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SODIUM AND WATER FLUXES IN FREE-LIVING CROCODYLUS POROSUS IN MARINE AND BRACKISH CONDITIONS¹

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Radioactive sodium and water were used to determine total body water (TBW), exchangeable sodium (ExNa) and water and sodium fluxes in free-living *Crocodylus porosus* in marine (hyperosmotic; salinity = 25‰–35‰) and brackish (hypoosmotic; salinity = 2‰–7.5‰) sections of the Tomkinson River in northern Australia. At capture, size-corrected TBW and ExNa pools in 62 crocodiles (hatchlings, juveniles, and subadults; weight, 0.108–54.4 kg) were independent of salinity history. To determine fluxes, all animals were released at their capture sites and left undisturbed until recapture. Thirty-seven were recaptured after 7–18 days. Fifteen of the 17 hatchlings recaptured from both salinity categories grew and maintained their condition and hydration status. In contrast, all 20 juveniles and subadults lost weight in the same period, and juveniles in hyperosmotic conditions showed significantly lower hydration and condition factors. Water effluxes in hatchlings were ~80 and ~160 ml · kg^{-0.63} · day⁻¹ in marine and brackish conditions, respectively. Comparable sodium effluxes were 7.5 and 4.4 mmol · kg^{-0.63} · day⁻¹. All crocodiles in hyperosmotic conditions had consistently lower water effluxes (≈ ×0.5) and higher sodium effluxes (≈ ×1.6) than did crocodiles in brackish water. In both salinity categories, hatchlings had greater water turnover (≈ ×1.3, ×1.6) and sodium turnover (≈ ×1.5, ×1.25) than did juveniles and subadults. Interpretation of the field data is complicated by integumentary exchange of sodium and water, a size-related aphagia apparently induced by disturbance, and difficulties of adjusting for allometric differences across a wide range of sizes. Nevertheless, it is clear that *C. porosus* is able to effect considerable economies of water turnover in hyperosmotic salt water and that the secretory capacity of the lingual glands, as measured in the laboratory, is more than enough to account for the highest sodium effluxes that we measured in *C. porosus* in the field.

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INTRODUCTION

In a series of recent papers, many aspects of the water and electrolyte economy of the estuarine or saltwater crocodile, *Crocodylus porosus*, have been described. It is now known that adults, juveniles, and hatchlings all maintain water and electrolyte homeostasis over salinities ranging from 0 to ≥60‰ (seawater [SW] = 35‰) (Grigg 1981; Taplin 1982, 1984a). Most significantly, *C. porosus* has lingual salt glands (Taplin and Grigg 1981), and these are known to play a major role in the salt and water economy of unfed crocodiles in the laboratory (Taplin 1985). Indirect evidence suggests that the salt glands are crucial in the survival of *C. porosus* in hyperosmotic conditions in the field also. Free-ranging hatchling *C. porosus* survive and grow routinely in hyperosmotic estuaries—where they have no access to fresh or brackish drinking water (Grigg et al. 1980)—as long as an adequate supply of food is available

(Taplin 1982, 1984a, 1984b). Direct evidence that the lingual glands are effective in coping with the sodium loads imposed during life in salt water has not been presented previously. In this paper we report radioisotopically measured water and sodium fluxes of free-living crocodiles acclimatized over a range of salinities. The data show that water turnover is reduced considerably in salt water, that sodium turnover is much higher, and that the secretory capacity of the salt glands, as measured in the laboratory, is more than enough to account for the sodium effluxes that occur in salt water in the field.

We know of no other such study that has been undertaken on a free-living aquatic reptile. Hence, although the methodology has proved successful, we herein discuss in detail some of the limitations that we encountered, because they provide useful insights into the practical difficulties surrounding the interpretation of such data and highlight some of the problems of integrating laboratory and field studies of osmoregulation.

MATERIAL AND METHODS

PILOT STUDY

A preliminary study was conducted in Sydney in 1978 to determine equilibration times for tritiated water (THO) and sodium-22 (^{22}Na) in the body fluids of crocodiles and the approximate efflux rates for these isotopes in fresh water (FW), SW, and air. Six *Crocodylus porosus* (body weight [BW] = 1.58–2.90 kg) were injected intraperitoneally with 170 MBq of THO and 0.3 MBq of $^{22}\text{NaCl}$ and held in air at 25 C. Blood samples were removed from the caudal vein at intervals of 4, 8, 14, 24, and 31 h and allowed to clot. Serum and cellular fractions were separated and stored frozen until analyzed. A period of 4–31 h was found to be suitable for THO equilibration and 8–24 h for ^{22}Na equilibration. Total body water (TBW) and the exchangeable sodium pool (ExNa) were calculated following methods described by Green and Dunsmore (1978). Further blood sampling over an 18–21-day period showed that both ^{22}Na and THO fluxes could be described appropriately by assuming a single-isotope pool to be exchanging with the external

medium. Measurement of THO dilution in *C. porosus* is known to lead to an overestimation of TBW by ~8% relative to desiccation methods (Taplin 1984a), but TBW and water flux measurements reported here have not been corrected for this overestimation.

TWO FIELD STUDIES

Field study 1.—The main field study was carried out at the Tomkinson River in Australia's Northern Territory (lat. 12°9' S, long. 134°11' E) between July 16 and August 2, 1979, midway through the northern dry season. Habitat descriptions and background information on the Liverpool-Tomkinson river system are available in Magnusson, Grigg, and Taylor (1978); Messel, Wells, and Green (1979); Taylor (1979); Grigg et al. (1980) and Grigg (1981).

