runoff from the slightly elevated sand banks surrounding the inner lagoon. The youngest terrapins seem well adapted for utilizing even the sporadic rainfall of the dry season. However, in Florida Bay they probably have at least one very rainy month (if they hatch by the end of September) before the beginning of the dry season. Very little is known at present about any possible synchronization between laying, hatching, and rainfall patterns in Florida. There is an indication that the Florida Bay population lays later (in July) than the Virginia terra-

pins, but the present data on laying times in Florida Bay are quite inadequate.

FOOD SODIUM AND WATER CONTENT

Terrapins appear to be rather omnivorous and may even scavenge for food; they are often caught in crab pots. Their normal diet is primarily marine invertebrates. Coker (1906) reported stomach contents containing the gastropod molluscs *Littorina* and *Melampus*, the annelid worm *Nereis*, and various crabs. I have collected feces from Virginia specimens that contained hundreds of opercula from mud snails (*Nassarius*). Since most of the present experiments were conducted with a vertebrate food (fish), it is worth considering how the natural prey may differ in water and salt content from this experimentally convenient, but perhaps atypical, food. Table 5 lists the results of such analyses. If one discards the shell, the molluscs do not differ much in water or potassium concentration from the fathead minnow. However, the clams and gastropods contain about 800 μmol sodium/g dry mass of flesh, a value about three times that of fathead minnows.

A series of growth tests were performed to determine whether food salt concentration or water salinity were more important in affecting sodium intake, and thereby eflux, of terrapins. The turtles used were about 1 yr old and readily accepted the different diets offered. Sodium eflux was quite

TABLE 6
EFFECT OF DIET AND WATER SALINITY ON SODIUM EFFLUX AND GROWTH
OF YOUNG *Malaclemys* FROM FLORIDA BAY

Initial Wct Mass, g (n)	Salinity, % SW	Days	Mass Change, % Initial/Day	²² Na Efflux (μ mol/100 g wct mass·h)
Unfed:				
48 \pm 10 (5)	75	6	. . .	4 \pm 4
Fathead minnow diet:				
44 \pm 8 (5)	75	13	+ .57 \pm .27	58 \pm 6
~50 (5)	75	4	. . .	49 \pm 7
Squid diet:				
51 \pm 10 (5)	0	13	+ .84 \pm .24	7 \pm 1
55 \pm 12 (5)	75	13	+ .34 \pm .07	38 \pm 9
Clam diet:				
60 \pm 14 (5)	0	14	+1.68 \pm .26	10 \pm 4
78 \pm 13 (4)	75	14	+ .73 \pm .19	74 \pm 7
51 (1)	75	14	-.18	16

NOTE.—At 28 C. Means \pm SD; n in parentheses.

low in unfed terrapins in 75% seawater or in those fed high-salt diets in fresh water (table 6). High rates of efflux occurred only when terrapins were fed minnows or clams in 75% seawater. Differences in maximum rates of sodium efflux achieved in 75% seawater while eating minnows, squid, or clams are most likely related to the relative amounts of each food ingested and thereby incidental swallowing of saline water. This is illustrated by the smallest terrapin offered clams in 75% seawater; it apparently ate little, lost mass, and had a sodium efflux considerably less than that of its siblings, which gained mass. Thus a maximum of 13.5% (10/74) of the sodium efflux of terrapins feeding on the most salty diet (whole clams) could be attributed directly to sodium taken in through food. This assumes that the animals were in a steady state and that they ate comparable amounts in the two salinities.

GENERAL DISCUSSION AND CONCLUSIONS

The diamondback terrapin (*Malaclemys*) occupies a unique position among the relatively few reptiles that can live in saline waters. It is restricted to estuarine, coastal waters of high salinity (Dunson 1970) and is the only member of its family (Emydidae) to possess a salt gland. The only other chelonians with salt glands are the two families of true sea turtles (Cheloniidae, Dermochelyidae; Dunson 1984). The terrapin salt

