

Osmoregulation in Crocodilians¹

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SYNOPSIS. Recent crocodilians live primarily in freshwater habitats. However two species (*Crocodylus acutus* and *C. porosus*) are estuarine specialists; two others (*C. niloticus* and *C. johnstoni*) that are primarily found in fresh water, have estuarine populations. Routes of uptake of water and sodium include drinking, feeding and associated incidental drinking, and integumental and buccal diffusion. Routes of loss include faeces-cloacal fluid, lingual salt glands, integumental and buccal diffusion, and respiratory loss. The least understood route of salt and water exchange is that of the oral and buccal epithelia, which are much more permeable to water and sodium than the general integument. The freshwater alligator (*Alligator mississippiensis*) osmoregulates in a manner typical for an amphibious reptile. Body sodium turnover is low and the general integument is quite low in permeability to sodium. Water turnover is more rapid (in terms of molar exchange) but still relatively low for an aquatic reptile. Most water exchange occurs across the integument and buccal epithelia. The presence of lingual salt glands in freshwater crocodilians remains enigmatic, as does the failure of these exocrine glands in estuarine species to respond to saline loading. Secretion does occur after injection of the parasympathetic stimulant methacholine chloride. The "salt water crocodile" (*C. porosus*) possesses a suite of osmoregulatory adaptations similar to those found in other estuarine reptiles. Water and sodium balance are maintained primarily by an extremely low general permeability to sodium, by economies in water loss, and by excretion of excess sodium by the lingual salt glands. Further work is needed to examine newly hatched *C. porosus*, and the possibility of ontogenetic change in lingual gland function in *C. acutus*. The importance of incidental drinking of sea water during feeding (recently discovered in turtles) needs to be evaluated in crocodilians. The use of osmoregulatory data in interpretation of the evolutionary history of the genus *Crocodylus* needs to be viewed with caution. The hypothesis that all species of *Crocodylus* originated from the transoceanic migration of a saline-tolerant form may not be the most parsimonious explanation.

INTRODUCTION

Osmoregulation is essentially similar in all aquatic reptiles (Dunson, 1979; Dunson and Mazzotti, 1989; Taplin, 1988). The most pervasive aspect of crocodilian osmoregulation is the amphibious habit of all extant forms, in combination with a uniform external morphology. Even the most aquatic species spend a great deal of time basking on land and/or resting in subterranean refugia or dens. Unlike some of the fossil forms (Williston, 1914), no extant species is truly marine or pelagic.

Living crocodilians do differ in the use of either fresh or saline water habitats. This has major consequences for osmoregulatory mechanisms because crocodilians maintain body fluid concentrations essentially the same as other vertebrates (about one-third that of sea water). Probably an equally important, but very poorly known, aspect of crocodilian osmoregulation is the exposure of many species to periodic/seasonal droughts. Little information on the physiological consequences of such dehydrating conditions exists, but they must be considerable. The study of possible aestivation in crocodilians should receive a high priority since it has great potential for furthering our understanding of the evolution of the lingual salt glands found in *Crocodylus*.

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TABLE 1. Potential routes of intake and loss of water and salts in crocodylians.

Intake	
1. Drinking without feeding	
2. Drinking "incidental" to feeding	
3. Feeding	
4. Integumental diffusion—general body surface	
—buccal area	
—eyes	
Loss	
1. Faeces/urine—cloacal fluid	
2. Respiratory water loss	
3. Lingual salt glands	
4. Integumental diffusion—general body surface	
—buccal area	
—eyes	

This review will summarize selected literature on crocodylian osmoregulation, and compare it to other aquatic reptiles. A primary emphasis will be to document the essential similarity in osmoregulation among all aquatic reptiles.

Osmoregulatory data are now being used to support evolutionary theories of the origin of the *Crocodylus* group. Proponents of the "transoceanic migration hypothesis" suggest that a single salt-tolerant form of *Crocodylus* gave rise to all extant species (Densmore, 1983; Taplin, 1984a; Taplin *et al.*, 1985). An alternative explanation, as is proposed for marine snakes and turtles, is that a gradual freshwater to estuarine transition led to marine forms (Dunson, 1986; Dunson and Mazzotti, 1989). Another possible scenario could involve the origin of lingual salt glands under the dehydrating conditions of periodic droughts in freshwater systems. We will argue here that current data are inadequate to establish any of these theories as the preferred hypothesis. What is needed is a broader perspective on crocodylian osmoregulation in the context of studies on other reptiles, and a great deal of new information specifically targeted to discriminate between alternative hypotheses. First, however, one must thoroughly understand the present data base dealing with water and salt balance.

ROUTES OF EXCHANGE

The osmoregulatory "problems" posed by life in fresh or saline waters are directly

related to the amounts of water and salts that are exchanged across various body surfaces. In crocodylians, potential routes of exchange are the same as in other aquatic reptiles (Table 1; Dunson, 1979; Minnich, 1979). Of course, the relative importance of each route varies with external salinity and the specific body plan of each reptile. For example, crocodylians are especially prone to expose buccal areas of epithelium lining the mouth to the exterior. This also occurs in turtles (where the pharynx and cloaca also may be effectively exteriorized), but not in snakes which are tightly sealed. Since these bucco-pharyngeal areas are higher in permeability than the general integument (Dunson, 1967; Dunson and Mazzotti, 1988) they may play a significant role in overall water and salt balance.

A recently discovered route of salt and water uptake in reptiles is "incidental drinking" (Shoemaker and Nagy, 1984; Dunson, 1985). This represents water swallowed along with the food, as distinct from water swallowed without any feeding (true drinking). Although as yet directly measured only in turtles (Dunson and Mazzotti, 1989), incidental drinking is clearly a route of major significance, especially in estuarine forms. Dunson (1985) has suggested that this is the single most important source of salt intake in estuarine-marine reptiles, is related to the geometry of the food consumed, and explains variations in salt gland size among marine reptiles. Special mechanisms probably have developed among sea turtles (and presumably among other marine-estuarine reptiles) to minimize such sea water uptake, by expelling most of the water swallowed with food.

Another major route of water exchange that is poorly understood is the integument. Direct measurement of crocodylian integumental permeability has only rarely been attempted (Dunson, 1981; Dunson and Mazzotti, 1988). The general body shape of crocodylians is quite similar to that of lizards, as is the total surface area relative to mass (Table 2). It is interesting that reptiles of this body form have relatively low total surface areas; snakes have a total surface area that is much higher (Table 2). However as important as body form may be in general adaptation, it appears to have

TABLE 2. The relation between total exterior surface area (A in cm^2) and body mass (M in g) in various reptiles (arranged in order of declining area of a 100 g hypothetical reptile).*

Species	Equation	A for M = 100 g	Reference
<i>Pelamis platurus</i> (sea snake)	$A = 12.78M^{0.717}$	347	Dunson, 1978
<i>Nerodia sipedon</i> (water snake)	$A = 14.17M^{0.679}$	323	Dunson, 1978
<i>Trionyx</i> sp. (softshell turtle)	$A = 16.61M^{0.614}$	281	Dunson, 1986
<i>Alligator mississippiensis</i> (alligator)	$A = 13.7M^{0.654}$	261	Davis <i>et al.</i> , 1980
<i>Crocodylus</i> sp. (crocodyle)	$A = 11.7M^{0.654}$	223	Dunson, 1982
"Standard lizard"	$A = 10M^{0.67}$	219	Minnich, 1979
<i>Chelydra serpentina</i> (snapping turtle)	$A = 8.62M^{0.678}$	196	Dunson, 1986

* Pharyngeal/buccal areas inside the mouth are not included.

a minor role in osmoregulation. An analogy with thermoregulation is instructive (Dunson, 1986). As Scholander (1955) has eloquently pointed out, it is the heat transfer across a surface that is the primary determinant of thermoregulatory adaptation. The same is true of crocodylian surface area and osmoregulation. It is not so much the integumental surface area per se as its permeability that determines the role in osmoregulation. The buccal area of crocodylians is a case in point. Although much remains to be learned about the role of this oral epithelium, it appears to be highly permeable to water and to play a significant role in overall exchange, despite an obviously minor surface area in comparison with the general body integument (Dunson and Mazzotti, 1988). Such a local area of higher epithelial permeability probably also occurs in the ocular membranes.

