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Behavioural osmotic control in the euryhaline diamondback terrapin *Malaclemys terrapin*: responses to low salinity and rainfall

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(With 4 figures in the text)

Diamond terrapins, *Malaclemys terrapin* Latreille, inhabit salt marshes and estuaries where they may encounter sustained high salinities for weeks or months. Terrapins can discriminate between salinities. When salt-loaded they avoid drinking high salinities (27·2–34·0‰), drink small amounts of salinities which are a little more concentrated than the blood (13·6–20‰), and drink copious quantities of lower salinities (0–10·2‰). After seven days in full sea water (34‰) they can rehydrate themselves in < 15 min if given access to fresh water. Terrapins are capable of drinking from the thinnest of freshwater films (1·6 mm), exploit menisci and have specific postural responses to collect small quantities of fresh water from horizontal and vertical surfaces. Specimens of *Malaclemys terrapin* respond to the vibration of simulated rainfall by rapid emergence followed by drinking from thin films, either on the exposed substratum or from the surface of the water column. Under simulated conditions of heavy rainfall they collect rain directly from above.

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Introduction

The medium-sized emydid diamondback terrapin *Malaclemys terrapin* Latreille is one of the few reptilian species which is able to survive in brackish-water habitats. Distributed from Cape Cod to southern Texas on the eastern coast of North America, the diamondback terrapin occurs in warm temperate and subtropical lagoons and estuarine waters, especially in salt marshes. A typical habitat is found in the extensive flat, low-lying salt marshes around Beaufort, North Carolina, where terrapins were farmed during the later phases of the species' commercial exploitation between the 1880s and 1920s. For long periods during the summer (when river outputs are low), terrapins live in a marine habitat with water column salinities above 28‰. Their diet is essentially marine throughout the year, terrapins browsing upon small marine animals (e.g. littorinid gastropods (*Littorina irrorata*), small fiddler crabs (*Gelasimus* sp.) and nereid worms (*Nereis irritabilis*) according to Coker, 1906; mussels (*Mytilus edulis*) according to Hurd, Smedes & Dean, 1979). In captivity, terrapins will survive in full sea water for many months (e.g. Gilles-Baillien, 1973*a*) and readily feed upon invertebrate food which contains similar salt concentrations to sea water). However, terrapins cannot survive indefinitely in sea water since they do not possess salt glands as powerful as those of such marine reptiles as sea turtles (Family Cheloniidae) and the marine iguana *Amblyrhynchus cristatus* (Dunson, 1976). Instead, they rely upon a low integumentary permeability to salts and water, plus reduced urinary output, to delay dehydration when in salt water (Dunson, 1979, 1984). Gilles-Baillien (1970, 1973*b*, *c*) showed that terrapins held in sea water exhibited a progressive increase in plasma urea concentration. She held that this response was adaptive since it reduced the osmotic gradient between blood and the external medium; Dunson (1979) believed that the increase in urea concentration simply resulted from strong antidiuresis since there is a general rise in tissue urea and NH_3 as well. Whichever view is correct, it is clear that diamondbacks are not in a stable physiological state when in sea water.

The investigation reported here stemmed from observations made by one of us (JD) in the Beaufort area during summer. Although salinities in the salt marshes were high and river inputs low, the area featured frequent coastal storms during which several centimetres of rain often fell within short periods. Since the water of the salt marshes is non-turbulent (mainly because of the vegetation which slows currents and protects against wind-driven turbulence) the occurrence of heavy rain suggested the possibility of stratification of the water column, with transient thin films of near-fresh water forming on the surface. In addition, direct observation showed that freshwater films formed on the surface of exposed intertidal mud during storms. It was thought that diamondback terrapins might be able to exploit such sources of fresh/low salinity water to replace water lost by osmotic dehydration, particularly as Dunson (1970) reported that a correspondent had told him that he had observed terrapins drinking rain water from mud banks. It was hypothesized that they would need to be able to drink quickly from thin films, would be able to discriminate finely between waters of different salinity, and might even be capable of responding to the vibration or sight of rainfall. The study described in this paper was designed to test these hypotheses.

