Osmoregulation of the Australian freshwater crocodile, *Crocodylus johnstoni,* in fresh and saline waters

L.E. Taplin, G.C. Grigg, L. Beard

Abstract. An unusual saltwater population of the "freshwater" crocodilian, *Crocodylus johnstoni,* was studied in the estuary of the Limmen Bight River in Australia's Northern Territory and compared with populations in permanently freshwater habitats. Crocodiles in the river were found across a large salinity gradient, from fresh water to a salinity of 24 mg.ml^{-1}, more than twice the body fluid concentration. Plasma osmolarity, concentrations of plasma Na$^+$, Cl$^-$, and K$^+$, and exchangeable Na$^+$ pools were all remarkably constant across the salinity spectrum and were not substantially higher or more variable than those in crocodiles from permanently freshwater habitats. Body fluid volumes did not vary; condition factor and hydration status of crocodiles were not correlated with salinity and were not different from those of crocodiles from permanently fresh water. C. *johnstoni* clearly has considerable powers of osmoregulation in waters of low to medium salinity. Whether this osmoregulatory competence, extends to continuously hyperosmotic environments is not known, but distributional data suggest that *C. johnstoni* in hyperosmotic conditions may require periodic access to hypoosmotic water. The study demonstrates a physiological capacity for colonisation of at least some estuarine waters by this normally stenohaline freshwater crocodilian.

Key words: Osmoregulation - Salt glands - Salinity - Homeostasis - Crocodile, *Crocodylus johnstoni*

Introduction

Two species of crocodilian occur in northern Australia - the freshwater crocodile, *Crocodylus johnstoni,* and the estuarine or saltwater crocodile, *C. porosus.* The latter species is found commonly in tidal estuaries and in the sea but is common also in fresh waters. *C. johnstoni* is found predominantly in fresh water but numerous occurrences in saline waters have been recorded (Messel et al. 1980, 1981, 1986). Clearly, both species are euryhaline to some degree.

The physiological and ecological basis of euryhalinity in *C. porosus* has been studied extensively. Hatchlings and juveniles are able to maintain plasma homeostasis and grow in salinities of 0-45 mg.ml^{-1} at least (Grigg et al. 1980; Grigg 1981; Taplin 1984, 1988). This capability derives in part from active lingual salt glands (Taplin and Grigg 1981) which account for a substantial fraction of Na$^+$ efflux in sea water (Taplin 1985; Grigg et al. 1986).

An intriguing feature of lingual salt glands in crocodilians is their presence and surprisingly high secretory capacity in all of the extant Crocodylidae (true crocodiles) examined and their apparent absence from alligatorids (Taplin et al. 1985). These observations led Taplin et al. to propose that the extant `freshwater' crocodiles may be descended from saltwater-competent ancestors, rather than the prevailing view that `saltwater' crocodiles have derived from freshwater ancestors. The hypothesis has been explored from a zoogeographical perspective by Taplin and Grigg (1989).

Evaluation of this hypothesis depends in part on investigation of osmoregulation in the numerous `freshwater' crocodiles known to occur in saline waters. These include C. *cataphractus, C. novae-guineae, C. palustris, C. rhombifer, C. niloticus* and *Osteolaemus tetraspis* (Taplin 1988). Debate between American and Australian scholars over the significance of lingual salt glands in *C. acutus* of the southern USA has highlighted the difficulty of reconciling laboratory and field observations in some environments (Taplin 1988).

The full scope of the present study was to examine the extent of homeostasis in a saltwater population of *C. johnstoni* in the Limmen Bight River system in Australia's Northern Territory, and the role of the lingual salt glands. As part of the study the distribution and abundance of C. *johnstoni* in the river system were determined in relation to the area's complex tidal and seasonal salinity regime. This

paper reports an essentially descriptive analysis of the homeostasis shown by C. *johnstoni* in this estuarine river system and compares the results with data from permanent freshwater locations in the same geographical region. Preliminary results describing the function of the lingual salt glands and their apparent contribution to plasma homeostasis were reported by Taplin et al. (1985).

Material and methods

The Limmen Bight environment. Relevant information on the climatic and salinity regimes of northern Australian river systems is provided in Messel et al. (1979-1986), Grigg et al. (1980, 1986), Grigg (1981), and Taplin (1984). Background information specific to the Limmen Bight River System is provided by Messel et al. (1980) and Taplin et al. (1985).