OSMOREGULATION IN FRESH WATER

The alligator is by far the best studied of the freshwater crocodylians (Ellis, 1981a; Coulson and Hernandez, 1983; Ellis and Evans, 1984), although there are some data also on estuarine species in fresh water (*C. acutus*: Schmidt-Nielsen and Skadhauge, 1967; Evans and Ellis, 1977) (*C. porosus*: Taplin, 1982, 1984b). There seem to be few differences among crocodylians in their abilities to adapt to fresh water. Thus the discussion below will be organized into physiological categories of salt and water balance.

Plasma composition of several species of crocodylians in fresh water is unremarkable in that it resembles that of freshwater reptiles generally (Dill and Edwards, 1931;

Dessauer, 1970; Minnich, 1979; Coulson and Hernandez, 1983; Ellis and Evans, 1984). These animals face the classic problem of hyper-osmoregulation (maintenance of a body fluid concentration far above that of the environment). Thus they would be expected to possess mechanisms for conservation of solutes such as sodium, and excretion of excess water.

Sodium turnover

Ellis and Evans (1984) have characterized sodium exchange in *Alligator mississippiensis*. Total body sodium was 82 $\mu\text{moles/g}$ wet mass, similar to that of *C. porosus* at 75 $\mu\text{moles/g}$ wet mass (Taplin, 1984b), but higher than the value for *C. acutus* (43 $\mu\text{moles/g}$ wet mass; Evans and Ellis, 1977). Ellis and Evans (1984) attributed the difference between *Alligator* and *C. acutus* to effects of the technique used to solubilize the body (either acid digestion in the former or homogenization in the latter). This seems unlikely to be the case since Dunson (1982) recorded a similarly low value for a single *C. acutus* (39 $\mu\text{moles/g}$ wet mass) when the animal was dissolved in acid, not homogenized as was done by Evans and Ellis (1977). It is also quite possible that differences in whole body sodium concentration are related to differential amounts and degrees of calcification in bone. Dunson and Heatwole (1986) documented large differences in total body sodium of turtles that were related to shell size and body size of the animal. Such effects seem to be primarily related to relative sizes of the extracellular and intracellular spaces.

For sodium flux calculations it is important to know how much of the total body sodium is exchangeable. In juvenile rep-

tiles generally all of the body sodium is exchangeable (Dunson and Heatwole, 1986). However, with growth (and presumably progressive calcification of the bones) the relative amount of exchangeable sodium in turtles diminishes (Dunson and Heatwole, 1986). This effect is thought to be due to binding of sodium to bone crystal. Exchangeable sodium pools account for all of the body sodium in small *Alligator* (Ellis and Evans, 1984) and small *C. porosus* (Taplin, 1984b). It is very likely that the proportion of sodium that is exchangeable in crocodylians diminishes with age/size, but this is yet to be studied. Data are also needed on the skeletal mass and composition of crocodylians of varying sizes. We believe that Ellis and Evans (1984) were incorrect in assuming that hatchling *Alligator* have a nonexchangeable bone sodium pool.

The rate constant (fraction of body sodium exchanged per unit time) for sodium efflux of hatchling *Alligator* in fresh water is 0.00068/hr; thus sodium efflux is about 5.5 $\mu\text{moles}/100 \text{ g}\cdot\text{hr}$ (recalculated from Ellis and Evans, 1984, using total body sodium rather than their estimated exchangeable value; Table 3). This is quite similar to sodium efflux from the freshwater turtle *Trachemys scripta* (1.6–3.3 $\mu\text{moles}/100 \text{ g}\cdot\text{hr}$; Dunson, 1967). Sodium efflux for *C. acutus* in fresh water was 2.5 $\mu\text{moles}/100 \text{ g}\cdot\text{hr}$ (Evans and Ellis, 1977). The value for the softshell turtle, *Trionyx* (1.5 $\mu\text{moles}/100 \text{ g}\cdot\text{hr}$; Dunson, 1979), is somewhat lower. It is unclear if such relatively minor differences are biologically significant without further understanding of overall body sodium balance. In *Alligator*, 46% of the sodium efflux was from the head, neck and forelimbs, 43% from the body, tail and hindlimbs, and only 11% from the cloaca (Ellis and Evans, 1984). This suggests that sodium conservation in renal/cloacal excretory processes is fairly effective, and that obligatory epithelial diffusion may be the main route of loss of sodium. In *C. acutus*, 69% of sodium efflux in fresh water is from the head, neck and forelimbs, 12% from the body, hindlimbs and tail, and 19% from the cloaca (Evans and Ellis, 1977).

Small unfed *C. porosus* in fresh water had net sodium losses of 0.6–0.9 $\mu\text{moles}/100 \text{ g}\cdot\text{hr}$; efflux was 2.4–3.0 $\mu\text{moles}/100 \text{ g}\cdot\text{hr}$ (Taplin, 1982). There was no evidence of net sodium uptake, even when bath sodium concentrations rose as high as 1 mM. Most (90%) sodium loss was integumentary, with the remainder being cloacal. Virtually all sodium influx was integumentary. The rates of sodium exchange in the crocodylians *C. porosus*, *C. acutus* and *C. johnstoni* were all similar (half lives of 50–75 days; Evans and Ellis, 1979; Taplin, 1982), but higher than values for the freshwater turtles *Trionyx spiniferus* and *Trachemys scripta* (Dunson, 1967, 1979). Taplin (1982) suggested that this is due to lower integumentary sodium permeability in turtles, which seems reasonable. Whatever the explanation for the difference, it appears that crocodylians are less efficient or specialized in conservation of sodium in fresh water than are turtles.

