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Osmoregulatory mechanisms of the Australian freshwater crocodile, *Crocodylus johnstoni*, in freshwater and estuarine habitats

Abstract. The estuary of the Limmen Bight River in Australia's Northern Territory is home to an unusual salt water-adapted population of the Australian `freshwater' crocodile, *Crocodylus johnstoni*. Crocodiles were captured from tidal reaches of the estuary ranging in salinity from 0.5-24$^{0}/_{00}$ and from several permanent fresh water reaches more or less remote from saline waters. *C. johnstoni* is an effective osmoregulator in moderately saline waters and has osmoregulatory mechanisms very similar to its more marine-adapted relative, the estuarine crocodile *Crocodylus porosus*. Fasted *C. johnstoni* in brackish water appear to lose little sodium in cloacal urine, relying on their lingual salt glands for excretion of excess sodium chloride. The lingual glands show clear evidence of short-term and long-term acclimation to salt water. Like estuarine crocodiles, *C. johnstoni* drinks fresh water and will not drink sea water. Gross sodium and water fluxes in brackish water are very similar to those in other crocodilians, suggesting differences in integumental permeability are not a major influence on osmoregulatory differences between crocodilians. The data reinforce the hypothesis that crocodylids differ fundamentally from alligatorids in the structure and function of the renal-cloacal-salt gland complex and are of interest in current debate over the evolutionary and zoogeographical history of the eusuchian crocodilians.

Key words. Crocodylidae – Osmoregulation - Salt glands - Physiology - Zoogeography
Abbreviations. *FW* fresh water - *SW* salt water - *THO* tritiated water - *TBW* total body water

Introduction

Within the Crocodylia the two main families, Alligatoridae and Crocodylidae, are conspicuously different in their ability to cope with saline habitats (see review by Taplin 1988). It has been proposed that underlying differences in their osmoregulatory systems have influenced the historical and present day distributions of alligatorids and crocodylids (Taplin and Grigg 1989) and reflect more deep-seated divergences in the natural history of the two groups than has been recognised in the past. Pidcock, Taplin and Grigg (1997) identified that the renal: cloacal complex of the estuarine crocodile has markedly different capabilities from that of the American alligator which are linked to the presence of lingual salt glands in the former and their absence from the latter. They suggested, on the basis of the limited comparative data then available, that these structural and functional differences may be consistent discriminators of alligatorids from crocodylids.

The present study aimed to identify the key osmoregulatory mechanisms employed by Australian freshwater crocodiles, *Crocodylus johnstoni*, in fresh water (FW) and saline habitats, taking advantage of a quite unique natural population in the Limmen Bight River. Descriptions of the study site, the distribution of crocodiles within it, and of osmoregulatory homeostasis in the population have been reported in Taplin et al. (1985, 1993). In particular, it aimed to identify whether *C. johnstoni* used osmoregulatory mechanisms similar to those of the estuarine crocodile, *Crocodylus porosus*, with which it lives sympatrically in the system or to those employed by *Alligator mississippiensis* and *Caiman latirostris* in estuarine areas (Mazotti and Dunson 1989; Grigg et al. in press).

Materials and methods

Crocodiles used in this study were captured from the remote Limmen Bight River system. The geography and environmental setting of the study site, background to the study and the Limmen Bight's unusual population of *C. johnstoni* are set out in some detail in Taplin et al. (1985, 1993); that material is not repeated here.

Crocodiles were captured over a series of nights in November 1984 and the range of experiments described below was performed in a crude bush laboratory established on the bank of the river.

Sampling of plasma and urine

Crocodiles were captured by hand at night and returned to base camp where they were sampled the following morning for plasma and cloacal urine (see Taplin 1985 and Taplin et al. 1993 for methods). Plasma and urine were tested for osmolarity in the field and then frozen for return to Sydney University where they were analysed for Na, K, and Cl concentrations by flame photometry or coulometric titration.

Salt gland function
Several of the crocodiles captured from each region of the river system were tested for salt gland function. Each was injected with methacholine according to the protocol outlined by Taplin et al. (1985). Experiments were carried out in the early hours of the morning to allow stabilisation of the crocodiles' body temperatures at approximately 28 °C.

Lingual gland function in an acclimatised crocodile from the Albert River, Queensland
A single crocodile was captured opportunistically in June 1985 in remarkably hypersaline waters ($43^0/_{00}$) of the Albert River system in Queensland, during routine population surveys by one of the authors (LET). This animal was shipped immediately to Sydney, tested for salt gland function on 24 June 1985 using methacholine injection, and then held in FW for 5 months. It was tested again after 28 days and 133 days exposure to determine whether any reduction in the lingual glands' response to methacholine would occur.

Lingual gland responsiveness to acute exposure to brackish water
Three crocodiles captured in FW and weighing 0.5, 3.5 and 4.9 kg were exposed to $87^0/_{00}$ salt water (SW) for periods of 3, 4 and 6 days respectively. Longer exposures were used for the larger animals to compensate for expected slower responses. Each was tested for salt gland function before and after exposure by injection with methacholine at a dose rate of 1 mg kg^{-1}. Plasma samples were collected before and after exposure. Only a limited sample size was possible because comparatively few crocodiles could be tested and each gave only a small number of samples.

The distribution of sodium concentrations in a run of methacholine-induced samples from a single crocodile tended to be skewed and to have some high- or low-concentration outliers, usually the first and/or last samples collected. The concentration data were, therefore, analysed by Mann-Whitney U-test for each crocodile independently.