Two study areas were selected on the basis of river salinity and population density of crocodiles. The downstream study area was in lower mangrove habitat (Magnusson et al. 1978) where the salinity varied from 25‰ to 35‰ according to the state of the tide. This is within the SAL4 category (Grigg 1981), in which salinity is hyperosmotic to crocodile body fluids throughout the tidal cycle. The upstream study area was in upper mangrove habitat with salinities from 2‰ to 7.5‰, corresponding to Grigg's SAL2 category (in which the water is brackish for at least part of the tidal cycle and is always hypoosmotic to crocodile plasma). No FW (SAL1) study area was available within the navigable limits of the river at this stage of the dry season. Salinity profiles were measured at both high and low tide at the beginning and end of the study, using an American Optical salinity refractometer reading to 0.5‰. No differences between top and bottom salinities were found anywhere within the river system.

Crocodiles were caught by hand at night at low tides. A sample of 48 *C. porosus* (40–140 cm in total length and 0.1–7.6 kg BW) was taken, and the capture locations were marked with streamers tied to fringing mangroves. Each crocodile was weighed, measured, sexed (juveniles and subadults only), and marked individually by scute clipping. They were then injected intraperitoneally with 0.2 ml (hatchlings) or 0.5 ml (juveniles) of THO at 740 MBq/ml and

with the same volume of $^{22}\text{NaCl}$ at 0.74 MBq/ml. After 8 h equilibration in air, 0.2–0.5 ml of blood was withdrawn via heart puncture. Serum and red cell fractions were sealed in separate plastic vials and held frozen until analyzed.

The crocodiles were released at their original capture sites within 48 h of capture and were left undisturbed for 15–17 days, after which time 30 were recaptured. A second blood sample was removed from each of these 30 by heart puncture, and a second dose of THO was injected to assess any changes in TBW that had occurred during the interval. ExNa was not redetermined because of the high cost of ^{22}Na . A third blood sample was removed after a further 8 h THO equilibration.

Field study 2.—In the subsidiary study conducted in October 1981, 14 larger (2.11–54.4 kg) crocodiles were captured, most of them by harpooning (Webb and Messel 1977), and then weighed, measured, injected, equilibrated, and sampled as above. Sodium was injected into only 12 of these animals. Each was released at its point of capture and left undisturbed, as in the earlier field study. Seven were recaptured 7–16.5 days later, reweighed, and blood samples taken.

ASSESSMENT OF TBW, ExNa, AND WATER AND SODIUM FLUXES

Tritium activity was counted in 5- μl (hatchlings) or 100- μl (juveniles and subadults) samples extracted from the cellular fraction of the blood by freeze-drying and dissolved in 7 ml of Aquasol (New England Nuclear Corp., Boston) scintillation cocktail. Concentration of ^{22}Na was determined in 50- μl samples of plasma following the method of Green and Eberhard (1979). Samples were counted on a Packard scintillation counter. Plasma sodium concentrations were determined by flame photometry. Water and sodium effluxes were calculated from the general equation

efflux (ml or $\mu\text{mol/day}$)

$$= \frac{(W_2 - W_1) \ln(H_1^+ \cdot W_1/H_2^+ \cdot W_2)}{\ln(W_2/W_1)t}$$

derived from equation (4) of Nagy and Costa (1980), where W_1 and W_2 = initial and final exchangeable pool sizes (ml or

μmol), respectively, H_1^+ and H_2^+ = initial and final specific activities of isotope in plasma (cpm/ μmol), respectively, and t = time elapsed (days).

This equation includes terms to account for changes in plasma sodium concentration and assumes linear changes in sodium and water pools over time. In crocodiles that grew in length and weight during the experiment (hatchlings), it was assumed that ExNa increased in proportion to the change in TBW. This was more appropriate than assuming constancy of sodium pools in animals that grew and showed no signs of sodium or water imbalance. In crocodiles that appeared not to feed during the experiment (juveniles and subadults; see below), ExNa was assumed to remain constant despite changes in TBW in SAL4 crocodiles. This is in accord with laboratory data of Taplin (1982, 1985), which shows that unfed *C. porosus* in SW lose body water linearly over time but maintain stable ExNa pools.

Net flux was calculated from the change in exchangeable pool size (i.e., $[W_2 - W_1]/t$), and influx was determined by difference:

influx (ml or $\mu\text{mol/day}$)

$$= \text{efflux} + (W_2 - W_1)/t.$$

SIZE CLASSES

Because of the great size range of animals involved in this and other studies of crocodiles, we find it convenient to distinguish five size categories—neonate, hatchling, juvenile, subadult, and adult. The terms are not used to distinguish strictly either between animals of particular age classes or between mature and immature individuals, but they do provide convenient names for groups of crocodiles that should be considered separately in discussions of osmoregulatory capabilities in which effects of allometry may be significant. We regard newly hatched *C. porosus* (usually weighing 60–100 g) with imperfectly healed umbilical scars as neonates. The term hatchlings refers to animals of 100–500 g BW; juveniles weigh in the range of 500 g to ~ 15 kg (approximately 0.6–1.7 m total length); and subadults range from 1.7 m to ~ 3 m in length (approximately 15–80 kg). The categories are not defined rigidly and need not

be. The subadult size class, for example, includes a proportion of mature females, but sex-related differences are unlikely to be of greater consequence to osmoregulatory capability than the large differences in surface area:body mass ratios between subadults, juveniles, and hatchlings.

ADJUSTING FOR SIZE EFFECTS: CONDITION FACTOR (CF), HYDRATION FACTOR (HF), AND SODIUM FACTOR (NaF)

In analyzing the experimental data we recognized five groups of crocodiles; SAL2 hatchlings, SAL2 juveniles, SAL4 hatchlings, SAL4 juveniles (all from the July 1979 study), and SAL4 juveniles and subadults (October 1981 study). Members of each group showed, for the most part, similar changes in weight, hydration status, and plasma sodium concentration over the 15–17 days between release and recapture. The analysis of these changes proved to be a complex problem requiring appropriate compensation for the very great size differences between animals and for the effect of size on sodium and water pools, net water loss in SW, and gross sodium and water fluxes. To complicate the issue further, both groups of hatchlings grew in length and weight, requiring allowances for growth increments in body water and BW as well as for any increments attributable to changes in hydration status.