The Limmen Bight River lies in the south-west corner of Australia's Gulf of Carpentaria (Fig. 1), in a region of hot, moist climate characterised by a marked winter dry season. Winter rainfall is extremely low - typically less than 20 mm from May to October (Hall et al. 1981). Arrival of the wet season is usually presaged by localised rain storms in late October or November, rainfall peaking in January. Low winter rainfall and correspondingly low freshwater runoff lead to intrusion of saline waters into the upstream reaches of the Limmen Bight River as the dry season progresses. Our expeditions to study C. *johnstoni* were timed to coincide with the ends of the dry seasons of 1982 and 1984.

The tidal regime in rivers of the south-western gulf can be very complex with confusing patterns of two and three tides per day, as highlighted by Messel et al. (1980, 1986). Representative salinity regimes in the river system at the times of our expeditions are shown in Fig. l, together with the distributions of C. *johnstoni* captured in 1982 and 1984. It was not possible to determine fully the range of salinities experienced by crocodiles distributed through the various tributaries of the system in the time available. However, generalised low-tide salinity profiles were determined and salinities were measured at the time and point of capture of all crocodiles sampled.

A distinction is drawn in this paper between salinity regimes in which salinity is continuously hypo- or hyper-osmotic to crocodilian plasma and those in which salinity is periodically hypo- and hyper-osmotic according to the state of the tide. The relevance of this distinction to consideration of salt and water balance in crocodilians (and other taxa) has been outlined by Grigg (1981) and Taplin (1984, 1988). The approximate boundaries between continuously and periodically hypo- and hyper-osmotic waters on the Limmen Bight mainstream at the times of our expeditions are shown in Fig. 1. Continuously or periodically hypoosmotic conditions would have been encountered by crocodiles in the downstream Cox and Nathan Rivers also, but their boundaries were not identified in this study because of the limited salinity data collected for these tributaries.

Crocodiles inhabiting the middle reaches of the river system (25-45 km, Fig.1) can be expected to experience the widest range of salinities because the salinity gradient there is steep and there are few barriers to tidal flow. A range of 10 mg.ml^{-1} between high and low tides would not be surprising based on experience in the Liverpool-Tomkinson River system (Grigg 1981). In the upstream reaches the salinity range is certain to be much narrower. Saline water carried on ˉa rising tide is blocked at various upstream points by ledges a metre or more in height. Significant ledges are located at km 44.8 on the Cox River, at km 41.4 on the Nathan River, and at km 63.3 on the Limmen Bight River.

Salinity regimes in 1982 and 1984 were very similar in the Limmen Bight mainstream and Nathan River. Only in the Cox River was there a marked difference between years (Fig.1). This difference is believed attributable to a ledge at km 44.8 (see above), which acts as a partial barrier to tidal inflow and to differences in tidal and rainfall/runoff regimes between the two years.

Surveys of crocodile distribution in the Limmen Bight River. The distributions of C. *johnstoni* and C. *porosus* on 127.3 km of the Limmen Bight system were determined by spotlight survey in May 1979 and October 1985 by the University of Sydney (Messel et al. 1980, 1986). We conducted comparable surveys between 30 October 1982 and 5 November 1982, covering 104 km of mainstream and side creeks but excluding the West Arm and side creeks (Fig. 1). The survey techniques of Messel et al. were employed. Crocodiles were identified as 'eyes-only' if only distant sightings were possible - these crocodiles could not be reliably identified to species.

Abundance of crocodiles is reported here as the observed numbers of non-hatchlings sighted per kilometer of waterway, referred to as the `sighting index'. An 'eyes-only' component of 0.05 crocodiles per kilometre has not been allocated to either C. *porosus* or *C. johnstoni.* No hatchlings of either species were identified in tidal areas.

Formal surveys were not conducted during the 1984 expedition which was devoted principally to field experimentation. However, the distributions of C. *johnstoni* and C. *porosus* in the upstream reaches of the river system were recorded systematically throughout the expedition.

Crocodile captures. Seventy-one C. *johnstoni,* ranging in size from 0.18 to 8.4 kg, were captured at night by hand during the two expeditions, 28 during the first trip and 43 during the second. The distribution of sexes and size classes between years and between brackish and fresh waters was uniform overall, providing a broadly representative sample of the population (Tables la, b). Thirty-six individuals were captured in areas subject to tidal influence, as shown in Fig. 1. Another 35 were captured from four areas of permanent fresh water above the limit of tidal and saline influence. Of these 26, 8 were captured in 1982 from the Wilton River (14° 32' S, 134° 33' E), a tributary of the Roper River some 120 km northwest of the Limmen Bight. Eleven were taken from Midgley Billabong, a lagoon on the upstream Limmen Bight River (15° 42' S, 135° 26' E) and nine from a nearby tributary, Eastern Creek (15° 51' S, 135° 26' E). These three sites all lay between 25 and 35 km upstream of the limit of tidal influence in the river systems. Another seven crocodiles were taken in 1984 from a lagoon on the Nathan River (km 41.7, Fig. 1), where they were less than 1 km from brackish water but separated from it by a high rocky ledge.