Materials and methods

Collection and maintenance

Twelve male terrapins (about 5 years old judging from their size and scute ring numbers) were purchased from a biological supplier. They were held in water of about 5‰ at 25 °C and fed 3 times a week on fish.

mussels or squid until used in experiments. All experiments were carried out under appropriate Home Office licences.

Drinking discrimination

To determine whether diamondbacks would drink some salinities but not others, all of the animals were held for 7 days in full sea water (34‰). Each animal was dried superficially and weighed to the nearest 0·1 g. It was then held in one of the following salinities for 30 min: 0, 6·8, 10·2, 13·6, 20·4, 27·2 and 34·0‰. At the end of 30 min the animal was dried and reweighed. The whole experiment was repeated until 3 animals had been offered each salinity.

Length of drinking bouts

To obtain more detailed information about the effect of environmental salinity upon drinking behaviour, 12 animals were held in full sea water (34‰) for 7 days. They were next placed individually in clear, dry plastic tanks and left for 3 h to settle down. Then 500 ml of water was poured into the bottom of the tank, to form a layer *c.* 3 mm deep. Water of 6 different salinities (0, 3·4, 6·8, 10·2, 13·6, 17·0‰) was used, so that 2 animals encountered each salinity level. Drinking activity (i.e. gulping throat action combined with a submerged, open mouth) was timed until no such activity had been seen for 30 min. The whole experiment was repeated twice more so that a total of 6 animals was offered water of each salinity.

Drinking from thin films

To determine the minimum thickness of surface-water film that terrapins could drink from, the following experiment was carried out. A cubical perspex tank (side 30 cm) was constructed, using chloroform as an adhesive so that there were no internal beads of glue. Weighed amounts of distilled water were placed in the tank to produce water films of known thickness. Terrapins were salt-loaded by holding in full sea water (34‰) for at least 2 days, were dried using paper towels and weighed to the nearest 0·1 g. Each was then placed in the tank with a water film of known thickness and observed (photographically as well as visually). If drinking activity appeared to have taken place, the terrapin was reweighed to check that a weight increase had indeed occurred. Experiments were carried out with film thicknesses of 4, 3, 2 and 1·6 mm (the last being the minimum achievable).

To determine whether terrapins could drink from a layer of fresh water lying on top of a saline water column, the same cubical perspex tank was used. To perform an experiment, the tank was filled to a depth of 20 cm with sea water (34‰). A weighed, salt-loaded terrapin was placed in the tank and allowed to settle down for 30 min. A 3 mm-thick layer of distilled water (visualized by adding a small amount of methylene blue dye) was gently added to the tank. The terrapin was observed for signs of drinking behaviour and reweighed. A total of 3 terrapins was studied in this fashion.

Responses to simulated rainfall

To investigate responses to simulated rainfall, a varnished plywood platform, featuring 2 sunken drinking chambers, and reached by a ramp (crosscut to allow easy climbing by the terrapins) was placed in the centre of a high-sided, opaque holding tank which contained water of a depth of about 10 cm. Water in droplet form (either fresh or sea water) could be delivered to the whole apparatus (through an overhead watering-can rose), without otherwise disturbing the animals.

Five experiments were carried out, using all 12 available terrapins in each case. First, the animals were held in full sea water within the apparatus for 7 days, being fed in the usual fashion. When all terrapins were swimming or resting in the sea water, the drinking chambers were filled, one with fresh water, one with sea water. Simulated rainfall (fresh) was initiated and the animals were observed for 30 min. Secondly, after an interval of about 4 weeks, the experiment was repeated, but in this case the simulated rainfall consisted of sea water and no fresh drinking water was available. Thirdly, the terrapins were held in fresh water within the apparatus for 7 days and the initial experiment was repeated using freshwater 'rain'. Fourthly, the initial experiment was repeated, but in this case simulated rainfall (fresh) was delivered in complete darkness.

Finally, terrapins were salt-loaded in full sea water for 7 days before being exposed to extremely heavy simulated rain (fresh) for about 15 min; their behaviour was observed during the rainfall.