gland secretes sodium at a slower rate than that of the true sea turtles (Dunson 1976) and only achieves maximum stimulation in Na-K ATPase levels when the plasma sodium concentration nears 200 mM (Dunson and Dunson 1975). Adult terrapins have a complex set of physiological adaptations for hypoosmoregulation, including expansion of the interstitial fluid to very high levels (up to 19% wet mass), perhaps as a means of "storing" water for periods of dehydration (Robinson and Dunson 1976). Urea accumulation in the plasma (up to one-fourth of the total osmotic pressure) assists in conservation of water by decreasing the gradient for osmotic water loss between plasma and seawater (Gilles-Baillien 1970). Adjustments in intracellular osmotic pressure also occur via increases in amino acids and ammonia (Gilles-Baillien 1973). In early stages of dehydration terrapins can rely on their ability to decrease net water loss and net sodium uptake to a minimum (Robinson and Dunson 1976). However, to a degree quite unusual among marine reptiles, terrapins appear to rely on drinking fresh or brackish water to replenish body water stores. The desirability of supplying fresh water to captive terrapins was noticed very early when attempts were made to cultivate them for food (Coker 1906). Terrapin trappers who hold the animals in pens of seawater for later sale also learned that it was advisable

to "water" them occasionally with fresh water (Dunson 1970). Indeed, terrapins that are dehydrated show an avid interest in fresh drinking water, so much so that they will drink from a puddle or from a spigot in captivity. An experienced Virginia trapper (Miles Hancock) reported that terrapins will drink rainwater as it runs off the marsh (in Dunson 1970). Thus terrapins possess a suite of specific physiological and behavioral adaptations for surviving in seawater, a habitat that is extremely rigorous for reptiles. They appear to be the most specialized living example of a reptile that is neither a fully marine nor a fully freshwater species.

The present study has contributed to our knowledge of the effect of salinity on hatchling terrapins in several ways. It was not previously known that hatchlings fail to grow in seawater without an exogenous source of water other than the food. An additional new finding was the presence of an optimum in growth of hatchling terrapins continuously immersed in water of constant salinity. The optimum occurred at 25% SW; growth was significantly less in either fresh water or 50% SW (fig. 1). This suggests that hatchling terrapins require a greater quantity of salt for growth than do freshwater turtles. A similar effect has been recorded for estuarine populations of snapping turtles (Dunson, unpublished data). It appears then that the process of adaptation to growth in saline waters, presumably involving a shift in various physiological mechanisms, predisposes estuarine species to be less efficient at growth in fresh water.

The present laboratory data present an apparent paradox in that hatchling terrapins are unable to grow at salinities that are found near nest sites. However, a clue is provided by the experiments in which fresh water was periodically offered at feeding times for terrapins otherwise immersed in 100% SW. Such periodic access to fresh drinking water permits growth and is an obvious simulation of rainfall events that are common in the late summer and early fall after hatching. One would predict that sites offering access to rainwater, in the form of runoff from banks or collection in brack-

ish pools, would be most favorable for hatchling growth.

The concept that incidental swallowing of saline water during feeding is the major source of sodium influx in terrapins is an important new finding. It suggests that feeding adaptations might develop in marine reptiles to diminish the uptake of water with the food bolus. Turtles feeding on soft-bodied food would be predisposed to high levels of salt intake. Indeed the peculiar, backward-pointing spines in the esophagus of sea turtles have been suggested to act as a filter to retain food particles during ejection of excess seawater from the stomach (Den Hartog and Van Nierop 1984). The spines are present in both *Chelonia*, which feeds on plants, and in *Dermochelys*, whose main food appears to be pelagic coelenterates. In contrast, snakes that swallow entire small fish might gain only minimal amounts of saline water during the swallowing process. In fact, the mangrove snake (*Nerodia fasciata compressicauda*) is commonly found along with terrapins in highly saline mangrove areas, yet it manages to osmoregulate without a salt gland (Dunson 1980). Small crocodiles (*Crocodylus acutus*) also appear to be able to grow under saline conditions without a functional salt gland; they feed on whole prey (often small fish), as do mangrove snakes. A similar explanation might pertain to the puzzling variation in sizes of salt glands and their maximum secretory capacity among sea snakes (Dunson and Dunson 1974; Dunson 1976). Species of *Hydrophis* and *Laticauda* tend to have extremely small salt glands; they feed on elongate eels which are individually much larger prey than fish taken by a large salt glanded form such as *Pelamis*. Consequently *Hydrophis* and *Laticauda* might ingest much less seawater during feeding and require less capacity for extracloacal salt excretion. The sea snake with the highest known rate of salt gland secretion is *Aipysurus eydouxii* (Dunson 1975), which feeds only on fish eggs (Limpus 1975) and must ingest relatively much more seawater per unit of "prey" mass than snakes that prey on fish.

LITERATURE CITED

COCKER, R. E. 1906. The natural history and cultivation of the diamond-back terrapin. N.C. Geol. Survey Bull. no. 14. 67 pp.