Physiological measurements. Blood and urine samples were taken from all crocodiles on the day following capture. Blood, taken by cardiac puncture, was mixed with Li heparin and the plasma separated for later analysis. All crocodiles captured in 1982 and 17 crocodiles captured in 1984 were then injected with ^{22}Na and THO for determination of ExNa and TBW pools. The techniques used have been described by Taplin (1984).

Plasma osmolarity was determined using a Knauer freezing point osmometer. Plasma Na and K were determined using a Corning Model 435 flame photometer. Chloride was determined by coulometric titration using a Radiometer CMT10 chloridometer.

Absolute values for ExNa pools in 1984 were compromised by a systematically low bias in ExNa values. The bias was attributable solely to an inexplicably low value for the ^{22}Na standard (some 60% lower than expected) which resulted in artificially and uniformly low ExNa values. However, these data have been used for comparative purposes by assuming that crocodiles captured from permanent fresh waters in 1982 and 1984 had the same mean ExNa value and adjusting the 1984 data by the appropriate proportionality constant (2.63). Data for crocodiles from tidal waters were then adjusted by the same proportion. That these adjustments are appropriate is illustrated clearly by the internal consistency of other measurements made on the 1982 and 1984 groups (see Results and Discussion).
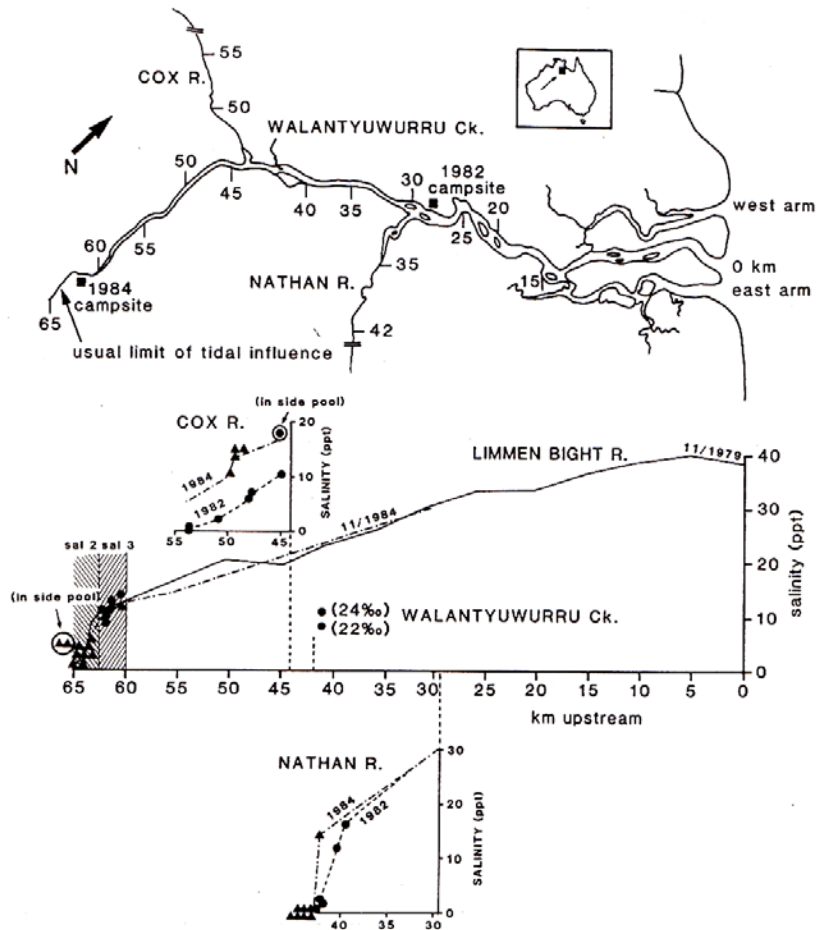
Fig. 1. Geography and salinity profiles of the Limmen Bight River study area and capture sites of crocodiles in 1982 and 1984. Salinity profiles for the Limmen Bight River mainstream in 1982 and 1984 were essentially identical. Only the 1984 data is plotted: The November 1979 salinity profile for the Limmen Bight mainstream is reproduced, with permission, from Messel et al. (1980) to show the hypersaline nature of the lower reaches of the system in the late dry season. Crocodile locations are plotted as data points on the salinity profiles: 1982 (●), 1984 (▲). Cross-hatched areas on the mainstream salinity profile refer to segments of the stream where the salinity either did not exceed the osmotic concentration of the plasma (Sal 2) or was alternatively above or below plasma concentrations (Sal3) as tides cycled (*sensu* Grigg 1981). Downstream of 60 km, salinities in the mainstream were consistently at a higher osmoticity than the plasma at all stages of the tidal cycle (Sal 4). Three crocodiles were captured in side pools immediately adjacent to the rivers (their data points are circled). Data for crocodiles on Walantyuwurru Creek are shown as points only - the stream is too short for a meaningful salinity profile to be displayed. Redrawn after Messel et al. (1980)