Anatomy and posture

During the behavioural experiments visual and photographic observations were made of the postures employed by terrapins during drinking. Drawings were made from 35 mm colour slides with the aid of a *camera lucida*. The anatomy of the mouth of a dead terrapin was inspected to determine whether there were any special modifications of the mouth to facilitate drinking from thin films (comparisons were made with a related freshwater emydid terrapin, *Mauremys caspica caspica* (Gmelin)).

Results

Drinking discrimination

Weight changes in animals offered various salinities after salt-loading are shown in Table I. From these data it may be seen that diamondbacks avoid drinking high salinities (34·0 and 27·2‰), drink small quantities of media containing more salt than the blood (20·4 and 13·6‰) but drink copiously in more dilute media (0–10·3‰). The data are quite variable, particularly in the case of terrapins drinking fresh water; this variability stems from defecation by animals during the 30 min period during which drinking took place.

Length of drinking bouts

The observed lengths of drinking bouts are presented in Table II. From these data it may be seen that there was a progressive decrease in length of drinking bout as the offered salinity was increased. In media of 13·6 or 17·0‰ the terrapins only drank for a few seconds, suggesting that

TABLE I

Drinking behaviour of salt-loaded terrapins: salinity discrimination

Salinity offered (‰)	% weight gain (mean)	% weight gain (range)
0·0	7·8	2·4–13·6
6·8	11·2	9·0–13·6
10·2	8·8	5·5–12·3
13·6	3·8	1·2–6·4
20·4	1·4	0·0–2·4
27·2	0·0	0·0–0·0
34·0	0·0	0·0–0·0

TABLE II

Drinking behaviour of salt-loaded terrapins: length of drinking bouts

Trial no.	Length of drinking bout (min) — Salinity offered (‰) 0·0	3·4	6·8	10·2	13·6	17·0
1	14·47	11·77	6·08	0·03	0·08	0·12
2	10·55	9·63	3·81	0·38	0·17	0·25
3	9·87	8·98	5·95	7·35	0·10	0·00
4	13·03	11·87	4·90	7·87	0·72	0·03
5	12·98	8·45	1·47	6·05	0·05	0·00
6	11·45	14·25	10·40	10·40	0·52	0·12
Mean	12·06	10·82	5·43	5·35	0·27	0·09

media must be tasted for discrimination. There was no significant difference between the periods of drinking salinities of 0 and 3·4‰ ($P > 0·05$; t-test for small samples, preceded by F-test for comparability of variance), but there was a significant difference between the periods of drinking salinities of 0 and 6·8‰ ($P < 0·01$), indicating some reluctance to drink the more saline medium. It appeared that 10·2‰ was close to the critical salinity at which animals refused to drink substantial quantities, since four animals (out of six) drank for several minutes while two drank only for a few seconds. The data also demonstrate that drinking (and presumably rehydration) is completed in less than 15 min.

Drinking from thin films

All animals offered fresh water in the form of thin films on land showed gulping behaviour and substantial weight increases whatever the thickness of film offered; diamondbacks are clearly capable of drinking from the thinnest water films available (1·6 mm). Animals offered thin freshwater films on top of saline water columns also showed gulping and weight increases.

Responses to simulated rainfall

Within a few seconds of the start of simulated rainfall, all salt-loaded terrapins were aroused and those resting on the bottom swam to the surface. All climbed out of water within about 30 s, but their behaviour thereafter varied. Some turned round and drank from the water's edge, most adopted a typical 'drinking posture' (see below) and drank from the surface film on the plywood land area of the apparatus, while a few climbed out far enough to drink from the drinking chambers. In the last case, terrapins only drank from the freshwater chamber; the contents of the seawater chamber were tested, but thereafter ignored. Drinking continued for 15–30 min as animals jockeyed for position. This experiment demonstrated that diamondbacks responded to rain, but it did not distinguish between a response to vibration, a visual recognition of rain, or a response to lowered salinity.

In the experiment in which simulated rainfall consisted of sea water, the terrapins again showed arousal and climbed out of water; they assumed drinking postures, but no gulping was observed and the response died away after about 5 min. Taken with the results of the first experiment these observations indicated that terrapins respond initially to the vibration/sight of rainfall, rather than its freshwater content.

