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Plasma Homeostasis and Cloacal Urine Composition in *Crocodylus porosus* Caught Along a Salinity Gradient

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Summary. Juveniles of the Estuarine or Saltwater Crocodile, *Crocodylus porosus*, maintain both osmotic pressure and plasma electrolyte homeostasis along a salinity gradient from fresh water to the sea. In fresh water (FW) the cloacal urine is a clear solution rich in ammonium and bicarbonate and containing small amounts of white precipitated solids with high concentrations of calcium and magnesium. In salt water (SW) the cloacal urine has a much higher proportion of solids, cream rather than white in colour, which are the major route for excretion of potassium in addition to calcium and magnesium. Neither liquid nor solid fractions of the cloacal urine represent a major route for excretion of sodium chloride. The solids are urates and uric acid, and their production probably constitutes an important strategy for water conservation by *C. porosus* in SW. These data, coupled with natural history observations and the recent identification of lingual salt glands, contribute to the conclusion that *C. porosus* is able to live and breed in either fresh or salt water and may be as euryhaline as any reptile.

Introduction

Until recently it was thought that the marine and/or estuarine Crocodilia, *C. porosus* and *C. acutus*, lacked functional extra-renal salt glands (Dunson 1969, 1970; Dunson and Moll 1980). This apparent lack led to doubt about the ability of these animals to maintain electrolyte balance in salt water, so they came to be regarded as having estuarine rather than marine capabilities (Schmidt-Nielsen 1975). Geographic distribution and various observations of large crocodiles at sea (Neill 1971; Allen 1974) were explained in terms of a relatively impermeable skin, a small surface area/mass ratio and a fish diet (Dunson 1976).

However, the occurrence of juvenile and hatchling *C. porosus* as residents in hypertonic estuarine conditions in northern Australia (personal observation) suggested physiological competence rather than shortcomings. Accordingly a study was made in 1975 to determine the extent to which juvenile *C. porosus* are homeostatic along a natural salinity gradient. As will be described in this paper, the animals maintain a very high degree of homeostasis, but whether or not this homeostasis depends on fresh water for drinking was not known. In a subsequent field study, in July 1979, Grigg et al. (1980) found that hatchling *C. porosus* survive and grow in sea water, without access to fresh water. All these data implied an active salt gland and the existence of such glands was confirmed recently in both *C. porosus* (Taplin and Grigg 1981) and *C. acutus* (Taplin et al., in preparation).

A more complete understanding of the water and electrolyte physiology of *C. porosus*, and the related problem of nitrogen excretion, requires information on typical levels of electrolytes and metabolites in both plasma and cloacal urine from crocodiles captured over a wide range of salinity. The present paper reports these data, examines the extent to which plasma homeostasis is maintained by juveniles, and describes cloacal electrolyte and nitrogen excretion over a wide natural salinity gradient.

Materials and Methods

The study was carried out in the Liverpool and Tomkinson Rivers near Maningrida, northern Australia, during July 1975. Blood and cloacal urine were taken from as many non-hatchling captives as possible. Stomach contents also were taken from many of the same animals (Taylor 1977, 1979). Some supplementary data are included from a field trip to the same area in July 1979.

Study Area, Salinity Profiles and Crocodiles. Detailed descriptions of the Liverpool-Tomkinson river system have appeared previously (Webb and Messel 1978; Magnusson et al. 1978; Messel et al. 1979)

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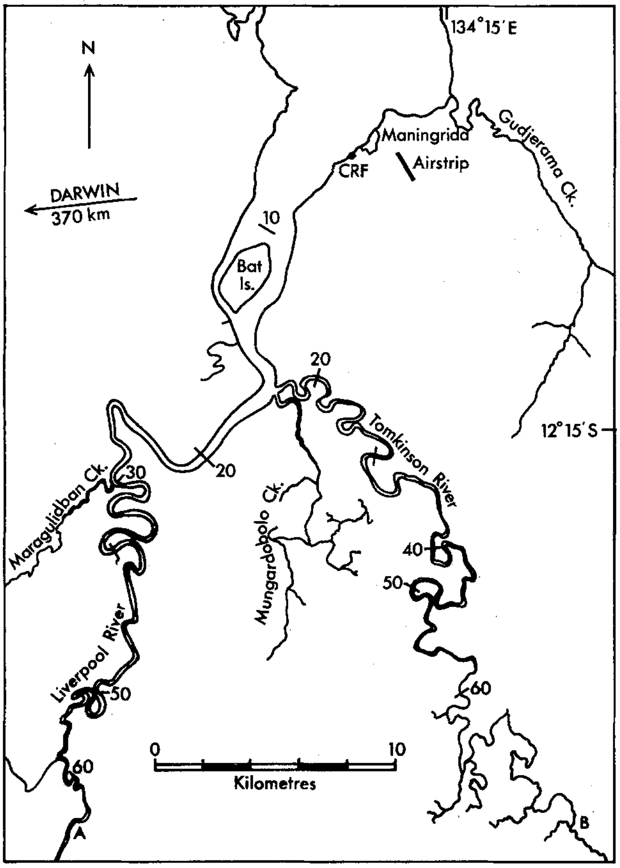

Fig. 1. Map of the study area

and a brief description only need be given here. The rivers meander across an extensive shared flood plain before they join and enter the Arafura Sea via a common estuary (Fig. 1). Three major (Mungardobolo, Maragulidban and Gudjerama) and several minor creeks form part of the system.