Table 1a,b. Breakdown of crocodiles captured in 1982 and 1984 by size class, sex, and capture site

(a)

Body weight (kg)	1982		1984		Totals
	M	F	M	F	
0.01– 0.3	2	2	3	3	10
0.31– 1.0	4	0	5	4	13
1.01– 3.0	5	10	6	9	30
3.01–10.0	3	1	6	7	17
Totals	14	13	20	23	70

(b)

Sex	Saline water sites					Fresh water sites					Overall totals
	A	B	C	D	Total	E	F	G	H	Total	
Female	10	3	7	0	20	3	4	4	5	16	36
Male	7	3	4	1	15	5	3	7	4	19	34
Totals	17	6	11	1	35	8	7	11	9	35	70

Refer to Fig. 1 and Materials and methods for locations and details of capture sites. One crocodile in the size class 0.31-1.0 kg captured at Site D in 1982 was not sexed and is omitted from the tables. Capture site labels are as follows: (A) Limmen Bight River mainstream; (B) Nathan River; (C) Cox River; (D) Walantyuwurru Creek; (E) Wilton River; (F) Nathan River lagoon; (G) Midgley Billabong; (H) Eastern Creek. M=male; F=female

TBW and ExNa are presented as both weight-standardised and length-standardised quantities to clarify their meaning. This technique has the advantage that it removes the confounding effect introduced when weight-specific TBW or ExNa is used for animals whose hydration status can vary substantially over short periods [see Taplin (1984) and Grigg et a]. (1985)]. Length-standardised measures have been termed "hydration factor" and "sodium factor", respectively, to highlight their similarities to the commonly used measure of length-standardised body weight known as "condition factor". These factors were calculated from empirically derived scaling relationships of TBW, ExNa and BW with SVL, determined by ANOCOVA. ANOCOVA was applied to data from five groups of crocodiles captured at different sites. Each group contained a sufficient number of animals across a sufficiently wide size range to provide a good estimate of the scaling coefficient. Sodium and hydration factors for small groups of crocodiles not included in the ANOCOVA were calculated subsequently using the regression coefficients derived from the other groups.

"Within-group" slopes of TBW, ExNa, and BW against SVL were homogeneous among the five groups. TBW and BW scaled with SV L to the powers 2.91 and 2.98, respectively. From the above relationships, TBW was calculated to scale with BW to the power 0.974. This exponent is negligibly different from the value of 0.984 determined previously for C. porosus (Grigg et al. 1986). The latter value has been used in calculating weight-specific TBW for C. *johnstoni* because it allows direct comparison with data for C. porosus and introduces an error of only 0.2-0.5% over the size range of *C. johnstoni* sampled. ExNa scaled with SVL to the power 2.69, and this value was used to calculate sodium factors for all crocodiles.

Unless otherwise indicated, all data are shown as mean + SE (n), where n is the number of samples included in the estimates.

Results

Distribution of C. johnstoni *in the river system*

C. johnstoni were distributed sparsely throughout most of the tidal waters in both 1982 and 1984. Where substantial numbers were found they were in the most upstream tidal reaches of the mainstream in water of less than 10 mg - ml^{-1} salinity. The largest numbers were found between 63 and 65 km on the mainstream at salinities of 1-6 mg - ml^{-1}. Forty-four crocodiles were counted in this area in 1984, of which 33 were definitely C. *johnstoni* and 11 were identified as 'eyes-only'. One *C. porosus* (approximately 3 m long) was definitely identified in this area. While not unequivocal, the apparently small size of many in this 'eyes-only' group and the dearth of *C. porosus* in the area suggest that the vast majority were *C. johnstoni,* giving a sighting index of 17-22 per kilometre in this area.