The question of scaling relationships between BW, TBW, and ExNa arose from our first efforts to interpret the data here presented and is considered in detail elsewhere (Taplin 1984a). Working from the empirical relationships between BW, TBW, ExNa, and snout-vent length (SVL) described in that paper, it can be shown that TBW (ml) scales with $BW^{0.984}$, and ExNa (mmol) with $BW^{0.943}$. Thus, expressing water pools in units of $ml \cdot kg^{-0.984}$ and sodium pools in $mmol \cdot kg^{-0.943}$ corrects for the effects of allometry and enables comparison between individuals without danger of confounding treatment effects with size effects.

Because BW is made up largely of water, any change in hydration status expressed per unit weight will tend to mask that change. For example, a 200-g crocodile might have an initial TBW of 160 ml (80% BW). If TBW falls by 25%, to 120 ml (75%

BW), the change in weight-specific TBW is only –6.3%. Similar problems arise with ExNa, because of the osmoregulatory linkage between sodium and water content of the body. One solution is to express pool size per unit length, making appropriate allowance for the effects of allometry and thereby producing a dimension that changes little in the short term. The procedure is essentially the same as that used in calculating condition factors (le Cren 1951) and requires information about the scaling of pool size with length. For *C. porosus* this information is known from work by Taplin (1982, 1984a), and the following hydromineral status factors can be defined:

$$CF = \left(\frac{BW \text{ (in g)}}{SVL \text{ (in mm)}^{3.16}} \right) \times 10^6;$$

$$HF = \left(\frac{TBW \text{ (in ml)}}{SVL \text{ (in mm)}^{3.11}} \right) \times 10^6;$$

$$NaF = \left(\frac{ExNa \text{ (in } \mu\text{mol)}}{SVL \text{ (in mm)}^{2.98}} \right) \times 10^6.$$

These status factors enable direct comparisons between crocodiles of different sizes and, furthermore, allow analysis of changes in hydration status of animals that grew in both length and BW during the experimental period. Both of the above methods of data presentation are used in this paper, in conjunction with simpler expressions such as absolute and fractional rates of change of BW and hydration status. Unless stated otherwise, the statistics given below are cited as means \pm SE (*N*).

RESULTS

HOMEOSTASIS OF TBW, ExNa, AND PLASMA SODIUM AT INITIAL CAPTURE IN HYPEROSMOTIC AND HYPOSMOTIC HABITATS

Size-corrected sodium and water pools and plasma sodium were essentially independent of salinity for four of the five groups of crocodiles (table 1). No significant differences between groups were detectable in TBW or ExNa, which averaged $765 \text{ ml} \cdot \text{kg}^{-0.984}$ and $58.9 \text{ mmol} \cdot \text{kg}^{-0.943}$, respectively. Levels of plasma sodium were similar in four of the five groups ($150.4 \pm 0.71 \text{ mmol} \cdot \text{liter}^{-1}$ [51]) but significantly higher in SAL2 hatchlings (174.5 ± 3.45 [11]). Such high values for plasma sodium

TABLE 1
COMPARISON OF WATER AND SODIUM STATUS OF HATCHLING, JUVENILE, AND SUBADULT *Crocodylus porosus* FRESHLY CAPTURED FROM HYPEROSMOTIC AND HYPOSMOTIC CONDITIONS

Salinity and Sample Status	BW ^a (kg)	NaB (mmol · liter ⁻¹)	TBW (ml · kg ^{-0.984})	ExNa (mmol · kg ^{-0.944})	CF	HF	NaF
SAL4 (Water hyperosmotic):							
1. Hatchlings, 1979	.22 ± .008 [.160-.270] (15)	151.3 ± 1.55 (15)	774 ± 8.4 (15)	57.2 ± 1.11 (15)	8.92 ± .175 (14)	9.25 ± .220 (14)	1.47 ± .033 (14)
2. Juveniles, 1979	3.5 ± .48 [1.08-7.65] (13)	149.5 ± 1.26 (13)	754 ± 4.1 (12)	60.7 ± .55 (12)	8.74 ± .115 (12)	8.84 ± .130 (12)	1.53 ± .027 (12)
3. Subadults and juveniles, 1981	16.1 ± 4.43 [2.11-54.4] (14)	152 ± 1.09 (14)	770 ± 8.8 (12)	59.7 ± 1.63 (12)	7.92 ± .188 (14)	8.14 ± .186 (12)	1.40 ± .059 (12)
SAL2 (Water hypoosmotic):							
4. Hatchlings, 1979	.16 ± .011 [.108-.196] (11)	174.5 ± 3.45 (11)	773 ± 8 (9)	59.5 ± 3.18 (11)	8.22 ± .267 (11)	8.46 ± .227 (9)	1.41 ± .075 (11)
5. Juveniles, 1979	1.3 ± .07 [1.14-1.76] (9)	147.5 ± 1.63 (9)	752 ± 9.2 (9)	57.8 ± .87 (9)	8.31 ± .114 (9)	8.38 ± .148 (9)	1.39 ± .024 (9)

NOTE.—Data are means ± SE. Numbers in parentheses are number of animals. BW = body weight; NaB = sodium; TBW = total body water; ExNa = exchangeable sodium; CF = condition factor; HF = hydration factor; NaF = sodium factor. Between-group statistical comparisons are as follows: TBW, .10 < P < .25 (ANOVA); NaB, gradual trend 1 = 2 = 3 = 5 < 4 (Kruskal-Wallis); ExNa, .90 < P < .95 (Kruskal-Wallis); CF, .005 < P < .01 (ANOVA) and gradual trend 1 > 2 > 4 > 5 > 3 (Kruskal-Wallis); HF, .005 < P < .01 and gradual trend 1 > 2 > 4 > 5 > 3 (ANOVA); NaF, .05 < P < .1 (ANOVA).