To understand the salinity environment of *C. porosus* and to assign a likely salinity history to crocodiles caught, one must understand the seasonal and tidal cycles which occur in these rivers and creeks. All are subject to a tidal rise and fall of 2–3 m approximately twice daily, even as far as 70–80 km upstream from the mouth. The water is mostly turbid with suspended material and its salinity varies in a distinct seasonal cycle, overlain by the tidal cycle. Extensive rainfall during the monsoon season (November-April) flushes the rivers, and potable water may be found as far downstream as Bat Island. With the cessation of the monsoon and reduction of flow at the headwaters, the marine influence moves progressively up river by mixing associated with the tidal flows until, by the end of the dry season, water may be too salty for humans to drink even as far upstream as A and B in Fig. 1. In addition to the seasonal cycle of salinity change, substantial changes often accompany each tidal cycle. These are most marked in the dry season when salinity along much of each river oscillates between high and low values with the tidal flow (Fig. 2).

Our sampling was carried out from 18–26 July, 1975, in mid dryseason. High and low tide salinity-distance profiles were determined in each river several times during the study, using a conductivity meter (Autolab). Seasonal changes in the nature of the salinity gradient during the sampling period were small enough to be ignored, but tidal changes were quite large (Fig. 2a, b). The different shape of the profile in each river reflects the lesser fresh water inflow to the Tomkinson river. Fortunately there was no top-bottom gradient in salinity and no 'salt-wedge' in either river, as occurs sometimes. This simplified the nature of the gradient considerably.

Crocodiles were caught on the night low tides, as described by Webb and Messel (1977) and the capture site of each was recorded. It was assumed that each animal had been exposed to the salinity range typical of its capture site and was acclimatised to that salinity regime. It is known that short term mobility of juvenile crocodiles is low (Webb and Messel 1978; Grigg et al. 1980; Messel et al. 1981).

The supplementary data collected in 1979 were gained at the same time of year (18 July–2 August) and high and low tide salinity-distance profiles determined as before. Thus, the salinity history of the 1979 animals was assessed in the same way as in 1975, allowing data to be pooled.

Blood and Cloacal Sampling and Analytical Procedures. Blood was sampled from 110 individuals in 1975, of which 65 gave a sample of cloacal urine. Eighteen samples of cloacal urine were collected in 1979. Blood was drawn from the caudal sinus (Grigg and Cairncross 1980) and lithium heparin was used to prevent clotting. Subsamples of whole blood were stored frozen for later analysis of blood glucose, lactate, hemoglobin and 2,3-diphosphoglycerate, reported elsewhere (Grigg and Gruca 1979; Gruca and Grigg 1980; Grigg and Cairncross 1980). Plasma samples were prepared by centrifugation at 3,000 r.p.m. for 5 min. Cloacal urine samples were centrifuged at the time of collection and the supernatant sub-sampled for osmotic pressure determination. Osmotic pressures of both plasma and cloacal urine were determined within two hours of sampling, using a Knauer Model M Semi-micro Osmometer. The remainders of plasma and cloacal urine samples (liquid plus solid) and all other samples were transported frozen to Sydney.

Sodium, potassium, chloride, bicarbonate, inorganic phosphate, ammonia, urea, uric acid and creatinine were analysed at St. Vincent's Hospital, Sydney, on an Autolab 12/60 auto-analyser. Calcium and magnesium were analysed by atomic absorption spectrophotometry (Perkin-Elmer) at Royal Prince Alfred Hospital, Sydney. Analysis of cloacal urine precipitates was effected following their digestion by acid.

Grouping of the Animals by Size, Sex and Salinity History. The crocodiles (69 males, 41 females) were all non-hatchlings in their second dry-season or older. Although they represented a fairly continuous size distribution from 0.89 (snout-vent length = 34.5 cm) to 46.4 kg, five weight classes were defined (Table 1), enabling some analyses using weight as a discontinuous rather than a continuous variable.

Based on knowledge of salinity profiles of the river at high and low tide and the mean osmotic concentration of *C. porosus* plasma (304 mOsm : 1^{-1}), four 'physiologically reasonable' categories of salinity history were decided upon (Fig. 2). These were as follows:

SAL 1 – fresh water above the limit of any measurable marine influence.
SAL 2 – some marine influence, but throughout the tidal cycle water salinity never exceeds the osmotic concentration of the plasma.
SAL 3 – tidal salinity fluctuations are such that osmotic concentration of the water fluctuates alternately above and below that of the plasma.
SAL 4 – water salinity at a higher osmoticity than the plasma at all stages of the tidal cycle.

Statistical Analysis and Data Management. Data management and analysis were undertaken using the SPSS system (Nie et al. 1975) and 5% levels of probability were adopted as significant. The main thrust of the analysis was to determine the effect of salinity on plasma and urine electrolytes and nitrogenous excretory products. Salinity was treated sometimes as a continuous variable (e.g. in correlation matrixes) and sometimes as a discontinuous variable