Maximum Na secretion rate (µmol 100 $g^{-0.7}$ h^{-1}) was used as the index of secretion rate. The maximum secretion rates at the start and end of the exposure period (which was varied from 3-6 days depending on the size of the animal) were paired for each crocodile and tested by a paired t-test.

Short-term acclimation to brackish water and water and Na fluxes
Fifteen crocodiles captured in salinities of $0.5\text{-}5^0/_{00}$ were held fasted in crudely constructed holding ponds for a period of days. A group of seven was exposed for 5 days to $8^0/_{00}$ salt water. A further three crocodiles captured in FW were added to this group 1 day later, and exposed for 4 days. A third group of five crocodiles captured in FW was held in FW for a period of 3 days. A measure of temperature control was achieved by constructing the holding ponds in pits on the river bank, under tree shade, and covering them continuously with wet sacking. Nonetheless, in the extreme conditions of the late dry season, daily maximum temperature in the tanks ranged from 36 °C to 41 °C and daily minimum temperatures ranged from 19 °C to 26 °C. Relative humidity tracked temperature quite precisely, ranging from 20% to 100% with regular diurnal fluctuations.

Tritiated water (THO) and/or ^{22}Na fluxes were tested in seven of the crocodiles held in SW and five of the crocodiles in FW. These animals were injected with 0.2 ml of THO at 740 MBq ml^{-1} , 0.2 ml of ^{22}Na at 0.74 MBq ml^{-1}, or both THO and ^{22}Na and then held for 8 h in moist containers, bled by heart puncture, and exposed to the experimental salinity regime. Blood samples were taken after 4-5 days exposure and the crocodiles reinjected with THO to determine any change in total body water. Exchangeable Na was not redetermined because of the limited amount of ^{22}Na available in the field - hence only the unidirectional efflux of ^{22}Na could be determined (on the assumption of a stable exchangeable Na pool). Analytical and computational techniques are as described in (Grigg et al. 1986). Fluxes were calculated as surface-area adjusted values expressed in ml $kg^{-0\text{-}63}$ day^{-1} or mmol $kg^{-0.63}$ day^{-1} in accordance with Grigg et al. (1986). This formula effectively adjusts for surface-area differentials between crocodiles of different size based on the scaling exponent of 0.637 for surface area:mass given by Dunson (1982).

Plasma and urine were sampled at intervals during the experiment for electrolyte analysis (refer to Results and Table 2 for timings of samples).

Drinking behaviour
Drinking behaviour was tested in crocodiles exposed to $8^0/_{00}$ and $18^0/_{00}$ SW for 11 h and then transferred to FW for 1 h. Nine crocodiles were used in this experiment - four captured in FW and five captured in brackish waters ($8\text{-}14^0/_{00}$). We sought to dehydrate the animals beforehand by holding them in bags in the cabin of a truck and using the vehicle's airconditioner to maintain a cabin temperature of about 30 °C and a relative humidity of 30-35%. These attempts were not wholly successful because of the difficult field conditions and limited period over which we could work. While we had hoped to achieve post-dehydration body weights of 85-90% capture weight, we achieved weights of only 96-100% of initial weights. Nonetheless, the

experimental outcomes were quite clear-cut (see Results). The animals were weighed before exposure to SW, immediately before being transferred to FW, and after 1 h in FW.

Results

Urine composition in wild-captured animals

Cloacal urine of *C. johnstoni* freshly captured from FW and SW was similar in composition to that several other crocodilians, being hypo-osmotic to the plasma, low in Na, K and Cl in FW and tending to have higher Na and Cl levels in SW (Table 1).

ANOVA showed there were no statistically significant differences in osmolarity between years but exposure to SW had a significant impact ($P < 0.001$). The same was true for cloacal fluid Na and CI levels ($P < 0.001$ in both cases). In the case of Na, however, the interaction effect between the year and salinity was close to statistical significance ($P \sim 0.06$). Further examination indicated that the interaction effect was driven principally by the contrast between 1982 and 1984 in urinary Na levels.

This result highlights a particularly interesting difference in cloacal urine composition between crocodiles captured from SW in 1982 and 1984 (Table 1). In 1982, they were characterised by relatively high Na and K concentrations of similar magnitude, when compared to animals from FW. In 1984, in contrast, urinary K was no different from its FW value while urinary Na was substantially higher and more variable. The pattern in urinary Cl follows that for Na between salinity regimes and between years.

Table 1 Composition of cloacal urine from *C. johnstoni* captured in fresh and brackish waters of the Limmen Bight River in 1982 and 1984. Data are expressed as mean ± SE *(n). (*FW fresh water, SW salt water)

	FW	SW
Osmolarity (mOsm 1^{-1})		
1982	175 f 15 (6)	233 ± 7.4 (18)
1984	180 ± 7.6 (22)	217 f 8.9 (12)
Na (mmol 1^{-1})		
1982	4.6 f 2.09 (8)	13.5 -d- 2.89 (17)
1984	2.5 f 0.63 (26)	26.3 f 6.84 (15)
K (mmol 1^{-1})		
1982	6.4 f 2.31 (8)	14.3 f 2.94 (17)
1984	6.1 f 1.39 (26)	6.2 f 1.87 (15)
Cl (mmol 1^{-1})		
1982	3.7 ± 0.84 (7)	24.7 f 5.16 (15)
1984	4.4 f 1.42 (23)	42.3 1 10.20 (13)

Closer examination of the data for Table 1 provides some further insight into the relationship between Na and K excretion in *C. johnstoni* (Fig. la). When urinary K and Na are plotted together for SW-captured crocodiles, it is apparent that both electrolytes can reach quite high levels but in none of the samples did they do so simultaneously. Urinary Na levels as high as 78 mmo1 1^{-1} were recorded and values over 20 mmol 1^{-1} were not uncommon. Four crocodiles had urinary K levels of 30 mmol 1^{-1} or higher and three others had values from 20-30 mmol 1^{-1}. In contrast, all crocodiles from FW had low urinary Na (< 20 mol 1^{-1}) and most were low in potassium (Fig. lb). A small number had urinary K values close to 20 mmol 1^{-1}. Significantly, no crocodile in either category showed high urinary Na and K simultaneously, suggesting that there may be some mechanism operating in the cloaca by which Na and K excretion are linked inversely.