DEN HARTOG, J. C., and M. M. VAN NIEROP. 1984. A study of the gut contents of six leathery turtles *Dermochelys coriacea* (Linnaeus) (Reptilia: Tes-

- tudines: Dermochelyidae) from British waters and from the Netherlands. Zool. Verh. no. 209, pp. 1-36.
- DUNSON, M. K., and W. A. DUNSON. 1975. The relation between plasma Na concentration and salt gland Na-K ATPase content in the diamondback terrapin and the yellow-bellied sea snake. J. Comp. Physiol. 101:89-97.
- DUNSON, W. A. 1970. Some aspects of electrolyte and water balance in three estuarine reptiles, the diamondback terrapin, American and "salt water" crocodiles. Comp. Biochem. Physiol. 32:161-174.
- . 1975. Salt and water balance in sea snakes. Pages 329-353 in W. DUNSON, ed. The biology of sea snakes. University Park Press, Baltimore.
- . 1976. Salt glands in reptiles. Pages 413-445 in C. GANS and W. R. DAWSON, eds. Biology of the reptilia. Vol. 5. Academic Press, New York.
- . 1980. The relation of sodium and water balance to survival in sea water of estuarine and freshwater races of the snakes *Nerodia fasciata*, *N. sipedon* and *N. valida*. Copeia 1980:268-280.
- . 1981. Behavioral osmoregulation in the Key mud turtle, *Kinosternon b. bairii*. J. Herpetology 15:163-173.
- . 1982. Salinity relations of crocodiles in Florida Bay. Copeia 1982:374-385.
- . 1984. The contrasting roles of the salt glands, the integument and behavior in osmoregulation of marine and estuarine reptiles. Pages 107-129 in A. PEQUEUX, R. GILLES, and L. BOLIS, eds. Osmoregulation in estuarine and marine animals. Vol. 9. Lecture notes on coastal and estuarine studies. Springer, New York.
- . 1985. Estuarine populations of the snapping turtle (*Chelydra*) as a model for the evolution of marine adaptations in reptiles. In preparation.
- DUNSON, W. A., and M. K. DUNSON. 1974. Interspecific differences in fluid concentration and secretion rate of sea snake salt glands. Am. J. Physiol. 227: 430-438.
- DUNSON, W. A., and J. D. LAZELL, JR. 1982. Urinary concentrating capacity of *Rattus rattus* and other mammals from the lower Florida Keys. Comp. Biochem. Physiol. 71A:17-21.
- DUNSON, W. A., and M. SEIDEL. 1986. Salinity tolerance, growth and osmoregulation of estuarine and island-dwelling Emydid turtles. (*Pseudemys nelsoni* and *Trachemys decussata*) in sea water. J. Herpetology (In press).
- EWERT, M. A., and J. M. LEGLER. 1978. Hormonal induction of oviposition in turtles. Herpetologica 34:314-318.
- GILLES-BAILLIEN, M. 1970. Urea and osmoregulation in the diamondback terrapin *Molacllemys centrata centrata* (Latreille). J. Exp. Biol. 52:691-697.
- . 1973. Isosmotic regulation in various tissues of the diamondback terrapin *Molacllemys centrata centrata* (Latreille). J. Exp. Biol. 59:39-43.
- LIMPUS, C. J. 1975. Coastal sea snakes of subtropical Queensland waters (23° to 28° south latitude). Pages 173-182 in W. DUNSON, ed. The biology of sea snakes. University Park Press, Baltimore.
- MAZZOTTI, F. J., and W. A. DUNSON. 1984. Adaptations of *Crocodylus acutus* and *Alligator* for life in saline water. Comp. Biochem. Physiol. 79A:641-646.
- ROBINSON, G. D., and W. A. DUNSON. 1976. Water and sodium balance in the estuarine diamondback terrapin (*Molacllemys*). J. Comp. Physiol. 105:129-152.
- ROESIADI, G., and E. A. CRECELIUS. 1984. Elemental composition of the hydrothermal vent clam *Culyptogena magnifica* from the East Pacific Rise. Mar. Biol. 83:155-161.
- SHEARER, K. D. 1984. Changes in elemental composition of hatchery-reared rainbow trout, *Salmo gairdneri*, associated with growth and reproduction. Can. J. Fisheries Aquatic Sci. 41:1592-1600.
- STOKES, G. D., and W. A. DUNSON. 1982. Permeability and channel structure of reptilian skin. Am. J. Physiol. 242:F681-F689.
- WOOD, R. 1981. The mysterious mangrove terrapin. Florida Naturalist, July-September, pp. 6-7.