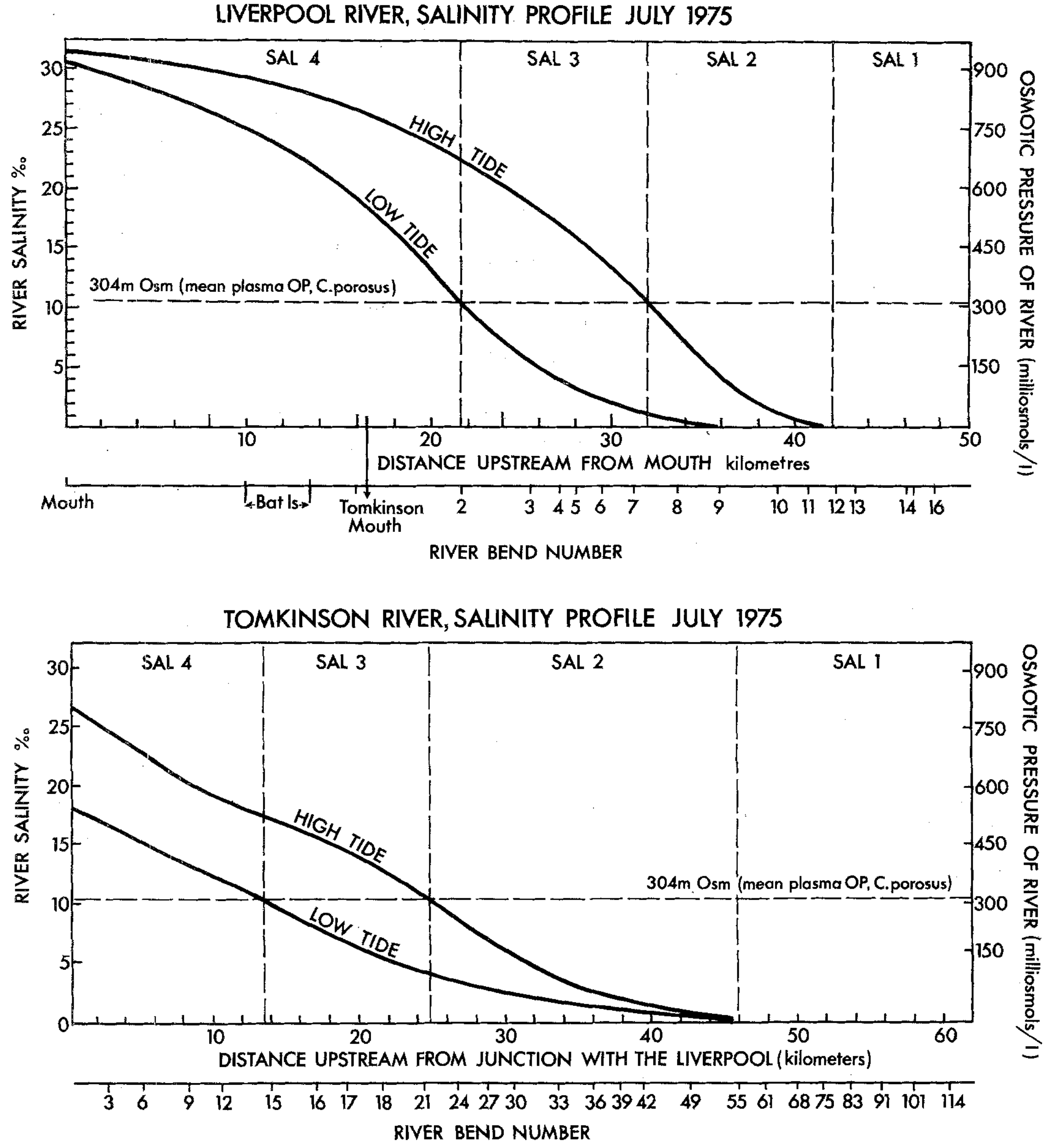

Fig. 2. Salinity profiles in the study area at high and low tide in July 1975. Mean plasma osmotic pressure was used to define four physiologically realistic salinity categories, SAL 1 to SAL 4 (see text)

(as in categorisation of animals by salinity history for analysis of variance and for tabulation of results). Despite a reasonably even spread of samples from both sexes over a wide range of salinity history and weights (Table 1) there was the possibility of confusion between effects of weight and/or sex and those due to salinity. Accordingly, the possibility of weight and/or sex effects was examined first, using analysis of variance techniques.

Results

Plasma Electrolytes and Osmotic Pressure; Effect of Salinity

(i) Effects of Size and Sex. Complete (or nearly so) sets of data were available for plasma from 99 individuals, and for urine from 58 crocodiles. In order to determine whether or not plasma electrolyte values depend upon size and/or sex, a two-factor analysis of variance was carried out using both sexes and five weight classes (Table 1) as factors for each electrolyte species and plasma osmotic pressure. Four replicates for each weight-sex cell in the analysis were selected at random (four being the smallest number of sets of observations in a single cell; females 8.01–10.2 kg). No differences due to sex were apparent. A significant dependence on weight was found only for plasma inorganic phosphate which was found to be significantly higher in larger crocodiles.

The question of correlates with weight and/or sex was pursued also using all data in a Pearson correlation matrix (a copy of which is available from the author, if desired). Again, a significant

Table 1. Breakdown by weight, sex and salinity category of 110 juvenile *C. porosus*

		Salinity category[a]				Totals
		SAL 1	SAL 2	SAL 3	SAL 4	
0.8– 2.4 kg	♂	5	8	4	3	20
	♀	1	6	3	2	12
2.41– 5.0 kg	♂	5	0	3	4	12
	♀	6	3	0	0	9
5.01– 8.0 kg	♂	4	1	4	3	12
	♀	4	1	2	0	7
8.01–10.2 kg	♂	1	2	1	6	10
	♀	2	0	0	2	4
above 10.2 kg	♂	8	2	3	2	15
	♀	7	1	0	1	9
Totals		43	24	20	23	110

[a] See text (Methods) and Fig. 2 for definition

increase in inorganic phosphate was indicated in larger crocodiles ($P<0.001$), though a scattergram showed that this result depends heavily upon data from the few individuals in the study which were approaching maturity. Plasma sodium, chloride, bicarbonate, magnesium, calcium, and lactate were again shown to be independent of both sex and weight, but plasma potassium, ammonia and osmotic pressure showed a significant increase in larger animals. Scattergrams of these latter relationships suggested that such a result depended upon the contribution of only a few points from the largest animals in the study. When the Pearson correlation was repeated without the eight crocodiles greater than 15 kg in body weight, inorganic phosphate and ammonia were still significantly weight-dependent, with calcium in addition.