The Na:K ratio in cloacal urine of crocodiles also varied quite distinctively between the two years in which they were sampled (Fig. 1 a). Crocodiles captured from SW in 1982 showed a predominance of high K concentrations while those captured from SW in 1984 showed a predominance of high Na concentrations. This distinction may be linked to the greater secretory responsiveness of the lingual glands among crocodiles captured in 1982 and differences in the extent and timing of their exposure to SW (see below).

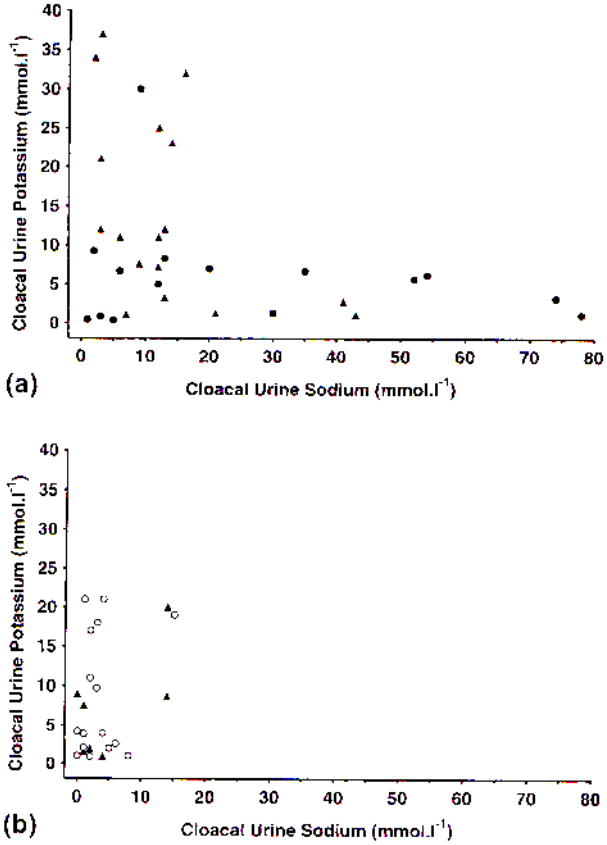

Fig. 1 Relationship between Na and K concentration in the cloacal urine of *C. johnstoni* wild-caught in **a** saline waters (SW) and **b** fresh waters (FW) of the Limmen Bight River. *(triangles* 1982 captures, *circles* 1984 captures)

Lingual gland secretions in wild crocodiles

Data from wild-caught crocodiles support the view that some acclimatisation of the lingual glands may be occurring. When crocodiles from estuarine waters and permanent FW were compared in respect of the concentration and volume of secretions, those from estuarine waters had consistently higher secretory rates and a tendency towards higher concentration in secretions also (Fig. 2). The groups clearly separate along these combined axes - the rate response is apparently more pronounced than the concentration response.

Among crocodiles captured from SW, there was no obvious and consistent relationship between position on these two axes and salinity measured at the point of capture (Fig. 2). There was a slight but discernible pattern of crocodiles captured in SW in 1982 lying further along the rate: concentration axis than those in 1984 (Fig. 2). This slight trend may be an effect of exposure to SW, because captures in 1982 were made later in the dry season than those in 1984, when saline influence had extended farther upstream.

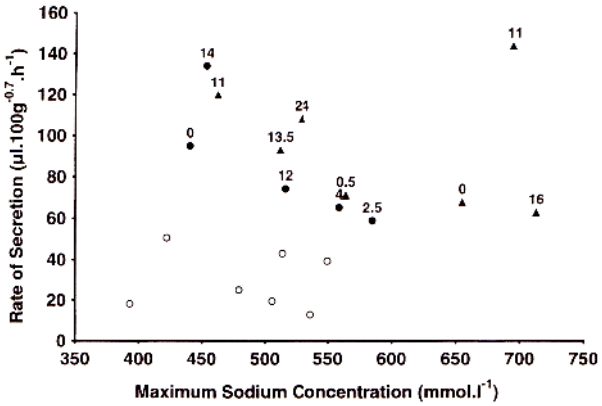

Fig. 2 Relationship between the maximum rate of secretion and the maximum concentration of Na in lingual gland secretions of wild-caught *C. johnstoni* from the Limmen Bight River. *(open circles* FW 1984, *closed circles* SW 1984, *triangles* SW 1982. Captures from SW are labelled with the salinity ($^0/_{00}$) measured at the animal's capture site

The two measures of glandular response were combined to give the maximum rate of sodium secretion, expressed as µmol 100 $g^{-0.7}$ h^{-1} (Fig. 3). Analysis of covariance (using salinity as the covariate) revealed no statistically significant difference in adjusted mean rates between crocodiles caught in 1982 and 1984 from SW. Data for the 2 years were therefore combined to test for any significant influence of salinity on secretory rate. The data point for one crocodile was found to be a statistical outlier in both the initial analysis of covariance and the subsequent regression analysis, so it was removed from the data set. For the subset of 12 crocodiles from SW, the slight positive gradient was statistically significant (P ~0.03), accounting for a rise in secretory rate from 37 µmol 100 $g^{-0.7}$ h^{-1} to 55 µmol 100 $g^{-0.7}$ h^{-1} between tidal FW and 800 mOsm 1^{-1} SW - an increase of nearly 50%.