Terrapins which had been held in fresh water for 7 days still responded to simulated (fresh) rainfall by arousal and some animals climbed out and drank briefly from the plywood surface.

Salt-loaded *Malaclemys* which were exposed to rainfall (fresh) in total darkness responded by arousal and drinking behaviour; when the light was switched on at the end of the period of darkness most had already climbed out of the water and were drinking from the plywood ramp and drinking chambers. This result indicates that terrapins respond to the vibration of rainfall rather than to visual cues.

Salt-loaded terrapins exposed to continuous heavy simulated rainfall (fresh) climbed out of water within a few seconds. Some drank from the substratum, but others drank from the surface of the shells or feet of other terrapins; two animals drank 'rain' directly by throwing their heads back.

Anatomy and posture

Figure 1 illustrates the posture usually used when drinking from thin water films. The thinner the film, the more arched was the neck. The establishment of a meniscus seal around the snout (see Fig. 2) was a necessary preliminary to gulping of water. In films of about 3 mm thickness menisci were easily established by placing the snout anywhere in the water surface, but in thinner films the terrapins usually exploited the thickness of the menisci at the sides of their vessel, or around their feet, to establish a seal. Commonly in such circumstances the neck was so arched that the head was near vertical until a seal was made, then the head was pushed forwards (with the snout against the substratum) until the neck was at a more comfortable angle before gulping started. In all cases the visible oral aperture was small (Fig. 2).

Under conditions of sustained heavy 'rainfall' the terrapins often drank from the marginal scutes of their fellows (Fig. 3). They also drank from the surfaces of their own limbs, and a single animal was seen to drink from the hind limb pockets of another terrapin. Some animals adopted the posture shown in Fig. 4 to drink 'rain' directly. In this case the neck was stretched upwards and bent backwards beyond the vertical. The mouth opened widely before vigorous gulping movements of the neck were observed.

Observation of the interior of the mouth revealed no special modifications for drinking from

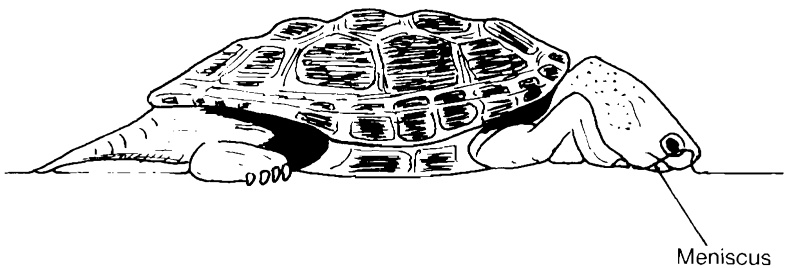

FIG. 1. Drinking posture employed by *Malaclemys* when drinking from thin water films.

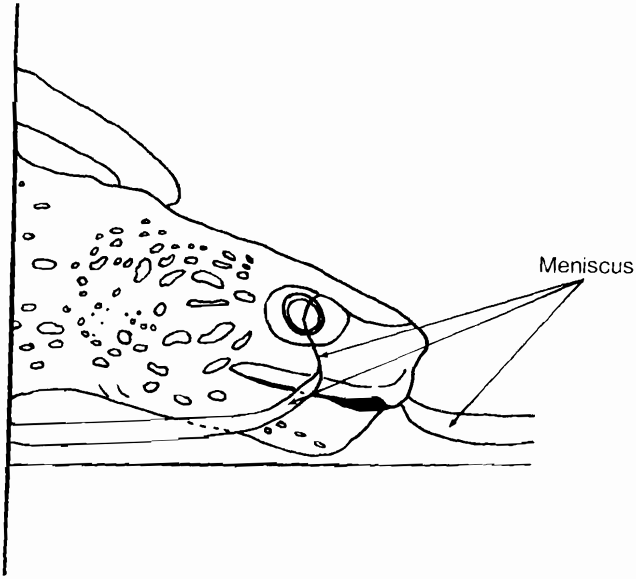

FIG. 2. Close-up view of diamondback terrapin drinking from meniscus at side of transparent vessel. Note subsidiary meniscus over snout and small size of oral aperture.