^a Numbers in brackets denote the ranges of values.

have been found previously only in dehydrated animals (Taplin 1982), yet careful examination of our records revealed nothing to suggest any difference in handling or treatment of this group that could account for the difference. Furthermore, size-corrected ExNa and TBW data indicate no sign of dehydration or hypernatremia in this group relative to others (table 1), so the high plasma sodium values in SAL2 juveniles remain unexplained at present.

HOMEOSTASIS DURING THE EXPERIMENTAL PERIOD
IN HYPEROSMOTIC AND HYPOSMOTIC HABITATS

1. *Hatchlings from SAL4, 1979.*—SAL4 hatchlings grew significantly in overall length, by $0.1\% \pm 0.11\% \cdot \text{day}^{-1}$ (10) ($.01 < P < .02$; paired *t*-test) (one animal had no final length recorded). Of the 11 hatchlings, nine maintained or increased their weight at rates of $0\% - 0.5\% \cdot \text{day}^{-1}$, whereas two suffered marked weight loss. The group of nine showed no significant change in CF or HF, but a statistically significant though slight fall in plasma sodium occurred during the 15–17-day exposure period (table 2). The substantial growth of these crocodiles, together with their maintenance of precapture condition and hydration status, shows that they must have been feeding actively between release and recapture.

The other two hatchlings (nos. 23 and 25; table 2) suffered marked declines in BW (-5% and -8% , respectively) and TBW (-9% and -8% , respectively), as well as in CF and HF. Plasma sodium rose in both by $\sim 7\%$. This suggests strongly that these animals must have fed poorly (if at all) after release and were unable to maintain condition or normal hydromineral balance. It is notable that the decline in TBW is mirrored by marked declines in HF (-15% and -13%) in each animal, whereas weight-corrected TBW ($\text{ml} \cdot \text{kg}^{-0.984}$) remained effectively constant. This illustrates dramatically the extent to which weight-specific portrayals of changes in hydration status can be misleading.

2. *Juveniles from SAL4, 1979.*—Members of this group showed a consistent pattern of marked weight loss, increase in plasma sodium, and decline in both CF and HF (table 2). Fractional rates of weight loss ($\% \cdot \text{day}^{-1}$) were correlated inversely with size and were similar in magnitude to those

measured by Taplin (1982, 1985) in non-feeding *Crocodylus porosus* in SW in the laboratory at 25 C. Even though we vacated the study area completely between the times of release and recapture, to minimize the possibility of disturbance artifacts, we think it likely that these animals ate less than usual or not at all after their release and hence suffered some dehydration through integumental, excretory, and respiratory water loss. Inhibition of feeding following capture and handling in juveniles but not in hatchlings would be consistent with other observations that juveniles appear to be more sensitive to disturbance than are hatchlings, which we have commonly seen feeding shortly after their release.

3. *Hatchlings in SAL2, 1979.*—SAL2 hatchlings, like those from SAL4, increased in SVL by $0.14\% \pm 0.102\% \cdot \text{day}^{-1}$ (6) ($.02 < P < .05$; *t*-test) and showed concomitant increases in BW and TBW (table 2). However, neither CF nor HF increased significantly in these animals, suggesting that the absolute increase in water content compensated for the dry-matter growth implied by the increase in SVL. It should be noted that initial and final TBW determinations were available for only four of the six recaptures. The small sample used here might preclude detection of small increases in TBW and HF.

The SAL2 hatchlings were notable for their unusually high plasma sodium levels at first capture (tables 1 and 2). At recapture, plasma sodium values were in the normal range for *C. porosus*, reflecting a 15% decline in concentration. If this had been accomplished by a direct increase in TBW, then HF would be expected to have risen by a like amount, to 9.88 (perhaps a little less if tissue growth was accomplished without retention of additional sodium). The observed mean of 9.20 (table 2) is too low to allow such an explanation. It appears more likely that a shift in the distribution of sodium and water between extracellular and intracellular fluid occurred or, alternatively, that some sodium had been excreted actively. Note, however, that the initial sodium status of these crocodiles (as measured by NaF; table 1) was little different from those of other groups.

4. *Juveniles in SAL2, 1979.*—Crocodiles in this group lost weight at approximately

TABLE 2

COMPARISON OF WEIGHT, CONDITION FACTOR, WATER, AND SODIUM STATUS IN FREE-RANGING *Crocodylus porosus* BEFORE AND AFTER 7-16 DAYS IN HYPEROSMOTIC AND HYPOSMOTIC CONDITIONS

Salinity and Sample Status	BW (kg)	NaB (mmol · liter ⁻¹)	TBW (ml · kg ^{-0.964})	ExNa (mmol · kg ^{-0.944})	CF	HF	BW Change (% increase · day ⁻¹)	TBW Change (% increase · day ⁻¹)
SAL4 (Water hyperosmotic):								
Hatchlings, 1979:								
Initial	.22 ± .009 (9)	152.8 ± 1.89 (9)	777 ± 12.6 (9)	56.8 ± 1.8 (9)	8.77 ± .218 (8)	9.12 ± .301 (8)		
Final	...	147.7 ± 1.03 (9)	756 ± 10.5 (9)	...	8.68 ± .563 (8)	NS	+ .25 ± .067 (9)	+ .07 ± .146 (9)
No. 23							***	
Initial	.22	146	724	58.2	9.42	9.14	- .32	- .57
Final	...	154	729	...	7.86	7.70		
No. 25								
Initial	.16	143	765	59.0	7.92	8.14	- .57	- .49
Final	...	153	764	...	6.91	7.10		
Juveniles, 1979:								
Initial	2.6 ± .46 (8)	150.3 ± 1.9 (8)	750 ± 5.1 (7)	61.4 ± .80 (7)	8.72 ± .170 (7)	8.76 ± .203 (7)		
Final	...	158.1 ± 2.42 (8)	732 ± 12.7 (7)	...	7.90 ± .180 (7)	**	- .57 ± .088 (8)	- .37 ± .206 (8)
Subadults and juveniles, 1981:							***	
Initial	17.4 ± 6.05 (7)	152.1 ± 1.16 (7)	766 ± 10.0 (5)	59.2 ± 4.64 (4)	7.79 ± .322 (5)	8.02 ± .354 (5)	- .12 ± .143 (5)	+ .23 ± .155 (7)
Final	...	156.1 ± 1.79 (7)	801 ± 14.8 (5)	...	7.67 ± .441 (5)	8.17 ± .391 (5)	NS	
SAL2 (Water hypoosmotic):								
Hatchlings, 1979:								
Initial	.19 ± .005 (6)	176.1 ± 3.81 (6)	777 ± 14.6 (4)	53.4 ± 3.94 (6)	8.33 ± .324 (6)	8.59 ± .170 (4)		
Final	...	149.6 ± 3.72 (6)	811 ± 16.1 (4)	...	8.45 ± .166 (6)	NS	+ .56 ± .063 (6)	+ .87 ± .317 (4)
Juveniles, 1979:							***	
Initial	1.4 ± .11 (5)	149.1 ± 1.66 (5)	767 ± 5.1 (5)	59 ± .89 (5)	8.32 ± .170 (5)	8.35 ± .139 (5)	- .19 ± .032 (5)	- .27 ± .08 (5)
Final	...	149.4 ± 1.45 (5)	755 ± 12.1 (5)	...	8.04 ± .145 (5)	**	***	***