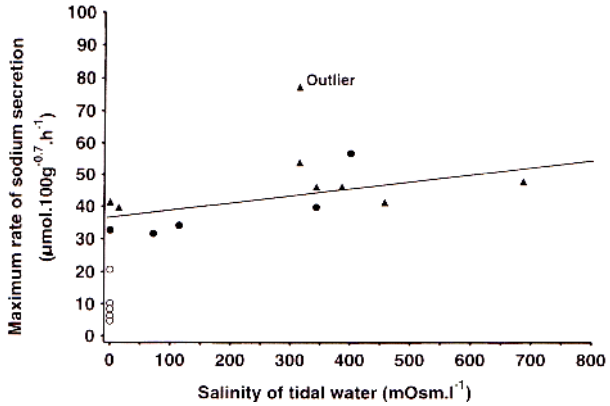

Fig. 3 Maximum rate of lingual gland secretion of Na in relation to the salinity of the capture site in wild-caught *C. johnstoni* from the Limmen Bight River. Symbols as for Fig. 2. Fitted line is a linear least square regression line fitted to the data for animals from SW (excluding the marked outlier)

K:Na ratios in lingual gland secretions and urine

K concentration in lingual gland secretions was approximately linearly related to Na concentration (Fig. 4). Potassium increased in relation to sodium from about 1.7% at the lowest Na concentrations encountered (approximately 160 mmol 1^{-1}) to about 3.7% at the highest concentrations (approximately 580 mmo1 1^{-1}). There was no evident relationship between K:Na ratio in plasma and that in lingual secretions. Indeed K:Na ratios in plasma were constant across the salinity spectrum, averaging 2.71% in SW and 2.73% in FW. It may be relevant that K:Na ratios in lingual secretions from crocodiles in FW were comparable to those in the plasma (mean = 2.54%) and significantly lower (t-test, P ~ 0.014) than those in crocodiles from SW (mean = 3.29%). No clear-cut relationship between K:Na ratio in lingual secretions and other environmental or physiological variables was apparent.

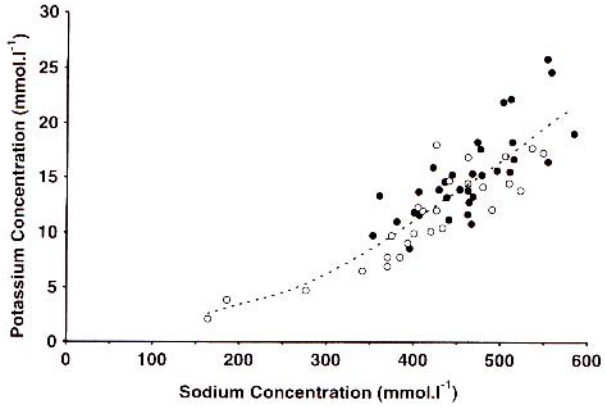

Fig. 4 Relationship of Na to K concentration in the lingual salt secretions of *C. johnstoni* from FW *(open circles)* and SW *(closed circles)* of the Limmen Bight River. The fitted line is a distance-weighted least squares regression

Field and laboratory acclimation experiments

Crocodiles exposed acutely to $8^0/_{00}$ SW showed evidence of short-term acclimation in their salt glands. Despite the small sample size, short duration of the experiment, and ten fold variation in size, all crocodiles showed increases in both the concentration of lingual gland secretions and the overall rate of secretion of Na (Table 2). Median Na concentrations in lingual gland secretions increased by 11, 16, and 3% respectively in three crocodiles after exposure to SW. Maximum secretory rates increased by 13, 10 and 11% respectively.

In only one crocodile was the increase in lingual gland Na concentration after exposure to SW statistically significant at the 95% confidence level. Likewise, the increase in Na secretory rate was not quite statistically significant (P ~0.074). Nonetheless, the fact that the changes in all three crocodiles were consistently in the same direction suggests the animals were showing some positive acclimation to the exposure. It is possible that the relatively short period over which they could be exposed and the small sample size influenced this result.

Table 2 Changes in the secretory response of lingual glands and in plasma composition following short-term exposure of three crocodiles, captured in FW, to $8^0/_{00}$ SW

Identifier	Body weight (kg)	Maximum Na secretory rate (% change)	Median lingual gland Na concentration (% change)	Plasma Na concentration (% change)	Plasma osmolarity (% change)
1	0.49	+13	+11	+11	+6
2	3.54	+10	+16	-1	-1
3	4.87	+11	+3	+4	-2

Plasma analyses suggest the measured increase in lingual gland Na throughput was not attributable solely to increased plasma Na concentration (Table 2). In the smallest animal, both plasma osmolarity and Na concentration increased quite markedly and the lingual gland Na throughput could reflect this change alone. The second crocodile (no. 2) showed marked increases in both indices of lingual gland throughput despite plasma osmolarity and Na concentration remaining stable. The third and largest crocodile (no. 3) showed a near stable plasma osmolarity and Na concentration, a corresponding increase in mean lingual gland Na concentration, but a proportionately much larger increase in the maximum secretory rate of the lingual glands, suggesting a greater volume of secretion in response to methacholine after exposure to SW.