Several studies have attempted to estimate integumentary sodium permeability indirectly (Bentley and Schmidt-Nielsen, 1965; Ellis and Evans, 1984), although this is fraught with difficulty. However, Ellis and Evans' (1984) *in vivo* estimate of body integumentary sodium efflux (0.01 $\mu\text{moles}/\text{cm}^2\cdot\text{hr}$) in *Alligator* is remarkably close to direct measurements on isolated skin (0.006 $\mu\text{moles}/\text{cm}^2\cdot\text{hr}$; Dunson and Mazzotti, 1988). It should be noted that this represents unidirectional efflux, not net loss. However in fresh water there may be only an insignificant influx of sodium. In *Caiman*, net loss of sodium across the integument (measured by holding the head out of the bath) was the same as in *Alligator* (about 0.01 $\mu\text{moles}/\text{cm}^2\cdot\text{hr}$; Bentley and Schmidt-Nielsen, 1965). However in *C. acutus* the body integument had a much lower estimated sodium efflux (0.001 $\mu\text{moles}/\text{cm}^2\cdot\text{hr}$; Evans and Ellis, 1977). This suggests that the euryhaline *C. acutus* has a lower integumentary sodium permeability than two freshwater forms (*Alligator* and *Caiman*). An additional finding was that some portion of the head-neck-forelimbs must be more permeable than the general body integument. This has now been confirmed by a few direct measurements of the

TABLE 3. Whole body sodium and water fluxes of unfed crocodylians immersed in fresh (FW) or sea water (SW) in the laboratory. Means \pm SD.

Parameter	<i>A. mississippiensis</i>			<i>C. ornatus</i>			<i>C. porosus</i>			<i>C. johnstoni</i>		
	Flux	Mass, g	Mass, g	Flux	Mass, g	Mass, g	Flux	Mass, g	Mass, g	Flux	Mass, g	Mass, g
Water efflux, ml/100 g·hr	0.25 \pm 0.04 (FW) ^a	310–586	260–520	0.3 \pm 0.03 (SW) ^a	216 \pm 24	108–221	0.56 \pm 0.06 (FW) ^a	133 \pm 50	108–221	0.50 \pm 0.05 (SW) ^a	133 \pm 50	108–221
	0.25 \pm 0.07 (SW) ^a	310–586	260–520	1.0 \pm 0.3 (SW) ^a	108–221	108–221	0.50 \pm 0.05 (SW) ^a	133 \pm 50	108–221	0.50 \pm 0.05 (SW) ^a	133 \pm 50	108–221
	0.6 (FW) ^b	32–72		0.96 \pm 0.06 (FW) ^a								
Sodium influx, $\mu\text{mol}/100 \text{ g}\cdot\text{hr}$	0.90 \pm 0.03 (FW) ^a	116 \pm 10		18.8 \pm 1.9 (SW) ^a	284 \pm 18		17 \pm 10 (SW) ^a	127 \pm 49				
	0.78 \pm 0.10 (SW) ^a	116 \pm 10		22.1 \pm 5.9 (SW) ^a	108–221							
	10.8 \pm 4.5 (SW) ^a	230–375		2.1 \pm 0.5 (FW) ^a	166 \pm 15							
Sodium efflux, $\mu\text{mol}/100 \text{ g}\cdot\text{hr}$	93 \pm 24 (SW) ^a	100 \pm 22		19.0 \pm 3.6 (SW) ^a	284 \pm 18		0.99 \pm 0.51 (FW) ^a	117 \pm 22				
	5.5 (FW) ^a	32–72		3.0 \pm 0.7 (FW) ^a	166 \pm 15							
	2.14 \pm 0.68 (FW) ^a	142 \pm 17										

^a Mazzotti and Dunson (1984).

^b Ellis (1981e); Ellis and Evans (1984)—recalculated assuming 100% exchangeable body sodium.

^c Dunson and Mazzotti (1988).

^d Evans and Ellis (1977).

^e Taplin (1982, 1985).

^f Dunson (1982).

sodium permeability of epithelia from the roof of the mouth and the tongue of *Alligator* (Dunson and Mazzotti, 1988). It appears likely that the oral epithelia of all crocodilians are considerably more permeable to sodium and water than the general integument (see *C. porosus* below).

Ellis and Evans (1984) have suggested that non-dietary sodium uptake can compensate for 86% of unidirectional sodium efflux in *Alligator*. We consider this unsubstantiated. Certainly present evidence shows that crocodilians cannot absorb net sodium from fresh water via an active uptake mechanism such as is known for some freshwater turtles (Dunson, 1979). We believe it is unwise to assume direct quantitative equivalence of isotopic efflux measurements and net loss data as Ellis and Evans (1984) have done, since differential technical artifacts are likely. We recommend further measurements of isotopic influx and efflux in the same animals, and a detailed examination of the *in vitro* transport characteristics of buccal epithelia.

Water turnover

Small *Alligator* have a body water concentration (74% wet mass, Ellis, 1981a) fairly typical of reptiles (Thorson, 1968). Thorson (1968) reported only minor variation in the total body water of four species of crocodilians (means of 72.9–73.1% wet mass; ranges of 71.0–74.4). Other reptiles (turtles) are known to show major variation in this parameter with changes in relative bone mass related to interspecific and age differences (Dunson and Heatwole, 1986). Body fat also influences body water concentration (Minnich, 1979). In contrast to the small amount of sodium efflux from *Alligator*, water efflux is substantial (about 0.3–0.9 ml/100 g·hr, Table 3). Variation in overall water efflux is considerable, but does not seem to be clearly related to external salinity. It is likely that water efflux is inversely related to body size in *Alligator* although there are too few data for a definite conclusion (Table 3). Ellis (1981a) calculated that 53% of unidirectional water efflux is integumentary, 24% respiratory, and 24% cloacal. Dunson and Mazzotti (1988) similarly found that a large pro-

portion of water turnover in *Alligator* occurs through the integument. They also made direct measurements of *in vitro* integumentary water permeability for the first time, establishing the previously suggested, but unproven, large difference in permeability of oral epithelia and general body integument. The exact rate of water efflux across the body integument of *Alligator* can now be directly compared with that of a wide variety of reptiles (Table 4). The results are somewhat surprising in that the skin of *Alligator* is considerably less permeable to water than that of many other freshwater reptiles. It is, however, similar to that of the amphibious snake *Aghistrodon*. Thus despite the relative significance of the integument in water balance of *Alligator* in comparison with other routes of water loss, water turnover in this amphibious crocodilian is less than that of highly aquatic freshwater reptiles. This seems in contradiction with the external morphology of *Alligator* which is specialized for life in water, but it may reflect the fact that a great deal of time is actually spent on land.

Small unfed *C. porosus* in fresh water exchange most of their water across the integument (about 85% of either unidirectional influx or efflux; Taplin, 1982). Integumentary influx is slightly greater than efflux, but net water intake via this route was only one-sixth the amount drunk. Buccal exchange was not separated from that of the general integument. In contrast to a net loss of sodium, water balance is maintained. Since cloacal water losses greatly exceed net integumentary uptake, drinking is necessary to maintain a steady state. Taplin (1982) concluded that the most estuarine crocodilian (*C. porosus*) is no less efficient in osmoregulating in fresh water than are the freshwater specialists *C. johnstoni* and *Alligator*.