NOTE.—All data except the entries for No. 23 and No. 25 are means ± SE. Numbers in parentheses are number of animals. Abbreviations are as in table 1.

* .01 < P < .05.

** .001 < P < .01.

*** P < .001.

one-third the rate of SAL4 juveniles (table 2) and approximately one-quarter the rate of SAL4 juveniles in the same size range. Water loss was slight and reflected in a small but significant fall in CF and HF, whereas plasma sodium remained constant. These findings are consistent with what one would expect to see in crocodiles living in mildly brackish water and feeding poorly or not at all. Metabolism would result in some loss of dry matter, whereas maintenance of normal hydromineral balance would pose no major problems.

5. *SAL4 juveniles and subadults, 1981.*—Crocodiles in this group showed responses to SW exposure similar to those of SAL4 juveniles captured in 1979. The changes were less clear-cut, however, because of the shorter period between release and recapture (7–16.5 days) and their larger size, which gives greater insulation from dehydration. BW and CF estimates were lower at recapture, but the apparent decline was statistically insignificant, whereas plasma Na increased slightly (table 2). The smallest of these recaptured crocodiles weighed 7.42 kg, and the largest 51.3 kg. All were considerably larger than any of the SAL4 juveniles recaptured in the 1979 study.

SODIUM AND WATER FLUXES

Hatchling crocodiles in both SAL4 and SAL2 grew during the experimental period, suggesting that they were feeding normally. In contrast, all 20 juveniles and subadults lost weight and presumably fed inadequately or not at all. Therefore, hatchlings have been treated separately from juveniles and subadults.

Estimates of the scaling relationships between isotope fluxes and size are available only for the SAL4 juvenile and subadult crocodiles, which span a large size range (fig. 1). Sodium and water turnover in these animals scale with $BW^{0.62}$ and $BW^{0.65}$, respectively ($r^2 = .91$ in each case). The slopes are not significantly different ($P > .5$; ANCOVA), and the best estimate of the allometric coefficient is $.63 \pm .08$ (means $\pm 95\%$ confidence limit). This suggests scaling of fluxes with surface area, as might be expected if these animals, which do not drink SW (Taplin 1984b), were feeding poorly or not at all. The derivation of this

scaling exponent assumes that the slope of the relationships shown in figure 1 is primarily a reflection of the influence of size on fluxes rather than of differences in behavior, physiology, or environmental conditions between 1979 and 1981. Given the evidence available we believe this assumption to be reasonable and the procedure to be more appropriate than assuming a scaling exponent of 1.0. For example, if water fluxes from the SAL4 1979 juveniles and SAL4 1981 juveniles and subadults are calculated in the units of $ml \cdot kg^{-1} \cdot day^{-1}$, significantly different flux rates emerge for the two groups (38.9 ± 3.16 [8] and 27.1 ± 4.23 [7], respectively; $.02 < P < .05$; *t*-test). This difference is removed by the scaling procedure used here.

The problem of removing the influence of size in flux expressions for juveniles in SAL2 and hatchlings in both salinity categories is more difficult, because *C. porosus* is thought likely to drink brackish water (Grigg 1981; Taplin 1984b) and the scaling relationships for food or water intake are not known. In the absence of adequate information, comparisons between groups have been brought to a common basis by scaling all fluxes to the power 0.63 (table 3 and fig. 2).

Water turnover was uniformly lower and sodium turnover uniformly higher in SAL4 relative to SAL2 crocodiles (table 3 and fig. 2). Water turnover in SAL4 hatchlings and juveniles was 50%–55% of that in SAL2 hatchlings and juveniles. Sodium turnover, on the other hand, was 70% higher in SAL4 than in SAL2 hatchlings but only 43% higher in SAL4 than in SAL2 juveniles. Comparisons may also be made between hatchling and juvenile crocodiles in the two salinity regimes. In SAL4, hatchlings showed sodium and water turnovers 45%–55% higher than did juveniles and subadults. In SAL2, however, hatchlings showed 57% higher water turnover and 26% higher sodium turnover than did juveniles. Although some of this difference may be attributable to the apparent difference in feeding between the two size classes, this appears not to be the complete explanation. Hatchlings 23 and 25, like the juveniles in SAL4, lost weight and dehydrated but still had sodium and water fluxes comparable to those of other hatchlings (table 3).