The possibility that the results above are indicative of some acclimation under changing salinity regimes is reinforced by the results of longer-term exposure to FW in the single *C. johnstoni* captured from hypersaline waters in Queensland. In just over 4 months, the methacholine-stimulated secretion rate of its lingual glands (expressed in volumetric terms) fell by 75%, while median and maximum Na concentrations fell by about 15% from initial values (Table 3). In contrast, plasma Na remained within the normal range for *C. johnstoni* from FW throughout the trial. Once again, there is evidence of both a volumetric and a concentration response to acclimation, with most of the change attributable to a reduced volume of secretions. It seems clear that the more equivocal results from experiments carried out in the field are probably attributable principally to the short exposures used.

Table 3 Long-term changes in plasma composition and the secretory characteristics of lingual glands in a single *C. johnstoni*, wild-caught in $43^0/_{00}$ SW and exposed chronically to FW in the laboratory. Secretory capability of the glands was measured as the maximum and median concentrations of Na in samples produced following methacholine injection and the maximum rate of fluid secretion observed over a 10 min interval

Exposure time (days)	Body weight (kg)	Plasma [Na] (mmol l^{-1})	Maximum [Na] (mmol l^{-1})	Median [Na] (mmol l^{-1})	Maximum secretion rate (µl 100 $g^{-0.7}$ h^{-1})
0	s.4s	155	618	543 (14)	124
28	s.65	148	578	478 (22)	69
133	5.00	155	507	471 (9)	32

Short-term acclimation - plasma and urine composition and THO/^{22}Na fluxes

Fasted animals exposed to FW or $8^0/_{00}$ SW for flux rate studies appeared strongly homeostatic in respect of their plasma composition. Plasma osmolarity, Na, K and CI were stable in all cases across the 4-5 days of exposure (Table 4). There were no significant differences across groups or sampling dates in any of the variables measured.

Table 4 Changes in plasma osmolarity and electrolytes in *C. johnstoni* exposed to FW and SW for 4-5 days in experimental tanks. Data are expressed as mOsm 1^{-1} or mmol 1^{-1}

	Pre-exposure	Post-exposure
Group 1: 5 days in $8^0/_{00}$ SW		
Osmolarity	299 ± 4.1 (7)	303 ± 6.5 (7)
Na	151 ± l.9 (7)	155 ± 3.3 (7)
K	4.0 ± O.16(7)	4.4 ± 0.31(7)
Cl	115 ± 1.7 (6)	120 ± 2.9 (6)
Group 2: 4 days in $8^0/_{00}$ SW		
Osmolarity	290 ± 3.8 (3)	288 ± 3.8 (3)
Na	150 ± 3.5 (3)	152 ± 3.2 (3)
K	4.0 ± 0.19 (3)	3.7 ± 0.21 (3)
CI	115 ± 4.6 (3)	119 ± 0.7 (3)
Group 3: 5 days in FW		
Osmolarity	286 ± 3.0 (s)	284 ± 2.2 (3)
Na	149 ± 2.5 (5)	153 ± 4.8 (3)
K	3.9 ± 0.24(5)	4.4 ± 0.33 (3)
Cl	113 ± 3.8 (5)	116 ± 0.6 (3)

Urine composition, in contrast, changed markedly over the course of the experiment in crocodiles exposed to SW and in directions opposite to the shifts seen in crocodiles held in FW (Table 5). In the group exposed to SW, all of which were captured in tidal SW, urine sampled immediately before they were placed in the tanks had essentially the same composition as that collected shortly after capture (Table 5 cf. Table 1). This is unsurprising given that the crocodiles were held out of water for the minimum time necessary. Exposure to SW resulted in a modest increase in urine osmolarity, a distinct decrease in Na to very low levels, a `complementary' rise in K, and relatively stable (but more labile) Cl concentrations. Overall, the combined concentration of Na, K and Cl ions rose by some 27 mmol 1^{-1} or 45%. Urine osmolarity tracked this combined ion concentration very precisely over the range of values encountered (Fig. 5) -adjusted r^2 for the linear regression of urine osmolarity on the combined ion concentration equalled 0.999.

In contrast, urine osmolarity in crocodiles held in FW for 72 h fell by only 4%, Na remained consistently low at levels comparable to those in SW-exposed crocodiles, K fell quite sharply, and Cl rose in a rather ‘complementary’ fashion. The combined ion concentration (Na + K) fell slightly (Table 5). More detailed examination of the results was not fruitful as the small number of animals available and the short period over which the experiment could be run limited the conclusions which could be drawn.

Table 5 Changes in urine composition of *C. johnstoni* captured in salinities of 0.5-5$^{0}/_{00}$ and exposed acutely to 8$^{0}/_{00}$ SW. Data are expressed as mean ± SE (*n*).