Evaporative water loss

Part of the discussion above has focused on exchanges of water across the integument of *Alligator* while it is submerged in fresh water. Yet it is well known that this species (as all crocodilians) spends considerable time out of water either basking and/or resting on land. Under these cir-

TABLE 4. Permeability of isolated reptilian skin to water when the outside solution is sea water and the inside is reptilian Ringer's solution (Dunson, 1978).*

Habitat and species	Water efflux	Reference
Fresh water—highly aquatic		
<i>Trionyx muticus</i> (softshell turtle)	1,049	Dunson, 1986
<i>Rogina septemvittata</i> (queen snake)	573 ^b	Stokes and Dunson, 1982
<i>Chelydra serpentina</i> (snapping turtle)	303	Dunson, 1986
Fresh water—amphibious		
<i>Nerodia sipedon</i> (northern water snake)	99	Dunson, 1978
<i>Nerodia fasciata pictiventris</i> (banded water snake)	70	Dunson, 1978
<i>Kinosternon subrubrum</i> (mud turtle)	70	Dunson, 1986
<i>Alligator mississippiensis</i> (<i>Alligator</i>)	46	Dunson and Mazzotti, 1988
<i>Aghistrodon piscivorus</i> (cottonmouth snake)	20–50 ^b	Dunson and Freda, 1985
<i>Eunectes notarius</i> (yellow anaconda)	28 ^b	Ljungman and Dunson, 1983
Estuarine—amphibious		
<i>Cerberus rhynchops</i> (dog-faced water snake)	36	Dunson, 1978
<i>Nerodia fasciata compressicauda</i> (mangrove snake)	21	Dunson, 1978
Marine—amphibious to pelagic		
Various species of sea snakes	40–133 ^b	Dunson and Stokes, 1983

* Efflux in $\mu\text{mole}/\text{cm}^2\cdot\text{hr}$.

^b Efflux measured between fresh water (inside) and 1 M NaCl (outside).

cumstances evaporative water loss occurs not only from the respiratory tract but across the integument as well. As in other reptiles, these integumentary evaporative losses would be expected to be much lower quantitatively than the water to water diffusion rates (Tercafs and Schoffeniels, 1965). Yet in the intact animal, rates of mass (water) loss of unfed *C. acutus* immersed in sea water (1.7% initial mass/day) are actually quite similar to the total evaporative loss in air at 96.5% relative humidity (1.3–1.6%/day) (Dunson, 1982).

The only detailed study of evaporative water loss in a crocodilian is that of Davis *et al.* (1980) on *Alligator*. Rates of loss were directly related to temperature and inversely related to body size. Respiratory water loss at 35°C was 0.12 $\mu\text{l}/\text{g}\cdot\text{hr}$; cutaneous water loss was 0.14 $\mu\text{l}/\text{g}\cdot\text{hr}$. The ratio of respiratory to cutaneous loss increased with decreasing temperature. The total evaporative water loss rate of animals weighing 2.5–6.6 kg was 0.61% mass/day (at 35°C, dry air) of which the cutaneous component was 54%. Skin resistance was 55 sec/cm, a value within the range reported for other reptiles. Davis *et al.* (1980) concluded that the rate of evaporative water loss of *Alligator* places it in a

transitional state between aquatic and terrestrial reptiles. This is most interesting since measurement of the aquatic water flux across *Alligator* integument led Dunson and Mazzotti (1988b) to a very similar conclusion.

Renal/cloacal excretion

In hatchling *Alligator* in fresh water, only 11% of sodium efflux and 24% of water efflux are cloacal (Ellis, 1981a; Ellis and Evans, 1984). Although the kidney and cloaca have only a minor role in sodium chloride excretion, they are the primary route of excretion of nitrogenous wastes. Coulson and Hernandez (1983) have reviewed kidney function of *Alligator* in great detail. Hydrated unfed *Alligator* excretes over half the urinary nitrogen as ammonium bicarbonate; upon dehydration uric acid is the primary means of nitrogen excretion. The five most common components of urine of unfed hydrated *Alligator* were (in declining order) ammonia, bicarbonate, uric acid, creatinine and phosphate. It appears at present that all crocodilians are similarly ammonotelic-uricotelic, but detailed interspecific comparisons of renal and cloacal function are lacking.

Even in the estuarine *C. acutus* the kidneys do not markedly regulate water and salt output. The osmotic urine/plasma ratio varied only between 0.7 and 0.9 when crocodiles were hydrated or dehydrated (Schmidt-Nielsen and Skadhauge, 1967). However a "downstream" regulation of water and salt absorption occurs in the cloaca. Sodium chloride was almost completely absorbed in the cloaca of hydrated *C. acutus* but was less completely absorbed after salt loading (Schmidt-Nielsen and Skadhauge, 1967). The precipitated uric acid in crocodilian cloacal fluid may contain significant amounts of some electrolytes bound as urate salts. Minnich (1972, 1979) has discussed the importance of this process in terrestrial reptiles faced with problems of water shortage. It is not yet clear whether urate salts are an important route of cloacal excretion in crocodilians. Sodium and potassium contents of cloacal uric acid solids in *C. acutus* and *C. porosus* were not affected by water salinity; only potassium was present at significant levels (393–671 $\mu\text{moles/g}$ dry mass) (Dunson, 1982). As Skadhauge (1977) has suggested, the crucial role of the cloaca in modifying crocodilian ureteral urine needs to be further examined with perfusion techniques.

OSMOREGULATION IN SALINE WATER

In contrast to fresh water, crocodilian physiological reactions to saline water vary among different species. Thus the following discussion will consider each species separately.

Alligator

There seems little doubt that *Alligator* is a freshwater species, with only a limited capacity to utilize estuarine habitats. It lacks the lingual salt glands that are found in *Crocodylus* (Taplin *et al.*, 1982). Yet *Alligator* is commonly seen in coastal areas and has reproducing populations in the Lower Florida Keys (Jacobsen, 1983) and on islands off the Georgia coast (Tamarack, 1988). Although data are limited, it is quite possible that recruitment in the Keys is mainly or entirely limited to areas of permanent fresh water, especially where mos-

quito control ditches have been dug into the fresh ground water lens. It also seems likely that the adults would have to return to fresh water periodically to drink. Clearly there is a need for a detailed study of island *Alligator* to determine whether they possess some physiological mechanisms for tolerating saline water or simply use behavioral means of osmoregulation. If they are to exist on islands where fresh water is extremely limited and there is very little for adults to eat in temporary freshwater ponds, they must forage in the sea (Tamarack, 1988).

Although we will have to await further studies on island *Alligator*, a most unique field study in North Carolina illustrates the behavior of *Alligator* when exposed to a dramatic increase in salinity. Birkhead and Bennett (1981) documented the movements of *Alligator* in a tidal creek before and after diversion of the freshwater headwaters during construction of nuclear power plant. Use by *Alligator* of the lower reaches of the creek (mean salinity 20 ppt) virtually ceased after the diversion. Although animals were not marked, it appeared that the population shifted to the diversion canal where they once again had access to fresh water. Thus although a rigorously controlled study remains to be conducted, it seems quite likely that *Alligator* is capable of behavioral osmoregulation. It may use the highly productive coastal marshes for feeding as long as it has access to fresh water periodically. However the limited physiological tolerance of *Alligator* to high salinities makes this a risky undertaking if brackish water of a low enough salinity is not available. Chabreck (1971) studied *Alligator* in nearly fresh (0.6 ppt) and saline (3–16 ppt) marshes along the Louisiana coast. Although the saline marshes had twice as much potential food, stomachs of animals in the freshwater marsh contained more than six times as much food as those from an adjacent saline marsh. Chabreck (1971) suggested that such a difference in food intake would result in diminished growth in saline marshes. His wild-caught animals did not in fact demonstrate any such obvious effect on body

condition, although their previous movements were not monitored.