DISCUSSION

HOMEOSTASIS IN *Crocodylus porosus*

Crocodylus porosus has been shown previously to have considerable powers of osmoregulation in salt water (Grigg 1981; Taplin 1982, 1984a). The data from the present study are, for the most part, in accord with those of other studies and emphasize homeostasis. Of the five size/salinity categories of crocodiles considered, all but SAL2 hatchlings showed uniform plasma sodium concentrations at first capture. Data from SAL2 hatchlings were inconsistent with those of all previous work. An explanation for this could come from future work on crocodiles at moderate salinities, for they may reflect some transient effect following first exposure to saline water

as the marine influence penetrates gradually upstream at the end of the wet season. More likely, however, these high sodium values derive from some unidentified artifact of handling in that subset of crocodiles and/or samples.

As has been discussed (Material and Methods), approaches to the question of homeostasis of sodium and water pools in animals as large as crocodiles cannot be made without considerations of allometric relationships. This is discussed in detail elsewhere (Taplin 1982, 1984a). The conclusion is inescapable that *C. porosus* is capable of precise regulation of its sodium and water pools. However, the present study illustrates the difficulties surrounding a demonstration of this fact and shows also some of the errors of interpretation that

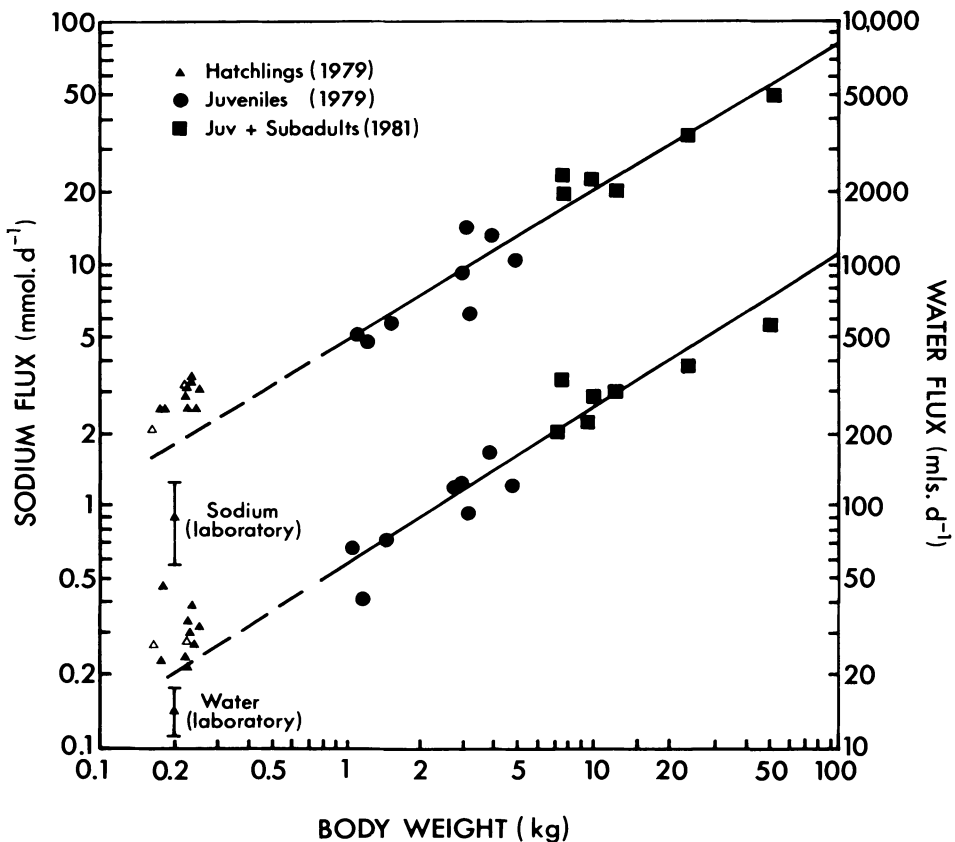


FIG. 1.—Water and sodium fluxes in free-living hatchling, juvenile, and subadult *Crocodylus porosus* in hyperosmotic conditions at the marine end of the Tomkinson River estuary, northern Australia (salinity = 25‰–35‰). For juveniles and subadults, water flux = $56.58 \text{ ml} \cdot \text{kg}^{-0.65} \cdot \text{day}^{-1}$ and sodium flux = $5.029 \text{ mmol} \cdot \text{kg}^{-0.62} \cdot \text{day}^{-1}$, ($r^2 = .91$ in each case). Hatchling fluxes were significantly higher. Values (mean \pm 95% confidence limit) obtained from hatchlings in seawater in the laboratory (Taplin 1985) are included for comparison. Open triangles represent hatchlings no. 23 and no. 25 (see tables 2 and 3).

TABLE 3
 FLUXES OF WATER AND SODIUM IN FREE-RANGING HATCHLING (FEEDING) AND JUVENILE/SUBADULT *Crocodylus porosus* IN HYPEROSMOTIC AND HYPOSMOTIC CONDITIONS

	FLUX TYPE							
	(ml · kg ^{-0.63} · day ⁻¹) Water				(mmol · kg ^{-0.63} · day ⁻¹) Sodium			
	N	Efflux	Storage	Influx	N	Efflux	Storage	Influx
Hatchlings (Presumed feeding):								
SAL4, 1979	9	80.1 ± 8.37	+3 ± .67	80.4 ± 7.96	9	7.53 ± .268	+02 ± .053	7.55 ± .277
No. 23		71.5 } 78*	-1.2	70.4		8.31 } 7.39*	...	=efflux
No. 25		84.5 }	-2.3	82.3		6.47 }	...	=efflux
SAL2	6	155.5 ± 9.75	+2.8 ± .93	157.3 ± 9.81	6	4.36 ± .390	+19 ± .075	4.55 ± .339
Juveniles and subadults (Presumed not feeding):								
SAL4, 1979	8	56.4 ± 4.24	-3.8 ± 1.70	52.6 ± 3.44	8	4.97 ± .456	...	=efflux
SAL4, 1981	7	62.8 ± 6.13	+5.9 ± 4.04	59.6 ± 9.56	6	5.15 ± .391	...	=efflux
SAL2, 1979	5	102.3 ± 7.08	-2.4 ± .81	99.9 ± 6.96	5	3.47 ± .322	-18 ± .060	3.28 ± .284