In summary, the results from the analysis of variance and the Pearson correlation showed that plasma electrolyte concentration and osmotic pressure are independent of sex, while size has little or no effect except that larger animals tend to have higher levels of plasma inorganic phosphate and ammonia. More data are needed from large animals to confirm whether levels of plasma potassium and osmotic pressure increase with increasing body size.

(ii) Effects of Salinity. There is a very high degree of plasma homeostasis along the salinity gradient. In the Pearson correlation not a single plasma electrolyte species was salinity-correlated and only plasma chloride came close ($P=0.080$). By analysis of variance, plasma chloride is just ($P=0.04$) significantly salinity dependent, with a trend to higher chloride in fresh water (Table 2). Plasma sodium shows a similar (though not significant) trend. According to the correlation matrix, plasma osmotic pressure is independent of salinity, though the analysis of variance indicated higher values in fresh water by a slight but statistically significant margin. Slightly higher values of plasma bicarbonate seem typical of crocodiles from salt water (Table 2) and may be an artifact of time between capture and sampling (see later) though the

Table 2. Plasma electrolytes and other data from *C. porosus* at different levels of salinity. Values are mmol/l unless stated otherwise. Mean values ($\bar{x}$) are given, followed by the number of observations (n). 95% confidence limits are given in brackets

	SAL 1		SAL 2		SAL 3		SAL 4		Probability (1-way analovar)	Overall mean	
	$\bar{x}$	n	$\bar{x}$	n	$\bar{x}$	n	$\bar{x}$	n		$\bar{x}$	n
Osmotic pressure (mOsm)	307 (305–309)	37	305 (302–308)	19	299 (295–302)	19	302 (298–305)	24	<0.001	304 (302–305)	99
Sodium	136 (133–139)	40	135 (130–139)	19	132 (129–135)	19	130 (126–135)	21	N.S.	134 (132–136)	99
Potassium	4.1 (3.7–4.4)	35	3.7 (3.4–4.0)	17	3.5 (3.3–3.7)	18	3.8 (3.6–4.0)	21	N.S.	3.8 (3.6–4.0)	91
Calcium	2.8 (2.7–3.0)	20	3.0 (2.9–3.1)	10	2.7 (2.5–2.9)	11	2.8 (2.6–3.0)	10	N.S.	2.8 (2.7–2.9)	51
Magnesium	1.4 (1.3–1.5)	21	1.6 (1.4–1.8)	9	1.4 (1.2–1.6)	6	1.5 (1.2–1.8)	9	N.S.	1.5 (1.4–1.6)	45
Ammonia	0.8 (0.5–1.1)	33	0.6 (0.3–0.9)	15	0.45 (0.3–0.6)	17	0.8 (0.4–1.1)	20	N.S.	0.7 (0.5–0.8)	85
Chloride	121 (117–125)	40	116 (114–118)	19	117 (114–120)	19	114 (111–117)	21	<0.05	118 (116–120)	99
Bicarbonate	14.9 (13.7–16.1)	40	16.7 (14.5–19.0)	19	18.9 (16.7–21.2)	19	17.8 (16.1–19.5)	20	<0.01	16.6 (15.7–17.5)	98
Inorganic phosphate	2.06 (1.92–2.21)	40	1.81 (1.53–2.10)	19	1.88 (1.68–2.08)	19	1.94 (1.72–2.15)	21	N.S.	1.95 (1.86–2.05)	99
Lactate	4.6 (3.4–5.9)	16	4.4 (2.1–6.8)	9	2.9 (1.0–4.7)	10	4.0 (1.9–6.1)	10	N.S.	4.1 (3.3–4.9)	45

Table 3. Electrolyte components of liquid cloacal urine of *C. porosus* at different levels of salinity. Values are mmol/l unless stated otherwise. Mean values ($\bar{x}$) are given, followed by the number of observations (n). 95% confidence limits are given in brackets

	SAL 1		SAL 2		SAL 3		SAL 4		Probability (1-way analovar)
	$\bar{x}$	n	$\bar{x}$	n	$\bar{x}$	n	$\bar{x}$	n	
Osmotic pressure (mOsm)	252 (240–265)	15	256 (246–266)	19	257 (250–266)	15	277 (259–296)	16	$P<0.03$
Sodium[a]	–		7.9 (0–17.5)	9	–		9.8 (2.4–17.2)	9	N.S.
Potassium	2.2 (1.6–2.9)	17	2.7 (1.5–3.9)	15	3.3 (1.5–5.2)	11	8.8 (5.5–12.0)	11	$P<0.0001$
Calcium	1.0 (0.6–1.4)	8	2.0 (0.6–3.5)	7	2.0 (0.6–3.3)	5	4.4 (0–9.0)	6	N.S. ($P=0.08$)
Magnesium	0.06 (0.03–0.08)	7	0.40 (0–0.87)	10	0.32 (0–0.86)	6	11.69 (0–28.2)	5	$P<0.01$
Ammonia	98 (85–111)	17	84 (66–101)	16	86 (69–104)	11	43 (31–56)	13	$P<0.0001$
Chloride[a]	–		9.1 (4.4–13.9)	7	–		9.7 (5.0–14.3)	9	N.S.
Bicarbonate	41.8 (33.7–50.0)	17	38.4 (23.3–53.4)	16	35.8 (18.6–53.1)	11	0.8 (0–1.84)	14	$P<0.0001$
Inorganic phosphate	7.76 (4.66–10.86)	17	6.75 (2.73–10.77)	16	7.30 (3.93–10.67)	11	9.90 (2.96–17.03)	14	N.S.