	Exposed to FW		Exposed to 8$^{0}/_{00}$ SW				
	Pre-exposure	72 h	Pre-exposure	24h	48h	96h	120h
Osmolarity (mOsm l^{-1})	232±19.4 (5)	222± 7.3 (5)	225±3.3 (4)	244 ± 11.8 (6)	248±3.4 (7)	247 ±5.2 (7)	259 ±3.8 (7)
Na (mmol l^{-1})	1.0±0.00 (5)	1.6±0.24 (5)	11.4±4.08 (5)	1.8±0.40 (6)	1.9±0.26 (7)	2.0±0.22 (7)	1.1±0.14(7)
K (mmol l^{-1})	18.4±4.29 (5)	3.5±0.92 (5)	7.2±1.63 (5)	18.1±2.65 (6)	27.9±2.13 (7)	28.5±6.11 (7)	20.0±4.36 (7)
Cl (mmol l^{-1})	4.0±1.82 (5)	11.8 ±3.44 (5)	42.3±13 (4)	42.7±21.51 (6)	38.3±13.74 (7)	35.4±8.99 (7)	66±9.05 (7)
Na + K + Cl (mmol l^{-1})	23.4±5.88 (5)	16.9±3.34 (5)	60.2±15.59 (4)	62.6±20.59 (6)	68±14.42 (7)	65.9±8.94 (7)	87.1±10.91 (7)

Weight-specific water fluxes were very consistent between crocodiles and between FW and brackish water groups (Table 6), doubtless reflecting the dominance of integumentary water exchange in the flux equation. Both groups were effectively in water balance over the duration of the experiment. Weight-specific Na effluxes in *C. johnstoni* exposed to 8‰ SW were consistent and low (Table 6) and some 80% higher than the rate in animals exposed to FW. Variability in Na flux rates was very low.

Table 6 Titrated water (THO) flux data are expressed as ml $kg^{-0.63}$ day^{-1} , ^{22}Na flux data as mmol $kg^{-0.63}$ day^{-1}, and as mean ± SE (*n*) with the range specified below each mean

Treatment	THO			^{22}Na
	Efflux	Net flux	Influx	Efflux
SW	145 ± 26.5 (5) (48-194)	-0.4 ± 20.1 (4) (-43 - ±74)	144 ± 13.0 (5) (118-184)	2.24 ± 0.102 (7) (1.98-2.63)
FW	163 ± 7.6 (4) (150-177)	-4.5 ± 5.50 (4) (-15 - ± 11)	158 ± 12.2 (4) (135-189)	1.27 ± 0.173 (5) (0.96-1.71)

Drinking behaviour

C. johnstoni from the study area appeared reluctant to drink SW but drank FW quite freely (Fig. 5). All nine crocodiles lost small amounts of weight over 11 h exposure to SW, averaging 0.08 ± 0.023% h^{-1}, and all gained weight rapidly at an average of 0.5 ± 0.133% h^{-1} on return to FW. One crocodile was a outlier gaining weight very rapidly at over 1.5 % h^{-1} , but the response among the other eight was quite uniform. The weight gain in FW in the non-outlier group equated to drinking rates of 0.8-7.0 ml h^{-1}. There was no evidence of any difference in behaviour between animals captured in FW and those from brackish water.

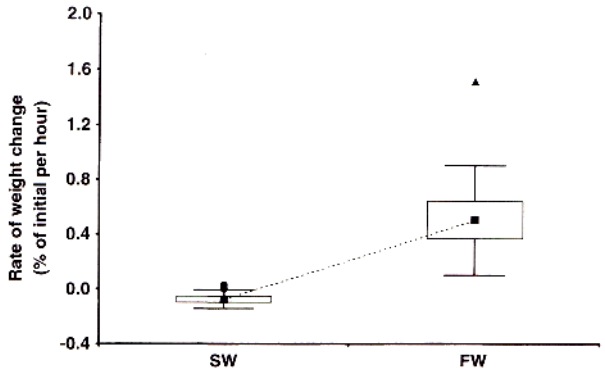

Fig. 5 Response of fasted and dehydrated wild-caught *C. johnstoni* to sequential exposure to SW and FW. Exposed to SW, crocodiles lost weight. Transferred to FW, all gained weight very rapidly indicating they were drinking the medium. [*Square* mean, *box* SE, *bars* 95% confidence limits, *circles* outliers, *triangle* (extreme value)]. The extreme value derives from one crocodile which drank promptly and copiously when returned to FW.

Discussion

It is clear from this study, and related work on plasma homeostasis in *C. johnstoni* reported in Taplin et al. (1985), that osmoregulatory responses to SW in the Australian FW crocodile are quite typical of those known from other Crocodylidae. *C. johnstoni* in FW produce cloacal urine very low in Na, K and Cl; essentially clear of precipitated urates and with an osmolar urine to plasma ratio of ca. 0.6. This helps reduce electrolyte losses and doubtless permits excretion of at least a proportion of excess nitrogen in the form of ammonium bicarbonate - as do other FW crocodiles and alligators. *C. johnstoni* has a low integumental permeability to water and Na compared with many other FW vertebrates and will drink FW. In these respects, *C. johnstoni* and other crocodiles present as rather similar to *Alligator mississippiensis* from FW.

Exposure to SW, however, draws out the several interesting features of its osmoregulation which distinguishes it (and other Crocodylidae) from alligators. Firstly, high levels of Na are the exception in cloacal urine of *C. johnstoni,* even in animals exposed to strongly hyperosmotic salinities in the field (Fig. la). Indeed, fasted *C. johnstoni* held in $8^0/_{00}$ salt water for 5 days showed the same remarkably low levels of cloacal Na (Table 5) found almost universally in wild-caught and captive *Crocodylus porosus* in SW (Grigg 1981; Taplin 1985, 1988). Secondly, cloacal K has the capacity to rise to quite high levels in SW (Table 5, Fig. la) and in ways which suggest Na and K excretion are quite tightly linked (Fig. la). Thirdly, the lingual glands of *C. johnstoni* have the capacity to excrete Na at rates and concentrations comparable to those in the estuarine-adapted *C. porosus* and appear to respond in both concentrating ability and secretory rate during exposure to saline waters (Table 2, Fig. 2). Fourthly, *C. johnstoni* can recognise SW and appears not to drink it (Fig. 5).