NOTE.—All data except that for No. 23 and No. 25 are means ± SE.
 * Mean of bracketed values.

may arise if scaling relationships are overlooked.

fluxes in crocodylians poses considerable difficulties. It was hoped at the outset (1978) that data from free-ranging crocodiles under natural conditions would shed light on the (then existing) problem of identifying a functional salt gland in *C. porosus*. That particular problem was subsequently solved

FLUXES

It is clear from the results presented here that the measurement of sodium and water

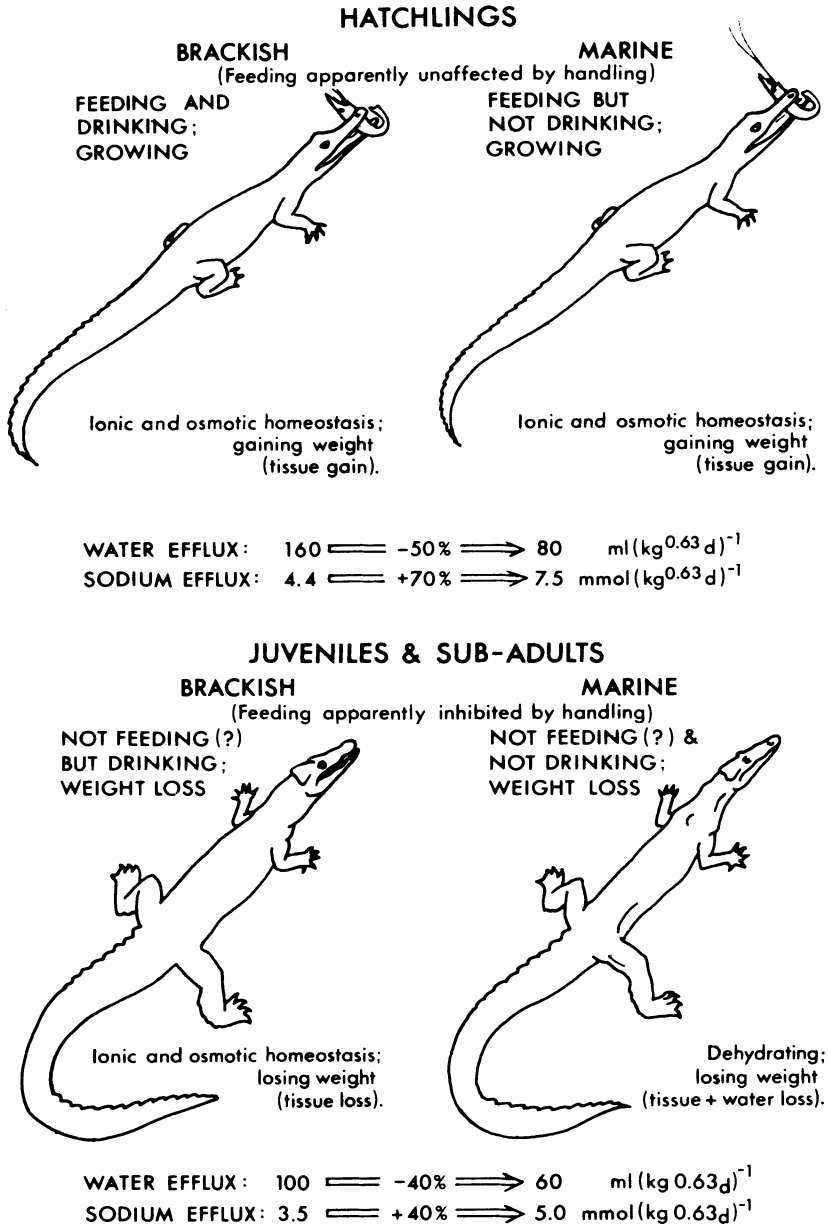


FIG. 2.—Summary model to explain our interpretation of the water and Na relations of free-living *Crocodylus porosus* in hyperosmotic and hypoosmotic sections of an estuary. Data published elsewhere have established previously that feeding juveniles and adults as well as hatchlings are easily able to maintain homeostasis in hyperosmotic conditions as long as there is adequate food intake (Grigg et al. 1980; Grigg 1981; Taplin 1982, 1984a). Hence, the weight loss of juveniles and subadults in the present study is thought to be an artifact, the result of feeding being completely or partly inhibited following capture, handling, and release.

in the laboratory (Taplin and Grigg 1981) during studies that also demonstrated that a very large proportion of the sodium and water exchange in *C. porosus* takes place across the integument (Taplin 1985). This large integumental exchange tends to mask the bulk flows (feeding, drinking, urination, and defecation) that are of particular relevance to studies of osmoregulatory mechanisms. It was hoped that laboratory measurements of integumental sodium and water exchange in fasted crocodiles would allow correction for this component of flux. However, gross sodium and water fluxes in laboratory-fasted *C. porosus* lie well below the extrapolated line for juveniles and subadults and the data points for hatchlings 23 and 25 (fig. 1), despite the fact that laboratory measurements were made on freshly captured hatchlings from the same section of river. In addition to this problem, the response of individual crocodiles to disturbance varies considerably and in a size-related fashion. Juvenile and subadult *C. porosus* all lost weight at rates similar to those seen in fasted crocodiles in the laboratory (Taplin 1982, 1985). The most likely explanation for the results is that the crocodiles were inhibited from feeding for some or all of the experimental period. This apparent aphagia during winter months cannot be considered normal, at least for *C. porosus* from highly saline water. *Crocodylus porosus* depends on feeding or periodic access to FW or brackish water for maintenance of salt and water balance in SW (Grigg et al. 1980; Taplin 1984a, 1984b, 1985). Access to FW or brackish drinking water is out of the question for many of the crocodiles in this study. Most of them were recaptured very close to their original capture sites, and extensive work in the area has shown no evidence of the sort of regular movements to and from discrete FW springs that would be required to explain homeostasis in nonfeeding animals (Grigg et al. 1980, unpublished data). Furthermore, Taylor (1979), using weight of food in the stomach and number of empty stomachs, found no evidence of significant shifts in food intake or loss of condition in juvenile and subadult *C. porosus* between wet (summer) and early dry (winter) seasons. Juvenile *C. porosus* from this river system do, in fact, suffer considerable loss of condition between early (June/July) and