[a] Data collected July 1979, other data July 1975

relationship did not show up in the correlation matrix. In neither method of analysis was there any suggestion of a significant salinity-related trend in the values of plasma sodium, potassium, inorganic phosphate, calcium, magnesium, ammonia or lactate.

(iii) Capture Artifact. The struggling of a crocodile at the time of capture can be expected to have an influence on plasma metabolites and electrolytes. Accordingly the time between capture and blood sampling was introduced as a variable in the correlation matrix analysis. Cognizance of any variables related to elapsed time is important in terms of reporting 'normal' values for *C. porosus* and may shed light on the physiological correlates of recovery from a struggle.

Following capture, significant trends towards lower plasma lactate, osmotic pressure and plasma calcium were found, while bicarbonate levels rose. In all cases, however, the variability was very great and correlation coefficients were too low for the relationship to have any predictive value.

Urine Electrolytes and Osmotic Pressure; Effects of Salinity

In both fresh and salt water, *C. porosus* excretes urine with some insoluble precipitate. Although the precipitated fraction is particularly substantial in salt water, even in freshwater the solids are a persistent and characteristic component (Fig. 3). The results of analysis of liquid and precipitated urine fractions will be given separately.

(i) The Electrolyte Composition and Osmotic Pressure of the Liquid Urine at Different Salinities. Whereas plasma electrolytes showed a high degree of homeostasis along the salinity gradient, a markedly different pattern emerged for electrolytes in cloacal urine (Table 3). Crocodiles from SAL 4 had significantly higher cloacal osmotic pressure, though always hypoosmotic to the plasma (Table 3). Large increases in potassium, calcium and magnesium were observed and in each of these cases the most dramatic increase was in SAL 4 with respect to SAL 1–3 (Table 3). Inorganic phosphate levels were broadly similar in all four salinity categories. A very large decline in both ammonia and bicarbonate was observed and again in this case the biggest change was in SAL 4 with respect to SAL 3 (Fig. 3).

The most striking result, however, is that sodium and chloride are present in only very small amounts and, furthermore, there is no suggestion of any increase in sodium and chloride in the liquid fraction of the cloacal urine towards salt water at higher salinities (Table 3).

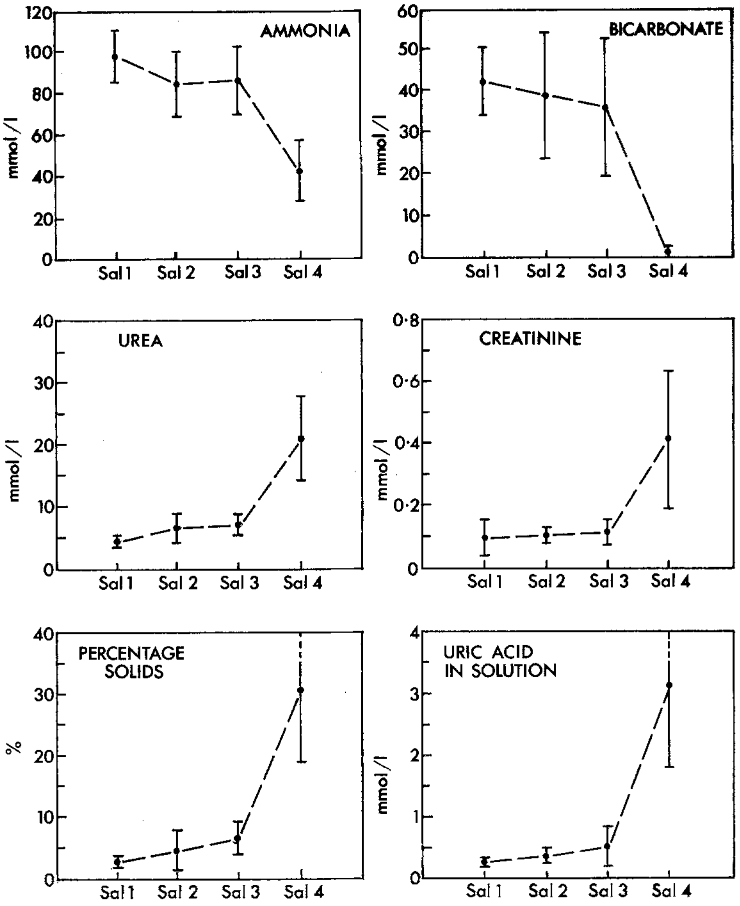

Fig. 3. Nitrogen excretion in liquid cloacal urine by *C. porosus* at different levels of salinity, and comparative percentages of solids in cloacal urine. Error bars are 95% confidence limits

Table 4. Composition of cloacal urine solids from *C. porosus* at different salinities. Values are mg/g solid unless stated otherwise. The mean ($\bar{x}$) is given and number of observations (n), with 95% confidence limits in brackets