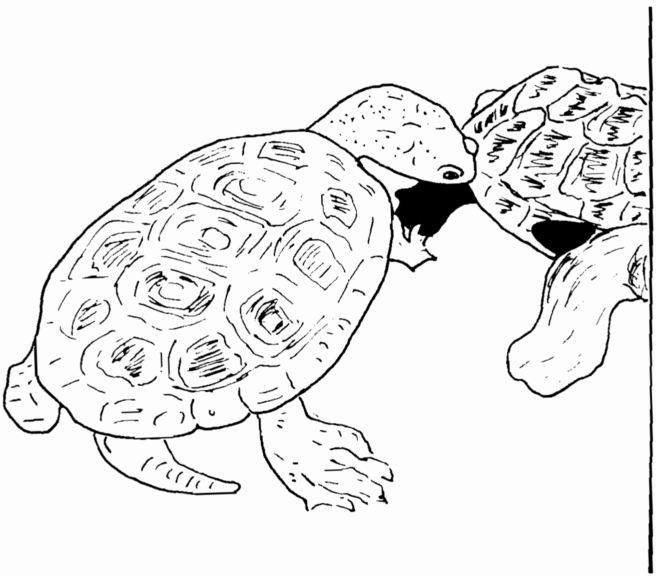

FIG. 3. Specimen of *Malaclemys* exposed to heavy simulated rainfall and drinking from the marginal scutes of another terrapin.

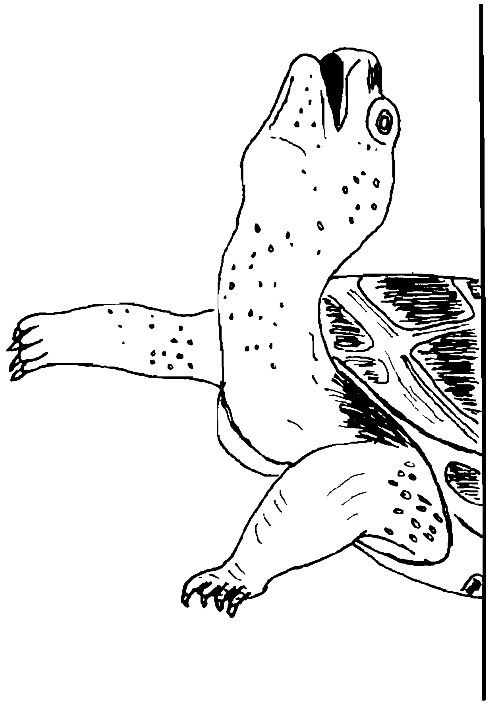

FIG. 4. Specimen of *Malaclemys* exposed to heavy simulated rainfall and drinking rain directly.

thin films; the anatomy of the mouth is similar to that of the Caspian terrapin *Mauremys caspica caspica.*

Miscellaneous observations

Over a period of many months it was established that diamondbacks fed equally voraciously in fresh or sea water; no fall-off of appetite was observed in animals held in sea water for 2–3 weeks. Salt-loaded terrapins simultaneously offered fish and mollusc flesh showed no preference for the less salty food (fish).

It was noticed that the terrapins spent much more time resting on the land area of their enclosure when only sea water was available than they did when they had access to fresh water. The significance of this observation to terrapins in the wild is difficult to assess, but such behaviour may slow dehydration in the humid environment of salt marshes.

When carrying out pilot experiments with simulated rainfall it was noted that salt-loaded animals which had emerged from sea water often sought out and drank droplets of fresh water from vertical surfaces. This behaviour was quite deliberate, the animals climbing and stretching their necks upwards to reach visible droplets out of immediate reach. Finally, when the terrapins' holding tank was drained for cleaning, it was observed that the animals always adopted the drinking posture briefly when the water level fell below the shell, whether they had been previously held in fresh water or sea water.