Laboratory experiments by Mazzotti and Dunson (1984) confirm that *Alligator* do not gain mass in 35 ppt sea water even when fed; they do gain mass in 4 ppt. The ability of *Alligator* to survive for considerable periods in saline water seems to be due to low rates of water loss and sodium uptake (Table 3). However the inability of *Alligator* to excrete excess sodium chloride limits its stay in highly saline habitats. Lauren (1985) found that *Alligator* (mean mass 381 g) stopped eating at salinities of 10 ppt and above, and lost considerable amounts of mass. He measured plasma and urine osmotic pressure at weekly intervals for four weeks. In fresh water and 5 ppt (where growth occurred but was low, about 0.3%/day), urine and plasma concentrations did not increase; at 10 ppt and above they did increase significantly to levels above 100 mM sodium. It is not clear whether the increased plasma osmotic pressure in *Alligator* was due to net sodium chloride intake, net water loss, or both (Lauren, 1985). Plasma chloride rose from about 120 mM in fresh water to above 180 mM after four weeks at 20 ppt. Plasma sodium underwent a lesser increase, from below 140 mM to about 180 mM. The faster rise in chloride than sodium is puzzling. At 10 ppt and above, plasma and urinary uric acid and plasma corticosterone increased. The stress of saline exposure at 10 ppt and above was associated with cessation of feeding. Some limited homeostatic responses such as increases in uric acid production and cloacal fluid sodium occur, but are presumably insufficient to counter net water loss and net sodium chloride uptake. The inability to feed at 10 ppt, a salinity which is only slightly hyper-osmotic to extracellular fluids, may be related to unacceptable levels of salt uptake via incidental drinking (Dunson, 1985). However the ability of these juvenile animals (381 g) to cease feeding and wait out their fate in saline solutions indicates a sufficient degree of physiological tolerance to allow behavior to get them away from potentially lethal salinities. Hatchlings are assumed to be even less

tolerant; it would indeed be interesting to examine the effect of body size on salinity tolerance across the entire size range of the species. An examination of possible inter-population differences in tolerance would also be useful to see if a tolerant ecotype has arisen in coastal habitat (as in turtles, see Dunson, 1986). Lauren (1985) suggested that the integumental water permeability of *Alligator* is too high to allow much saline tolerance in the absence of a salt gland. Ellis and Evans (1984) similarly concluded that integumental water permeability of *Alligator* is much higher than that of *C. acutus*. In the absence of direct measurements on *C. acutus* we are reluctant to come to a conclusion. Instead we point out that the mangrove snake lives only in saline estuarine areas, can tolerate long periods of immersion in 35 ppt sea water, apparently lacks a salt gland, has a higher mass-relative surface area, and yet has about one-half the integumental water permeability of *Alligator* (Table 4). This tends to corroborate the idea that the overall water permeability (especially buccal) of *Alligator* is too high to allow long term survival at high salinities.

Crocodylus acutus

In terms of breeding biology in Florida, this species is completely estuarine. *C. acutus* makes nests on islands, exposed shorelines, and creekbanks along the southernmost mainland (Mazzotti, 1983). However adults may spend prolonged periods in the non-breeding season in totally fresh water of interior wetlands. This predilection for coastal habitats in many parts of its range, and the presence of lingual salt glands in subadults (Taplin *et al.*, 1982), establish *C. acutus* as one of the two crocodilians which are estuarine specialists (along with *C. porosus*).

The earliest detailed study of osmoregulation in *C. acutus* is that of Schmidt-Nielsen and Skadhauge (1967) who thoroughly examined the renal/cloacal system. They found that the renal tubules had little ability to regulate the osmolality or electrolyte composition of the urine. Even after a salt load, the osmotic pressure of the urine

never exceeded that of the plasma. Although amphibious, the crocodile retained the ammonio-uricotelic system of nitrogenous excretion characteristic of its terrestrial ancestors.

The first study of salinity tolerance of *C. acutus* by Dunson (1970) pointed out differences in the responses of large and small animals. A 3.4 kg crocodile was kept in 35 ppt sea water for five months and continued to feed avidly on trout. Yet unfed hatchlings lost an average of 1.7% mass/day and seemed quite intolerant of exposure to sea water. Ellis (1981b) extended these experiments with very similar results. Relative mass loss of unfed animals was inversely related to body mass (also confirmed by Mazzotti and Dunson, 1984). Field-caught *C. acutus* from high salinity sites had plasma sodium concentrations (141–174 mM) typical of reptiles generally. Crocodiles injected with sodium chloride loads did not show any externally obvious secretions from the cephalic region. Taplin and Grigg (1981) and Taplin *et al.* (1982) subsequently discovered that the salt glands are in the tongue and, as far as is known, can only be stimulated by the injection of the acetylcholine mimic methacholine chloride. Avian, reptilian, and elasmobranch salt glands respond to salt injections as well as to methacholine chloride (Peaker and Linzell, 1975). The glands in crocodilians are indisputably present and their unique failure to secrete after salt loading may represent our technical inability to reproduce natural conditions leading to gland secretion.

A series of investigators have examined sodium and water turnover in *C. acutus* in saline water using modern radioisotopic techniques. Evans and Ellis (1977) measured sodium efflux in hatchlings placed in 25% sea water (about 9 ppt). Sodium efflux was quite low (5.6 $\mu\text{moles}/100\text{ g}\cdot\text{hr}$), although these animals were not salt loaded or dehydrated. However, even in 35 ppt sea water, sodium effluxes remained low (0.8, 2.3, 3.8 $\mu\text{moles}/100\text{ g}\cdot\text{hr}$; Table 3). The smallest salt gland known in snakes or turtles secretes at a rate of about 15 $\mu\text{moles}/100\text{ g}\cdot\text{hr}$ (*Cerberus*; Dunson and Dunson, 1979). The rate of lingual gland secretion directly from the tongue of *C. acutus*

(injected with methacholine chloride) is 13.5 $\mu\text{moles}/100\text{ g}\cdot\text{hr}$ (Taplin *et al.*, 1982). Thus measurements of sodium efflux from *C. acutus* in sea water to date do not support the notion that a salt gland is functioning under naturally dehydrating conditions. Two measurements of sodium influx in sea water were nearly identical (11.3, 11.5 $\mu\text{moles}/100\text{ g}\cdot\text{hr}$; Table 3), and were higher than efflux. This suggests that small *C. acutus* undergo a sizeable net uptake of sodium in sea water. This needs to be confirmed by actual measurements of body sodium, and extended over a wide range of body sizes. It seems clear that hatchling *C. acutus* are quite intolerant of exposure to 35 ppt, and that larger animals are much less so. There is little chance that the intolerance of hatchlings is based on some technical flaw (*i.e.*, small animals are more nervous and simply do poorly in captivity), since these baby crocodiles feed voraciously and seem to adapt readily to experimental protocols. The same may not be true of *C. porosus* as very little work has been done on hatchlings and they are said to be difficult to rear in captivity.