	SAL 1		SAL 2		SAL 3		SAL 4	
	$\bar{x}$	n	$\bar{x}$	n	$\bar{x}$	n	$\bar{x}$	n
Ca	21.5 (11.3–31.7)	14	7.9 (1.7–14.0)	8	8.5 (2.3–14.6)	8	1.3 (0.6–2.0)	12
Mg	34.0 (23.8–44.1)	14	16.4 (8.4–24.4)	8	16.0 (9.7–22.3)	8	5.0 (1.2–8.8)	11
Na	0.0	14	0.2 (0–0.7)	8	0.2 (0–0.5)	8	0.3 (0.1–0.5)	12
K	0.2 (0–0.4)	14	2.7 (0–7.9)	8	0.8 (0.2–1.4)	8	12.7 (4.1–21.3)	12
% by volume	2.7 (1.7–3.7)	17	4.5 (1.1–7.9)	16	6.6 (3.9–9.3)	11	30.5 (18.9–42.2)	14

(ii) The Electrolyte Composition of the Precipitated Urine at Different Salinities. Substantial changes in cation composition in urine solids are seen between the four salinity categories (Table 4, Fig. 4). While the amount of potassium increases more than tenfold, the proportional contribution of calcium and magnesium decreases between fresh water and salt water. The low concentration of sodium in the liquid urine is mirrored in the composition of the solids where sodium occurs in only trace amounts.

(iii) Total Urine Electrolyte Composition (Liquid+Solid) at Different Salinities. Combining data on liquid (Table 3) and solid (Table 4) urine and allowing a specific gravity of 1.3 for the precipitate, one can arrive at an approximation of electrolyte excretion

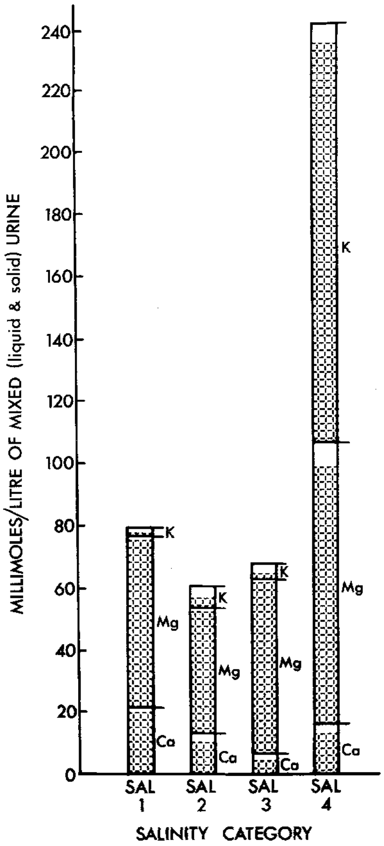

Fig. 4. Comparative contribution of liquid (open sections) and solid (shaded sections) urine to electrolyte excretion in each of the four salinity categories (calculated according to method described in text)

per litre of mixed cloacal urine in each of the four salinity categories (Fig. 4). This has been done using mean values only, not for each individual animal, and no statistical comparisons were made.

In fresh water the solids form a major vehicle for Ca and Mg excretion. In salt water, however, the additional role of the solids in potassium excretion is seen. The data suggest that the urine is not a major route for excretion of excess sodium.

Nitrogenous Excretory Products in the Plasma and Urine; Effects of Salinity

Levels of the four major nitrogenous excretory products in plasma and liquid urine from crocodiles in each of the four salinity categories are shown in Table 5. To enable easy comparisons, the data for ammonia are repeated from Tables 2 and 3 in which it appears as an electrolyte.

Homeostasis of plasma across the salinity categories is again emphasised, with no significant effect of salinity on ammonia, creatinine or uric acid. Plasma urea is, however, significantly higher in SAL 4 than in all the other categories.

In the liquid urine, ammonia decreases progressively from fresh to salt water whereas urea, creatinine and uric acid in solution are all increased significantly in SAL 4 by comparison with SAL 1–3. The large increase in the percentage by volume of solid precipitate in salt compared with fresh water has been referred to earlier. Fig. 3 summarises the levels of major nitrogen excretion-related variables at different salinities.

Discussion

Plasma Homeostasis

It is clear that *C. porosus* is able to maintain plasma homeostasis over a wide range of salinity (Table 2). No salinity-related changes were observed in plasma sodium, potassium, calcium, magnesium, inorganic phosphate or lactate. Very small, though statistically significant, trends downwards with increasing salinity were seen in osmotic pressure and chloride, paralleling an insignificant trend for sodium in the same direction. An increase in plasma bicarbonate was found at higher salinities, but this is almost certainly the result of a greater time elapsing between capture and blood sampling in the salt water areas (see later). Though statistically significant, total changes in each of these variables are very small and it is adequate to report average values (Table 2). Among nitrogen compounds in the plasma, only urea was significantly higher in SAL 4, though still present in only trivial amounts, while ammonia, uric acid and creatinine are low and independent of salinity (Table 3).

The low level of plasma urea in salt water (1–2 mmol/l) is in sharp contrast to the large increase in urea seen in the only other euryhaline reptile studied in detail, *Malaclemys terrapin*, from 22 mmol/l in fresh water to 115 mmol/l in salt water (Gilles-Baillien 1970). The same species shows a rise in plasma osmotic pressure from 160 to 400 mOsm/l in salt water, while both Na and Cl rise significantly.