Farther downstream, both C. *porosus* and C. *johnstoni* were distributed sparsely in both 1982 and 1984 in both mainstream and tributaries. Over the 104 km of water ways surveyed in 1982, the sighting index for C. porosus was 0.52 non-hatchlings per kilometer. No C. *johnstoni* were located downstream of the Nathan River junction in 1982. Between the Nathan River junction and km 63 on the mainstream, the sighting index for C. *johnstoni* was 0.35 per kilometre. No marked differences in the distribution and numbers of C. *johnstoni* and C. porosus were noted between 1982 and 1984.
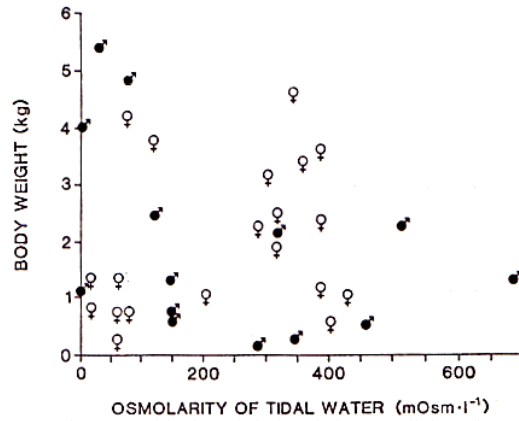
Fig. 2. Distribution of C. *johnstoni* by sex and body weight in relation to salinity. There is no evidence that distribution in the river system is strongly influenced by sex or that higher salinity areas are populated preferentially by larger crocodiles which would be better insulated from osmotic stress.
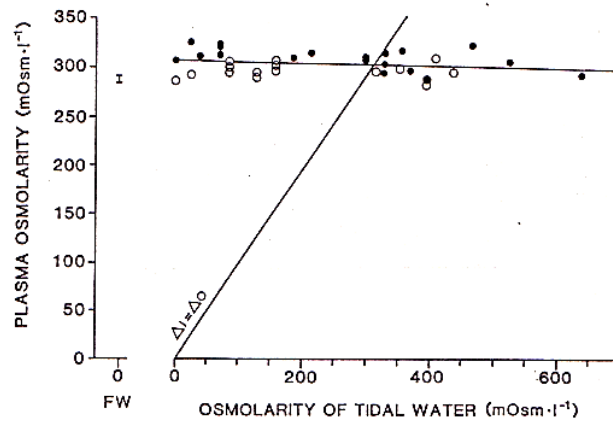

Fig. 3. Plasma osmolarity in C. *johnstoni* captured from saline tidal waters of the Limmen Bight River system: 1982 data *(closed symbols),* 1984 data *(open symbols).* Slopes of the regression lines relating these variables to the osmolarity of the water are not significantly different. Regression lines are fitted to data from crocodiles from tidal waters only. The very narrow 95% confidence limits for crocodiles captured from permanent fresh waters *(FW),* removed from tidal and saline influences, are plotted separately for comparison.
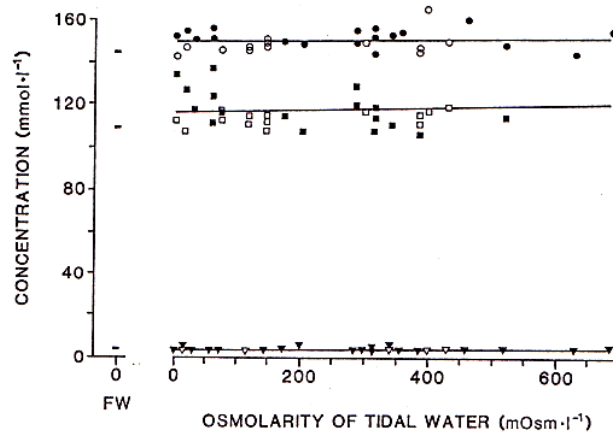

Fig. 4. Plasma sodium (●, o), chloride (■,□) and potassium (▼) concentrations in C. *johnstoni* captured from saline tidal waters of the Limmen Bight River system: 1982 data *(closed symbols),* 1984 data *(open symbols).* Slopes of the regression lines relating these variables to the osmolarity of the water are not statistically significant. Regression lines are fitted to data from crocodiles from tidal waters only. 95% confidence limits for crocodiles captured from permanent fresh waters *(FW),* removed from tidal and saline influences, are plotted separately for comparison but are so narrow that they appear as a single line