Hatchling *C. acutus* apparently do well in natural situations of high salinity (Dunson, 1982; Mazzotti *et al.*, 1986; P. Moler, unpublished observations). Yet in laboratory simulations, growth cannot occur in such highly saline conditions (Dunson, 1982; Mazzotti and Dunson, 1984). Instead very small *C. acutus* are thought to depend on periodic drinking of rain water to rehydrate, if salinities are above 20 ppt. Such a strategy was suggested also for hatchling *C. porosus* by Magnusson (1978), and for other estuarine reptiles (Dunson, 1970, 1985) and a mangrove-dwelling rat (Dunson and Lazell, 1982). Mazzotti and Dunson (1984) suggested that the major adaptations of *C. acutus* to saline water also include the ability to grow very rapidly during the wet season to a size much more tolerant of the high salinities likely to be encountered during the dry season.

Crocodylus johnstoni and *Crocodylus niloticus*

Lingual salt glands are present in all members of the subfamily Crocodylinae yet examined (Taplin *et al.*, 1982, 1985). This

includes some entire species, and inland populations of other species that never have any contact with estuarine conditions. Currently the one documented function of salt glands is extraoccal sodium chloride excretion. Since freshwater crocodiles do not have a need to excrete excess salt, the origin and purpose of the lingual salt glands in freshwater crocodiles remain obscure at present. *C. johnstoni* is primarily a freshwater form, but one unusual population that lives in saline water is known from the Limmen Bight River system of northern Australia. Taplin *et al.* (1985) compared lingual gland secretions (stimulated by methacholine chloride injection) and other aspects of osmoregulation in fresh and saline water populations. On the whole, body salt and water levels did not differ between the populations. Total body water was the same; exchangeable body sodium and plasma sodium and chloride concentrations were somewhat higher in the saline group. There was a much more striking difference in lingual gland excretion rates; saline exposed animals secreted about eight times as fast as those in fresh water (Table 5). This indicates that a crocodilian generally considered to be entirely of freshwater habits can adapt to saline environments, with a concomitant increase in the secretory capacity of the lingual salt glands. It would be especially interesting to determine whether this is a plastic response brought on by exposure to saline waters or whether the Limmen Bight population is an ecotype with a fixed, genetic, predetermination to develop more active glands. The occurrence of local estuarine populations in otherwise freshwater crocodiles may also occur in other species such as *C. niloticus* and *C. palustris* (Taplin *et al.*, 1982; Taplin and Loveridge, 1988).

Unfed *C. niloticus* in sea water lost mass at a rate of 1.4%/day (Taplin and Loveridge, 1988). Plasma sodium chloride, but not potassium, was markedly elevated after 134 hr. Cloacal fluid sodium concentration increased only to about 10 mM. When excretion of the lingual glands was blocked by glue applied to the tongue, the increase in exchangeable body sodium was 67% (over 92 hr), in contrast to a value of 43% in the controls. This seems to be clear evi-

dence that the lingual glands have an excretory function. However over this short period, homeostasis obviously was not achieved by the control animals (which were reared in fresh water and given an acute exposure to sea water).

The extent to which species of primarily freshwater crocodilians inhabit estuaries obviously deserves further study. The phenomenon may be more widespread than is currently recognized. If so, this would have an important bearing on theories of the evolutionary history of the genus *Crocodylus*. The possession of lingual salt glands in inland crocodilians would be easier to understand if there are coastal euryhaline populations of the same species. Such evidence would not, however, reveal which population was ancestral to the other.

Crocodylus porosus

This species has been extensively studied in northern Australia by G. Grigg, L. Taplin and their associates (Magnusson, 1978; Grigg *et al.*, 1980, 1986; Grigg, 1981; Taplin and Grigg, 1981; Taplin, 1982, 1984a, b, c, 1985, 1988). The amount of information amassed is impressive, especially in regard to data collected from animals under field conditions. We will focus our attention on a recent paper that summarizes the best available data on water and sodium budgets (Taplin, 1985).

An idealized sodium and water budget for a 250 g, unfed *C. porosus* in sea water is presented in Table 6. Current evidence indicates that *C. porosus* can remain in positive sodium balance without feeding or drinking fresh or brackish water. Sodium balance was not, however, achieved in all flux experiments. For example in one test in sea water, influx exceeded efflux by 4 $\mu\text{moles}/100\text{ g}\cdot\text{hr}$; exchangeable sodium increased by 14% (Tables 5, 6 in Taplin, 1985). In the most convincing experiment (Table 2 in Taplin, 1985), sodium efflux slightly exceeded influx and the exchangeable sodium pool remained constant. Of course, differences in efflux rate may be linked to effects of acclimation and the state of body fluid hydration. An additional factor is the amount of handling stress involved in such experiments which may interfere with normal maintenance of ion and water

TABLE 5. Secretory characteristics of salt glands of some marine and estuarine reptiles.*

Species	Habitat	Acclimation conditions, ppt	Body mass, g	Na excretion, $\mu\text{moles}/100 \text{ g}\cdot\text{hr}$	[Na] mM	Reference
Turtles (lachrymal salt gland)						
<i>Malaclemys terrapin</i>	E, S	0	469; 297	0.4	288	Dunson, 1970; Robinson and Dunson, 1976
<i>Malaclemys terrapin</i>	E, S	35	216; 234	43	682	Dunson, 1985
<i>Malaclemys terrapin</i>	E, S	In egg	8	65	—	Dunson, 1985
<i>Chelonia mydas</i>	M	35	68	134	685	Holmes and McBean, 1964
<i>Chelonia mydas</i>	M, S	35	24	590-950	644	Marshall, Cooper, and Saddler, unpublished observations
Lizards (nasal salt gland)						
<i>Varanus semiremex</i>	E, S	D	150-294	34	686	Dunson, 1974
<i>Amblyrhynchus</i>	M, S	35	80-428	146-255	<1,434	Dunson, 1969
Snakes (posterior sublingual salt gland)						
<i>Aipysurus fuscus</i>	M, S	35	—	24 ^b	749 ^b	Dunson and Dunson, 1974
<i>Hydrophis elegans</i>	M, S	35	—	35 ^b	520 ^b	Dunson and Dunson, 1974
<i>Acalyptophis peronii</i>	M, S	35	—	56 ^b	514 ^b	Dunson and Dunson, 1974
<i>Astroia stokesii</i>	M, S	35	—	133 ^b	520 ^b	Dunson and Dunson, 1974
<i>Aipysurus laevis</i>	M, S	35	—	157 ^b	791 ^b	Dunson and Dunson, 1974
<i>Lapemis hardwickii</i>	M, S	35	—	162 ^b	704 ^b	Dunson and Dunson, 1974
<i>Aipysurus egyptiacus</i>	M, S	35	—	222 ^b	749 ^b	Dunson and Dunson, 1974
Snake (premaxillary salt gland)						
<i>Cerberus rhynchops</i>	M, E	35	74-221	16	414	Dunson and Dunson, 1979
Crocodylians (lingual salt gland)						
<i>Crocodylus acutus</i>	E	0	5,100	13.5	455	Taplin <i>et al.</i> , 1982
<i>Crocodylus johnstoni</i>	F	0	3,600-8,100	2-4	365	Taplin <i>et al.</i> , 1982
<i>Crocodylus johnstoni</i>	E	10-24	180-3,350	18-44	530	Taplin <i>et al.</i> , 1985
<i>Crocodylus porosus</i>	E	>12	100-700	74	~700	Taplin 1985
<i>Crocodylus porosus</i>	E	>12	50,000	11	~400	Taplin 1985

* M = marine; E = estuarine; F = fresh water; S = salt loads; D = dehydrated.