Discussion

The results presented in this study demonstrate that diamondback terrapins have a considerable capacity for behavioural control over body hydration. Behavioural osmotic control in estuarine emydids has already been demonstrated for the herbivorous Asiatic species *Batagur baska* (Gray) and *Callagur borneoensis* (Schlegel and Muller) (Davenport & Wong, 1986), but those species appreciably more permeable to salts and water than *Malaclemys*, effectively isolate themselves from the environment when salinities are high, not eating, drinking, defecating or urinating. Their response is a short-term one, appropriate to the large estuaries of south-east Asia which feature tidal fluctuations in salinity and considerable freshwater input throughout the year.

The more complex behavioural and postural responses of diamondbacks appear designed to maximize exploitation of rainfall, permitting them to exploit the rich food supply of salt marshes, even during the summer when rainstorms provide the only source of fresh water. Specimens of *Malaclemys* are capable of detecting rainfall, even when submerged, they respond by drinking from freshwater layers formed above a saline water column, or by quickly emerging from water to drink from thin surface films on land. Under conditions of heavy rain they can capture water directly from above. They have fine salinity discrimination, being able not only to avoid drinking water of a much higher osmolarity than their body fluids, but also to drink greater quantities of lower salinities, especially pure fresh water. Rehydration after salt-loading is also accomplished much more quickly in *Malaclemys* than in the Asian emydids; diamondbacks finish drinking within 15 min, while *Batagur* needs at least an hour to reverse osmotic dehydration.

Physiological and biochemical studies have indicated that *Malaclemys* survives in sea water by virtue of strong antidiuresis, a relatively impermeable integument, resistance to dehydration and tolerance of osmoconcentration of the body fluids. All of these features are undoubtedly important to survival, but they suggest that a diamondback would spend much of the summer in a steadily deteriorating physiological state. The results described here paint a different picture, of a species well adapted to its environment throughout the year, supplementing physiological capabilities with effective behavioural responses.

REFERENCES

Coker, R. E. (1906). The cultivation of the diamondback terrapin. *Bull. N. Carol. geol. econ. Surv.* **14:** 1–69.

Davenport, J. & Wong, T. M. (1986). Observations on the water economy of the estuarine turtles *Batagur baska* (Gray) and *Callagur borneoensis* (Schlegel and Muller). *Comp. Biochem. Physiol.* **84A:** 703–707.

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.

Dunson, W. A. (1976). Salt glands in reptiles. In *Biology of the Reptilia* **5.** *Physiology A*: 413–455. Gans, C. & Dawson, W. R. (Eds). London, New York, San Francisco: Academic Press.

Dunson, W. A. (1979). Control mechanisms in reptiles. In *Mechanisms of osmoregulation in animals: maintenance of cell volume*: 273–322. Gilles, R. (Ed.). Chichester: John Wiley & Sons.

Dunson, W. A. (1984). The contrasting roles of the salt glands, the integument and behaviour in osmoregulation of marine species. In *Osmoregulation in estuarine and marine animals*: 107–129. Pequeux, A., Gilles, R. & Bolis, L. (Eds). Berlin: Springer-Verlag. (*Lecture notes estl estuar. Stud.* **9.**)

Gilles-Baillien, M. (1970). Urea and osmoregulation in the diamondback terrapin *Malaclemys centrata centrata* (Latreille). *J. exp. Biol.* **52:** 691–697.

Gilles-Baillien, M. (1973*a*). Isosmotic regulation in various tissues of the diamond terrapin *Malaclemys centrata centrata* (Latreille). *J. exp. Biol.* **59:** 39–43.

Gilles-Baillien, M. (1973*b*). Hibernation and osmoregulation in the diamondback terrapin *Malaclemys centrata centrata* (Latreille). *J. exp. Biol.* **59:** 45–51.

Gilles-Baillien, M. (1973*c*). Seasonal changes in inorganic ions in red blood cells of terrestrial and aquatic Chelonia. *Biochem. Syst.* **1:** 123–125.

Hurd, L. E., Smedes, G. W. & Dean, T. A. (1979). An ecological study of a natural population of diamondback terrapins (*Malaclemys t. terrapin*) in a Delaware salt marsh. *Estuaries* **2:** 28–33.