late (October/November) dry seasons (Taplin 1984a, unpublished data), a response presumably related to declining food supply during the drier months of the year; however, this loss of condition is not accompanied by any discernible imbalance of TBW, ExNa, or plasma electrolytes. The prolonged effect of the disturbance associated with the capture of larger crocodiles—which were left completely undisturbed for 2–2.5 wk after capture and first release—combines with the large integumental sodium and water exchange to limit the value of the field data collected. Nevertheless, some valuable conclusions can be drawn.

The marked reduction in water flux in SAL4 relative to SAL2 crocodiles over the whole size range (table 3 and fig. 2) is of particular interest given the similar water concentration gradients across crocodile skin in FW and salt water. The vast majority of the THO exchange across crocodile skin represents diffusional exchange rather than osmotic flux (Taplin 1982, 1985). That gross THO flux was reduced markedly in SW relative to brackish water suggests that there must be a considerable reduction in either the active components of flux or the diffusional water permeability (P_d) of the integument. Taplin (1982) found little evidence of marked shifts in P_d between FW and salt water in the laboratory. The likeliest explanation is, therefore, that influx owing to drinking and efflux owing to urination/defecation is reduced greatly in SAL4 relative to SAL2. Taplin (1982, 1984b, 1985) found that *C. porosus* avoids drinking SW but will drink relatively large volumes of FW. Unfed crocodiles in SW are virtually anuric in comparison with those in FW. No direct evidence is available regarding either the drinking behavior of *C. porosus* in brackish water or urination rates in feeding crocodiles. However, a marked change in drinking behavior between crocodiles in SAL2 and SAL4 habitats would conform with the observation of Grigg (1981) that the most obvious shifts in urinary electrolyte and NH_3 /urate composition occur between SAL3 and SAL4 (in SAL3 the water is alternately hypo- and hyperosmotic according to the state of the tide). SAL3 crocodiles produce largely liquid urine, rich in NH_4HCO_3 and low in solids. SAL4 crocodiles, on the other hand,

excrete nitrogen primarily in the form of urates, and the urine has a much greater solid content. The evidence from the present study suggests that the change from NH_4HCO_3 to urate excretion may contribute to a substantial decrease in water turnover. This may be essential in animals that are apparently unable to osmoregulate by drinking SW and that excrete excess sodium chloride via their salt glands.

The measurements of sodium fluxes in hatchlings known to be feeding in hyperosmotic conditions in the field provide an opportunity to compare previous measurements of salt-gland secretory capacity with the needs of the animal. Although the lingual glands are known to play a crucial role in osmoregulation in unfed crocodiles in SW, accounting for >96% of the actively excreted sodium (cloacal + salt-gland components) (Taplin 1982, 1985), it has not been possible previously to assess directly their ability to cope with the sodium load imposed by feeding on electrolyte-rich invertebrates while living in hyperosmotic media. The highest sodium effluxes that we measured in the field were in hatchlings in SAL4, where gross sodium efflux amounts to $7.5 \text{ mmol} \cdot \text{kg}^{-0.63} \cdot \text{day}^{-1}$ (table 3), an indeterminate fraction of which will reflect exchange diffusion across the integument and not impose any sodium load on the lingual glands. Nevertheless, the apparent maximum secretory capacity of the lingual glands in *C. porosus*, measured under methacholine stimulation, is $7.89 \text{ mmol} \cdot \text{kg}^{-0.63} \cdot \text{day}^{-1}$ (derived from Taplin [1982, 1985]), more than enough to account for the entire sodium load imposed by living and feeding in hyperosmotic salt water. Given the abundant evidence of precise osmoregulation over a very wide range of salinities, there can be no doubt that the lingual glands have the capacity to excrete the sodium load normally imposed on *C. porosus* in salt water.

In many studies, on a variety of animals (particularly carnivores), measures of ^{22}Na

turnover have been used to assess food intake. However, in crocodiles in salt water, the estimation of the contribution of feeding to sodium and water budgets is unlikely to be easy, since integumental exchange of sodium and water interferes with flux-partitioning studies using ^{22}Na , THO, or doubly labeled water. In freshwater populations, on the other hand, it may be feasible to estimate food turnover of *C. porosus* or other crocodylians using ^{22}Na and doubly labeled water. *Crocodylus porosus* shows no evidence of any capacity for active uptake of sodium from FW (Taplin 1982), so sodium influx in FW will reflect primarily the intake via food. Such a study would need to allow for the possibility of markedly different individual responses and the problems of allometry. Interpretation of data would be facilitated by either minimizing the size range of animals to be used or ensuring that scaling factors for all important physiological variables can be estimated. Extrapolation from one size class of crocodile to another must certainly be approached with caution. Even if consistent individual responses to treatment are evident, direct comparison of scaled values and even the derivation of scaling relationships can be problematical. Differences in diet, behavior, habitat preference, and other attributes have been demonstrated between hatchling and adult marine iguanas (Nagy and Shoemaker 1984), and these have a marked influence on water turnover. A component of the hatchling-juvenile/subadult difference in our study may reflect the operation of such factors over and above the more obvious feeding/nonfeeding component. The fitting of a power function to the data for juveniles and subadults in figure 1 relies on the assumption that there is no confounding of size with other aspects of the animals' biology across the size range. In the absence of data to support a more detailed interpretation, this assumption is arguably more appropriate and realistic than assuming isometry of fluxes with BW.

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