^b CI instead of Na.

balance. Acclimation or handling effects may also be involved in the large variation in measured water fluxes. There is no doubt that crocodiles in sea water suffer a severe net water loss; however, the overall size of the water fluxes varied between freshly captured (302 $\mu\text{l}/100 \text{ g}\cdot\text{hr}$) and captive-held crocodiles (1,006 $\mu\text{l}/100 \text{ g}\cdot\text{hr}$) (Taplin, 1985).

The partitioning of sodium and water influx and efflux in *C. porosus* in sea water (Table 6) has been established by flux measurements of crocodiles with cloacal bags, with the tongue surface sealed with glue, with the mouth closed, and with a divided chamber separating the water surrounding the head and the body (Taplin, 1982). These *in vivo* methods have revealed a little appreciated fact of crocodilian physiology, that the oral (buccal) epithelium is quite permeable to sodium and water. Indeed

sodium and water intake are primarily through this cephalic route, with the general integument the second most important route (Table 6). A greater proportion of sodium (80%) than water intake (60%) occurs in this fashion. The cloaca and drinking are insignificant in uptake of unfed crocodiles. In fed animals there would undoubtedly be a large amount of "incidental drinking" as occurs in turtles (Dunson, 1985). In contrast to uptake, routes of loss of sodium and water are divergent (Table 6). A majority (55%) of sodium loss occurs via the lingual salt glands, although a surprising amount passes across the cephalic epithelia (42%). The general integument and cloaca are insignificant as routes of sodium loss. In contrast, water efflux primarily occurs across the general integument (55%) and cephalic epithelia (36%).

For a hypothetical *C. porosus* of 250 g mass unfed in sea water (Table 6), the lingual glands contributed 55% (10.5 $\mu\text{moles}/100 \text{ g}\cdot\text{hr}$) of the total sodium efflux. For secretions collected directly from the tongue after methacholine injection, the rates were considerably higher, and inversely proportional to body mass (Taplin, 1985). The rates of sodium secretion for crocodiles weighing 100 g, 250 g and 50 g, respectively, are 74, 56, and 11 $\mu\text{moles}/100 \text{ g}\cdot\text{hr}$. The reason for the higher relative rate of secretion in smaller crocodiles is unknown, but may be related to allometric effects on rates of water loss and other factors relating to the imposed sodium load. This may be a general feature of the physiology of marine reptiles because relative salt gland mass (G as % wet body mass) shows an inverse relation to body mass (M in g) in the sea snake *Hydrophis ornatus* ($G = 0.1637M^{-0.470}$; Dunson, 1978). The maximum rate of lingual gland secretion stimulated by methacholine in a 250 g *C. porosus* is considerably higher than the efflux rate measured by radiosodium loss (56 vs. 10.5 $\mu\text{moles}/100 \text{ g}\cdot\text{hr}$). The difference probably does not represent an excess of secretory capacity because laboratory studies of sodium influx and efflux in fed crocodiles have not yet been performed. It is quite likely that drinking of sea water incidental to feeding will require a rate of excretion considerably in excess of the unfed efflux rate. In the marine iguana (*Amblyrhynchus*), Shoemaker and Nagy (1984) estimated that 63% of sodium influx came from ingestion of sea water incidental to feeding. Dunson and Mazzotti (1989) reported a 97-fold increase in sodium influx when diamondback terrapins (*Malaclemys*) were fed in sea water. Crocodiles are likely to undergo a lesser but still substantial increase, because they swallow their food whole instead of biting it into chunks as do turtles (Dunson, 1985). Indeed Grigg *et al.* (1986) have measured field sodium effluxes in *C. porosus* that almost exactly match the drug-stimulated maximal rate of lingual gland secretion. This latter study of field turnover of water and sodium in *C. porosus* represents the second such study of any aquatic reptile. The first field measure-

TABLE 6. An estimated sodium and water budget (unfed) for a 250 g *Crocodylus porosus* in 35 ppt sea water at 25°C.*

Route of exchange	Sodium	Water
Intake		
Cephalic (primarily buccal epithelium)	15.3 (80%)	144 (60%)
General integument	3.6 (20%)	96 (40%)
Cloaca	0	0
Drinking	0	0
Total	18.9 (100%)	240 (100%)
Loss		
Salt glands	10.5 (55%)	17 (6%)
Cephalic (primarily buccal epithelium)	8.0 (42%)	102 (36%)
General integument	0.2 (1%)	153 (55%)
Cloaca	0.4 (2%)	8 (3%)
Total	19.1 (100%)	280 (100%)
Net change	-0.2	-40

* Fluxes are in units of $\mu\text{moles}/100 \text{ g}\cdot\text{hr}$ (sodium) or $\mu\text{l}/100 \text{ g}\cdot\text{hr}$ (water) with percentage of total exchange in parentheses. Modified from Taplin (1984, 1985).

ments of aquatic water fluxes were made on the marine iguana by Shoemaker and Nagy (1984). Despite major problems in interpretation of aphagia apparently caused by handling stress, and negative or low overall growth rates, the measurements of fluxes in free-ranging crocodiles contribute to our understanding of the magnitude of naturally imposed salinity stress.

The greatest single unresolved issue in the osmoregulation of *C. porosus* is the status of newly hatched neonates. For *C. acutus* this age/size is the most intolerant of high salinity (as discussed above). Apparently the only study to date on salinity tolerance of neonates of *C. porosus* is that of Magnusson (1978). He found that recent hatchlings undergo drastic mass loss after 8-12 days of exposure to salinities of 15 ppt or greater. Yet these neonates showed no behavioral preference for different salinities. He suggested that drinking of low salinity water after rainfall might be crucial to survival of these young croco-

diles. Indeed there is a virtual absence of very small *C. porosus* from hypersaline rivers of the northern coast of Australia (Messel *et al.*, 1979–81). Grigg *et al.* (1980) demonstrated survival and growth of 6–10 small *C. porosus* (160–270 g) in the Tomkinson River of northern Australia. Salinities were 25–36 ppt; there was rainfall on only three days and no seeps of fresh water were found. Over periods of 16–17 days or 4 mo, growth occurred but was quite meager (mean of 0.44 g/day). Grigg *et al.* (1980) concluded that there was no evidence of stress in this habitat. Considering the very low rate of growth of these animals compared to other crocodylians (Mazzotti, 1983), this is not a convincing statement. Indeed the fact that some rainfall did occur leaves the possibility open that drinking of brackish water occurred. Even more significantly, recent hatchlings (about 70–75 g) were not studied during the period of early growth when they apparently have the least tolerance for exposure to saline water. Mazzotti and Dunson (1984) have hypothesized that for neonatal *C. acutus*, drinking of periodically available rainwater and very rapid growth over a 2–4 mo period to a more saline-tolerant size are important osmoregulatory strategies. Of course young crocodyles also have to be sufficiently impermeable to resist dehydration between episodic rainfall events. It is clear that there is a strong need for rigorous tests of the effects of salinity on growth of *C. porosus* starting with neonates (newly hatched) and extending to larger sizes. Ideally these experiments should be carried out in the laboratory and in field enclosures to test for any effects of handling stress. The value of such an approach can be illustrated by the demonstration that hatchling diamondback terrapins (*Malaclemys*), which possess a salt gland similar in maximum secretory capacity to that of *C. porosus*, have an optimal growth in water of about 9 ppt (Dunson, 1985). Growth can occur at a lower rate in 35 ppt, if fresh or brackish water is supplied periodically, mimicking rainfall events. In *Malaclemys*, the ability to grow at constantly high salinities also increases rapidly with body size/age.