There is a marked similarity in the values of major anions and cations in *C. porosus* with those reported in tabular form for other crocodilians by Bentley (1976) and Minnich (1979).

Capture Artifact

Capture led to high levels of blood lactate, presumably associated with the anaerobic metabolism of struggling, and concomitant fall in bicarbonate. Blood buffering has been discussed by Grigg and Cairncross (1980). Coulson and Hernandez (1964) discussed the increase in blood lactate following handling of alligators. They reported normal values ranging from 'trace' to 1.0 mmol/l (average 0.7) in alligators kept undisturbed and in a soundproof box prior to sampling. Thus our mean values of approximately 4 mmol/l are probably rather too high. The range

Table 5. Plasma and cloacal urine nitrogen compounds in *C. porosus* from different salinities. Values are in mmol/l, with 95% confidence limits in brackets. n = number of observations

	SAL 1		SAL 2		SAL 3		SAL 4		Probability (1-way analovar)
	$\bar{x}$	n	$\bar{x}$	n	$\bar{x}$	n	$\bar{x}$	n	
Plasma									
Ammonia	0.8 (0.5–1.1)	33	0.6 (0.31–0.9)	15	0.4 (0.3–0.6)	17	0.8 (0.4–1.1)	20	N.S.
Urea	1.2 (1.1–1.3)	39	1.1 (0.8–1.3)	33	1.1 (0.6–1.5)	19	2.2 (1.6–2.8)	21	$P < 0.0001$
Uric acid	0.62 (0.53–0.70)	40	0.69 (0.60–0.77)	19	0.67 (0.56–0.77)	19	0.73 (0.62–0.84)	21	N.S.
Creatinine	0.064 (0.057–0.071)	40	0.065 (0.052–0.077)	19	0.064 (0.051–0.076)	19	0.069 (0.061–0.076)	21	N.S.
Urine									
Ammonia	98 (85–111)	17	84 (66–101)	16	86 (69–104)	11	43 (31–56)	13	$P < 0.0001$
Urea	4.54 (3.77–5.31)	17	6.52 (4.08–8.97)	16	7.07 (5.30–8.85)	11	20.96 (14.05–27.87)	14	$P < 0.0001$
Uric acid	0.28 (0.24–0.33)	17	0.35 (0.22–0.49)	16	0.51 (0.17–0.85)	11	3.12 (1.80–4.44)	14	$P < 0.0001$
Creatinine	0.102 (0.042–0.162)	17	0.108 (0.087–0.130)	16	0.114 (0.072–0.157)	11	0.411 (0.188–0.633)	13	$P < 0.001$
Solids in urine (% volume)	2.7 (1.7–3.7)	17	4.5 (1.1–7.9)	16	6.6 (3.9–9.3)	11	30.5 (18.9–42.2)	14	$P < 0.0001$

was from 0.7 to 10 mmol/l with the mode at ≃3.0 mmol/l. Unfortunately, although the dependence of blood lactate on time elapsed between capture and sampling, is very marked, the correlation coefficient is too low to enable any confident prediction of mean levels either at capture or at, say, 24 h later. This example highlights the difficulty of reporting 'normal' levels.

Appearance of Cloacal Urine from Crocodiles at Different Salinities

Even to the casual observer the differences between cloacal urine produced by *C. porosus* in salt and fresh water are striking. In fresh water a clear fluid is produced, which has only a very small amount of white solid matter. In salt water however (Table 5) proportionally more solid is present; a cream-coloured mucous-rich granular/crystalline material which may represent by far the bulk of the cloacal contents. In some cases, almost no liquid is voided and the product is a loosely compacted moist solid. It should be noted that the samples in this study were not voided naturally but represent cloacal samples taken at random with respect to any 'secondary processing' which occurs in the cloaca. Hence, all reported values can be expected to show a wider range than the values of ureteral urine or cloacal urine voided voluntarily.

Electrolytes in Cloacal Urine

The observed stability of plasma osmotic pressure, electrolytes and other constituents throughout the salinity gradient can result only from active hyperosmotic and hypoosmotic regulation. In fresh water, crocodiles must retain salts, whereas in sea water they are likely to have an excess of salts, notably sodium and chloride. The problems of osmoregulation by reptiles in various environments, and the mechanisms by which regulation is effected, have been reviewed by Dunson (1976, 1979) Bentley (1976), Dantzler (1976) and Minnich (1979). Extensive review here would, therefore, be superfluous. The present results emphasise the overwhelming importance of the precipitated urine for excretion of K, Ca and Mg (Fig. 4).

Sodium Chloride. The results show that neither the liquid nor the solid fraction of the cloacal urine is a major vehicle for excretion of excess NaCl in salt water (Tables 3, 4). In the liquid cloacal urine, mean values of 8–10 mmol/l Na and Cl were found in both SAL 2 and SAL 4, with no significant differences between them (Table 3). These values compare favourably with Na values of 11 mmol/l in *Caiman crocodilus* (Bentley and Schmidt-Nielsen 1965) and 6 mmol/l in *Crocodylus acutus* (Schmidt-Nielsen and Skadhauge 1967) in fresh water. Only traces of sodium were found in acid digests of the precipitated urine (Table 4) and this has been confirmed by X-ray diffraction and electron probe analyses (Grigg et al., in progress). As the diet of *C. porosus* in salt water (Taylor 1979) probably provides an excess of sodium chloride, function of an extra-renal salt gland is implied and, as reported in the introduction, Taplin and Grigg (1981) have described lingual salt glands capable of producing a secretion of NaCl 3–4 times as concentrated as the plasma. Further studies are now in progress to elucidate the role of these glands in the electrolyte regulation of *C. porosus*.