In all of these characteristics, *C. johnstoni* mirrors the more euryhaline and well-studied *C. porosus* quite closely (Grigg 1981; Taplin 1985, 1988). Where tests have been conducted on other crocodylids (*Crocodylus niloticus, Crocodylus acutus, Crocodylus palustris* and *Crocodylus cataphractus*) the more limited results suggest key elements of the same osmoregulatory system are found in all - most particularly the lingual salt glands which appear to play a central role in Na regulation (Schmidt-Nielsen and Skadhauge 1967; Taplin et al. 1985; Taplin and Loveridge 1988). Indeed, the parallel responses in cloacal urine composition between *C. johnstoni* exposed to SW in this study and fasted Nile Crocodiles exposed acutely to SW by Taplin and Loveridge (1988) are surprising. Hatchling and juvenile Nile Crocodiles, derived (as best we can judge) from FW populations and having no prior experience of saline waters, showed no increase in clocal Na, modest increases in cloacal Cl and very marked increases in cloacal K - from 5 mmol 1^{-1} in FW to 79 mmol 1^{-1} after 92 h in SW. As in *C. johnstoni* and *C. porosus,* the lingual salt glands of *C. niloticus* appear functional and important in Na excretion.

The data presented here on *C. johnstoni* reinforce our view that the osmoregulatory system of crocodylids differs quite fundamentally in both structure and function from that of alligatorids. Studies of *A. mississippiensis* and *Caiman latirostris* (Lauren 1985; Taplin et al. 1982; Pidcock et al. 1997; Grigg et al. 1998) suggest neither species has lingual salt glands and that cloacal urine is a major route for excretion of excess Na and Cl. *A. mississippiensis* has little or no capacity to modify the composition of cloaca urine while the cloaca of *C. por*osus appears very active in decreasing cloacal Na and increasing cloacal K and urate (Pidcock et al. 1997; Kuchel and Franklin 1998). The cloaca of *C. acutus* appears similarly active in reducing the normally high Na content of ureteral urine during storage (Schmidt-Nielsen and Skadhauge 1967).

The data available suggest that integumental permeability to water and Na are not major influences on the osmoregulatory differences between alligatorids and crocodylids. Mazotti and Dunson (1989) summarise whole body Na and water fluxes from several studies of *A. mississippiensis, C. acutus, C. porosus,* and *C. moreleti.* Water effluxes and Na influxes (both likely to be dominated by integumental exchange; Taplin 1988) are of the same magnitude (0.3-1 ml 100 g h^{-1} and 1122 µmol 100 g ^{1} h^{-1}) in all four species in SW. Water and Na flux rates in *C. johnstoni* measured in this study are also of the same magnitude - some 0.6 ml - $100g^{-1}$ h^{-1} and 9 µmol $100g^{-1}$ h^{-1} respectively. Thus, the Na load accumulated passively in saline waters is likely to be rather similar in all species.

We think it likely that there are two principal influences underlying the differing osmoregulatory responses seen in alligatorids and crocodylids in FW and SW. The central difference derives from the different structure and function of the renal-cloacal-salt gland complex between the two groups. Lacking salt glands and the likely coevolved capability for cloacal Na reabsorption, alligatorids are limited to a renal response to saline loads. Cloacal Na levels in *Alligator* and *Caiman latirostris* may reach 100-150 mmol 1^{-1} (Lauren 1985; Grigg et al. 1998) but probably do not exceed plasma concentration. Thus, hypernatraemia is inevitable. If alligatorids respond to hypernatraemia by drinking highly saline water, they may flush more Na through the renal: cloacal system but will not regain Na and water balance. Crocodylids like *C. porosus* and *C. johnstoni,* in contrast, can exercise more influence over their Na and water balance by reabsorbing Na from the cloaca, excreting it at high concentrations from the lingual glands and gaining net free water to compensate for integumentary and excretory losses. Crocodylids may have an additional advantage over alligatorids in a greater capacity to excrete insoluble K urate, assisting further in water conservation. Grigg (1981) found very high levels of K in the solid fraction of cloacal

urine from hyperosmotic SW and little at lower salinities. Pidcock et al. (1997) showed that *C. porosus* exposed to both FW and hyperosmotic SW excreted high concentrations of urate (means of 270 mmol 1^{-1} and 290 mmol 1^{-1} respectively) in the liquid fraction of cloacal urine while alligator had mean and essentially invariant concentrations of 5 mmol -1^{-1} under identical fresh and saline conditions.

If we assume that Na is reabsorbed isosmotically from the cloaca and accompanied by Cl, then *C. porosus* or *C. johnstoni* should gain in the order of 2 ml of free water from the excretion of 1 ml of concentrate from the lingual glands (given plasma Na at 150 mmol 1^{-1} and lingual gland secretions at 450 mmol 1^{-1} Na). If the lingual glands are able to sustain a secretory rate in SW of 100 µL 100 $g^{-0.7}$ h^{-1} (Fig. 2), then a 1 kg crocodile could secrete 24 ml day^{-1} for a net free water gain of 48 ml. From measurements of water loss and net weight loss in fasted *C. porosus* in SW (Taplin 1985), we can estimate net water loss in a 1 kg crocodile to be in the order of 7 ml day^{-1}. We might expect rather similar water balance in fasted *C. johnstoni* given the dominance of integumental exchange in water balance (Taplin 1985) and the similar water flux measurements reported here. Superficially, therefore, we might expect that *C. porosus* or C. *johnstoni* could tolerate quite high salinities by drinking the medium. However, neither species does drink hyperosmotic SW (Taplin 1985; this paper), suggesting that wider dimensions of their salt and water equilibrium are important in limiting salinity tolerance.