EVOLUTIONARY IMPLICATIONS

Densmore (1983) was the first to propose that the current distribution of *Crocodylus* is the result of a relatively recent (post-Pliocene or about 4–5 million years) transoceanic migration of a sea water-tolerant form ancestral to all living species. Taplin and Grigg's (1981) discovery of lingual salt glands in *C. porosus* provided circumstantial proof that such an event may have occurred. Taplin (1984a) and Taplin *et al.* (1985) have elaborated considerably on this idea and produced further evidence by the presence of lingual salt glands in all seven species of *Crocodylinae* tested to date (stimulated with methacholine chloride injections). The presence of salt glands in many strictly freshwater species is paradoxical and the transoceanic migration hypothesis (TOMH) provides an appealing solution. We would like to point out alternative hypotheses which have merit and discuss ways in which further tests can be conducted. We also will consider untested assumptions inherent to the TOMH. Our goal is not so much to attempt to disprove or dispute the TOMH as it is to encourage the maintenance of an open mind on the subject until alternatives are carefully considered. Evolutionary hypotheses should be rigorously and experimentally evaluated from all perspectives despite their superficial attractiveness in reconciling disparate data.

One of the most important assumptions of the TOMH is that conclusions about the protein molecular clock must be valid (Densmore, 1983). Since differences in proteins among species of *Crocodylus* are quite small, the time since they diverged must also be small. However calibration of such a "clock" depends on comparisons with protein difference of other vertebrate groups and their known fossil histories. The rate of change in proteins might be slower in crocodyles than in other vertebrates, reflecting a conservative morphology (all crocodyles are basically the same in external structure). No slowing of the protein clock appears to have occurred between the divergence of the two species of *Alligator* (Densmore, 1983). However *A. mis-*

sissippiensis has been reported to have a lower than expected heterozygosity (0.021–0.022) and either a low (0.06) or normal (0.15) proportion of polymorphic loci (Gartside *et al.*, 1977; Adams *et al.*, 1980). This suggests a certain caution in interpretation of allozyme data from crocodylians. Another possibility that has yet to be suggested is that the common ancestor of modern *Crocodylus* species passed through a genetic "bottleneck" (reduction in population size to a very low level) that greatly reduced genetic diversity. Further examination of the genetic relatedness of *Crocodylus* species by new techniques (such as mitochondrial DNA) should help to resolve these issues.

A second important assumption of the TOMH is that the common ancestor of all *Crocodylus* species was capable of transoceanic migration and establishment on all continents. While *C. porosus* is undoubtedly highly salt-tolerant and has been found at sea on occasion, it is more significant to inquire where it has not been found. For example it breeds in the Palau Islands, and Papua New Guinea, but not the eastern Carolines such as in Ponape, or in Oceania. Even among the highly aquatic sea snakes (about 50 species which originated in the western Pacific), only one species (*Pelamis platurus*) has successfully colonized the New World; this must have occurred only since the Pliocene when the Central American isthmus last emerged to block migration into the Atlantic Ocean. Thus movement across the immense distances of the eastern Pacific Ocean is not easy, even for pelagic sea snakes. The hypothesized ancestral sea-crossing crocodile would have an even greater problem in that it must have been oviparous and there are apparently no islands suitable as breeding way-stations in Oceania. If one attempts to visualize what a true sea-going crocodile might be like, there are some ready examples in the fossil record (Williston, 1914; Neill, 1971). The Teleosauroidae are considered to be coastal crocodyles, whereas the Metriorhynchidae were highly modified pelagic forms. Neither of these families are considered directly ancestral to *Crocodylus* but they illustrate the potentialities of the crocody-

lian body form for evolution towards pelagic life. *Geosaurus*, a metriorhynchid from the upper Jurassic, was a fish eater (slender snout) with paddle-like limbs and an elongate tail with a fin on the end. Such a highly modified animal must have been totally aquatic except at egg-laying time. It must also have had a highly developed mechanism for extraocular salt excretion. This type of creature would have been able to cross the open ocean easily, in comparison with modern crocodyles. It might be considered too specialized morphologically to revert to the general crocodylian form. However a marine form less specialized than *Geosaurus* but more specialized than *C. porosus* may have done so and been the ancestor of modern *Crocodylus*.

A major alternative explanation of the presence of lingual salt glands in freshwater crocodyles is that the glands are needed during seasonal droughts (Taplin *et al.*, 1982). Very little is known about terrestrial dormancy or aestivation in crocodyles except that it may be an important factor in many species, such as *C. johnstoni*. Thus one can postulate that lingual salt glands evolved first as a means of excreting sodium chloride and conserving water in terrestrially dehydrating conditions. Secretion rates and numbers of gland tubule openings are lower in the freshwater than in estuarine forms (Taplin *et al.*, 1982, 1985). A gradual selection for higher secretion rates could have occurred in estuarine populations. This scenario is very similar to that proposed for the evolution of salt glands in the marine iguana from terrestrial ancestors with salt glands (Dunson and Mazzotti, 1989). It is also analogous to the estuarine evolutionary sequence proposed for the origin of salt glands in snakes and turtles (Dunson and Mazzotti, 1989). There is a very obvious way of testing the "dehydration-aestivation hypothesis" for the origin of crocodile salt glands. One simply needs to study completely freshwater crocodylians to determine what use if any is made of the glands under dehydrating conditions. If the glands are functional in osmoregulation during droughts, this alternative hypothesis gains credence. If the glands appear to be degen-

erate or vestigial with no apparent function, then the TOMH gains additional support.

Another possible way to approach this issue is to consider patterns of evolution in other aquatic vertebrates. The TOMH basically argues that crocodylians are secondarily freshwater species. In classic zoogeographic terms, such a secondary form would be chiefly found in fresh water, but would retain some salt tolerance (Darlington, 1957). Killfish of the genus *Fundulus* illustrate a fairly typical pattern of this sort (Griffith, 1974). There are 27 species in mainland North America of which eight are completely freshwater, ten are brackish, eight are principally freshwater but also occur in coastal brackish areas, and one is an inland saline lake form. The primarily estuarine species are tolerant of salinities in excess of 70 ppt. The inland species are less tolerant (generally with lethal levels below 29 ppt), but much more tolerant than typical freshwater fish. In some ways this resembles the situation with crocodiles, in that the freshwater forms have a reduced physiological capacity to excrete salts. In other ways, especially the presence of more freshwater than estuarine forms and the generally low level of pelagic adaptation in *C. porosus* and *C. acutus*, these two examples are not analogous. Thus this comparison could favor the hypothesis that the evolution of lingual salt glands occurred in the amphibious-freshwater species, not in the estuarine-marine forms.

In conclusion we propose that, until new data are obtained, there is at present no clear preponderance of evidence to decide between the alternative hypotheses presented above. We believe that a rigorous experimental examination of all possible ideas is necessary to realistically appraise the available hypotheses and to search for other explanations.

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