Potassium. Taplin and Grigg (1981) found only small quantities of K excreted by the salt gland. There can be little doubt that the major route for excretion of excess potassium is the insoluble precipitate in cloacal urine (Fig. 4) though confirmation of this awaits data on volumes of cloacal urine production. Preliminary results indicate that this occurs in the form of potassium urate (Grigg et al., in progress). As seen in Fig. 4, the liquid urine contains little K in either fresh or salt water. Similarly low values were reported by Schmidt-Nielsen and Skadhauge (1967) for *C. acutus* in fresh water.

Calcium and Magnesium. Although the levels of both Ca and Mg are higher in the liquid urine of crocodiles from SAL 4, by far the major part of urinary loss of Ca and Mg occurs in the insoluble fraction (Fig. 4). This is true in both fresh and salt water. Preliminary results show that Ca and Mg phosphates make up the bulk of the insoluble fraction of the urine in fresh water, whereas in salt water Ca and Mg are excreted mainly as urates (Grigg et al., unpublished). These results differ from those of Coulson and Hernandez (1964) who showed that alligators in fresh water lose Ca through the faeces, not the urine. Phosphate was, however, found in the urine. Dantzler (1976), reviewing this and other data on Ca in the urine of reptiles, concluded that Ca is excreted in the faeces, and P in the urine; otherwise insoluble calcium phosphate would tend to precipitate in the renal tubules. This seems to be the case exactly in *C. porosus*, with urinary excretion of precipitated calcium phosphate.

Ammonium Bicarbonate. As found for crocodilians in fresh water by other workers (Coulson and Hernandez 1964; Schmidt-Nielsen and Skadhauge 1967), the major ions in cloacal urine of *C. porosus* in SAL 1, SAL 2 and SAL 3 are ammonium and bicarbonate. In SAL 4, however, only traces of bicarbonate are present and ammonium is much reduced (Table 3). This represents a striking difference between SAL 4 and the other categories. Grigg (1978) confirmed, in fresh water at least, suggestions by Coulson and Hernandez (1964) that gasometric RQ of crocodilians would be low because of the urinary excretion of much CO_2 as bicarbonate, buffering NH_3. Values of $RQ = 0.49$ (range 0.32–0.74) were found for *C. porosus* in fresh water. In fresh water, some ammonium is lost also as ammonium urate (Grigg et al., unpublished).

Excretion of Nitrogen

Whereas in SAL 1–3 most nitrogenous waste is in solution as ammonium bicarbonate (Table 5), in SAL 4 the dominant nitrogen excretion is in the form of insoluble urinary precipitates, uric acid and its salts (Grigg et al., unpublished). There is also a significant amount of urea in the liquid urine in SAL 4. The presence of urea was not recorded in *Alligator* urine (Coulson and Hernandez 1964) but Khalil and Haggag (1958) report its presence in *C. niloticus* urine collected under unspecified conditions. Whether or not alligators would excrete urea in salt water is unknown.

The change-over in excretory products from ammonia to uric acid and its salts is undoubtedly a major strategy for water conservation in a hyperosmotic environment. Some preliminary data are available which allow the effectiveness of this to be gauged. Taplin et al. (unpublished), using radioisotope techniques, have measured water and sodium fluxes in 30 *C. porosus* (hatchlings and juveniles) under hypoosmotic (SAL 2) and hyperosmotic (SAL 4) field conditions in the Tomkinson River. Water flux in SAL 4 was approximately half that in SAL 2, whereas sodium flux nearly doubled. Comparable data are unavailable from other free-ranging euryhaline reptiles.

Osmoregulatory Strategy in SAL 3

Animals under SAL 3 conditions are exposed to ambient salinities which cycle with the tide between hypo- and hyperosmotic. Overall, the urine from animals caught in SAL 3 is more similar to urine from SAL 1 and SAL 2 than to SAL 4 urine. This suggests that strategies for hypoosmotic regulation are being employed more in SAL 4 than 3, although in SAL 3 the ambient water is hyperosmotic to plasma for part of every tidal cycle. Thus, crocodiles may show behavioural correlates with salinity as it changes during a tidal cycle. The most likely behavioural strategies would be for animals to leave the water at high tide in SAL 3 areas and/or drinking when hypoosmotic water is available at low tide. Messel et al. (1981) have analysed thousands of sightings of *C. porosus* but their data lend no support to the idea that crocodiles leave the water at high tide or to escape higher salinities.

Salinity Tolerance

C. porosus emerges from this and parallel studies (Grigg et al. 1980; Taplin and Grigg 1981) as a reptile which is far more euryhaline than has been thought previously. It is able to maintain the homeostasis of the body fluids over a salinity range from fresh water to salt water, has salt glands, and hatchlings can survive and grow in salt water without drinking fresh water. Many nests are located where fresh water is not available in the dry season, although there is some preference for sites with fresh water (Grigg et al., unpublished). Hence, *C. porosus* seems to have the capability to live and breed in either fresh or sea water. The maximum tolerance of *C. porosus* for high salinities is unknown, but 1–2 m animals have been seen at salinities up to 70⁰/₀₀, are seen commonly at 45⁰/₀₀ (Messel et al. 1980; Taplin, personal communication) and hatchlings have been seen in Mungardobolo Creek, a branch of the Tomkinson River, at 64⁰/₀₀ (Taplin, personal communication).

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