Extensive data reported by Grigg (1981) on cloacal urine composition in *C. porosus* across a wide range of salinities suggested that the most marked shift in electrolyte composition and nitrogenous excretion occurred when the crocodiles had no access to hypo-osmotic SW during the tidal cycle. Grigg found that ammonium bicarbonate fell markedly under these conditions, while uric acid in solution and as urine solids increased markedly. There was a simultaneous shift to very high levels of K in the precipitated urates while calcium and magnesium concentrations in the solids stayed fairly constant. This is consistent with the observation that the lingual glands appear to have no capacity to regulate the proportion of K and Na in secretions (Fig. 4, Taplin et al. 1985).

Together with data from the other studies of *C. porosus* and *C. johnstoni* cited above, the results strongly suggest that a second critical determinant of osmoregulatory function in crocodylids is their drinking behaviour. We strongly suspect that both *C. porosus* and *C. johnstoni* drink hypo-osmotic SW facultatively and, in these circumstances, may show a more 'alligatorid' response to SW reflected in the occasional observations of high cloacal sodium seen in the lower right of Fig. la and, we would expect, accompanied by a relatively high throughput of excreted urine. They may also, however, avoid drinking saline water, in which case they shift to the more commonly seen strategy of reabsorbing Na and water from the cloaca and excreting it through the lingual glands while cloacal K levels increase markedly (Fig. la, upper left). Fasted *C. johnstoni* exposed acutely to $8^0/_{00}$ SW in this study (Table 5) show the shift between these two quite different strategies. The aestivating *C. johnstoni* studied by Christian et al. (1996) also responded by showing an increase in cloacal K, while sodium remained constant, suggesting parallels with our non-drinking individuals.

This view of osmoregulation in crocodylids gains support from the work of Kuchel and Franklin (1998), who tested renal clearances and ureteral and cloacal urine composition in C. porosus exposed to FW, $10^0/_{00}$ SW and $20^0/_{00}$ SW. Glomerular filtration rate showed a distinct trend from 15 ml kg ^{1} day^{-1} in FW to 8 ml kg^{-1} day^{-1} in $10^0/_{00}$ and 6 ml kg ^{1} day^{-1} in $20^0/_{00}$. However, Na and Cl concentrations in both ureteral and cloacal urine were far higher in $10^0/_{00}$ than in either FW or $20^0/_{00}$. We think it likely that the shifts in Na and K excretion reflect changes in drinking behaviour. Some preliminary tests on drinking behaviour in *C. johnstoni* in $8^0/_{00}$ and $18^0/_{00}$, carried out as part of this study, support this possibility. Though inconclusive because of too few animals and variability in their behaviour, we found definite indications from weight changes that some *C. johnstoni* would drink at $8^0/_{00}$ but none would drink at $18^0/_{00}$, (L.E. Taplin, personal observation).

The fact that short-term exposure produced a small but discernible change in lingual gland output (Table 2) and that longer-term changes continued between 28 days and 133 days (Table 3) suggests that both neuroendocrine and structural influences are at work. This complements the findings of Franklin and Grigg (1993) who showed that the vascular volume of blood vessels supplying the lingual glands of C. porosus raised in $20^0/_{00}$ SW was three times greater than in animals from FW. We suggest, therefore, that the variation encountered in urine composition (Fig. 1) and salt gland secretory capability (Fig. 2) across parts of the Limmen Bight River system and between years reflect inter alia the timing of each animal's exposure to SW as it intrudes upstream in the dry season, its mobility and access to sources of FW or hypo-osmotic SW, and its behavioural response to SW as reflected in its drinking behaviour and dietary preferences. Clearly, disentangling these influences from field studies alone is impractical.

The finding that *C. johnstoni* has much in common with *C. porosus* and with all the other crocodylids studied to date has interesting implications for evolutionary and zoogeographic interpretations of crocodilian history (see Taplin and Grigg 1989; Pidcock et al. 1997). In particular, Willis and co-workers have published extensively in recent years on the history and systematic relationships of the numerous fossil crocodilians discovered in Australia - several of them from within the range of *C. porosus* and *C. johnstoni* in northern Australia (see Willis 1997 for review). Interestingly, Willis notes that the diverse mekosuchine crocodile fauna, which appears to have been dominant for much of the Tertiary, appears to have become extinct during the Pliocene in association with the collapse of the Australian megafauna. *C. porosus* appears suddenly in the fossil record from early Pliocene deposits while *C. johnstoni* appears in the Pleistocene, leaving open the possibility of its derivation from a *C.*

porosus ancestor (Willis and Archer 1990). In an extensive review of the phylogenetic relationships of crocodyloids, Salisbury and Willis (1996) suggested that the genus *Crocodylus* might be of post-Oligocene origin and that many earlier taxa referred to this genus (e.g. several North American and African forms) may be more distantly related. These interpretations support the possibility discussed by Densmore and Dessauer (1982) and Taplin and Grigg (1989) that many or all of the extant crocodylids might be derived relatively recently from marine-adapted ancestors with physiological capabilities similar to *C. porosus.*

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