All *C. johnstoni* in estuarine waters appeared to be situated in areas experiencing continuously or intermittently hypoosmotic conditions (Fig. 1). Seventeen crocodiles were captured in salinities of 10 mg - ml^{-1} or more. None of these individuals could be reliably assessed as living under continuously hyperosmotic conditions. However, while all of these crocodiles had access to hypoosmotic water within 1-2 km of their capture site, most of them would face a considerably longer journey to obtain low salinity water which could be ingested without a significant Na load. This is particularly true of individuals captured between 45 and 50 km on the Cox River, those taken from Walantyuwurru Creek, and those from 39 to 40 km on the Nathan River. Thus, we should expect these crocodiles, at least, to show some physiological impact from exposure to salt water. This expectation is heightened because none of these individuals was sufficiently large to be well insulated from its environment by size alone. Mean body weight in this group was just 2.4 kg, ranging from 0.25 to 8.4 kg. The two largest animals weighed only *8.4* and *5.4* kg, respectively.

No evidence was found that size or sex had a significant influence on the distribution of C. *johnstoni* in saline waters (Fig. 2). Small crocodiles were not distributed preferentially in low salinity waters, nor was there any tendency for males or females to distribute themselves preferentially in particular sections of the river system.
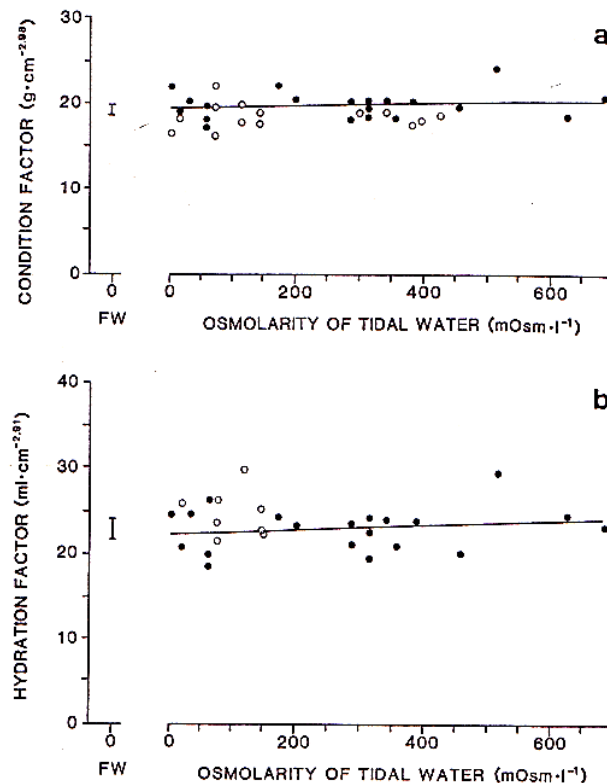

Fig. 5. Condition factors (a) and hydration factors (b) for C. *johnstoni* captured from saline tidal waters of the Limmen Bight River System: 1982 data *(closed symbols),* 1984 data *(open symbols).*

Condition factor (CF) is calculated as

$$CF = \frac{BW(g)}{SVL^{2.98}(cm)}$$

Hydration factor (HF) is calculated as

$$HF = \frac{TBW\ (ml)}{SVL^{2.91}(cm)}$$

The exponents 2.98 and 2.91 were derived from ANOCOVA of BW and TBW against SVL in the various sample groups. Regression lines are fitted to data for 1982 only as the 1984 data are from crocodiles concentrated at low salinities. Slopes of the regressions are not significantly different from zero (P=0.50 for CF; P=0.35 for HF). 95% confidence limits for crocodiles captured from permanent fresh waters (FM, removed from tidal and saline influences, are plotted separately for comparison

Osmoregulatory capabilities

There is no question that *C. johnstoni* from tidal waters of up to 24 mg - ml^{-1} salinity have the capacity to osmoregulate very precisely. Plasma osmolarity was remarkably constant across the entire salinity spectrum, as were concentrations of Na+, Cl$^-$ and K+ (Fig. 3, 4). The relationships of plasma

osmolarity and electrolyte concentrations to salinity showed no significant trends. Furthermore, none of the animals captured in tidal water deviated markedly in its plasma composition from the pattern for the tidal water group as a whole. The data suggest that *C. johnstoni* has no difficulty adjusting to a fluctuating saline environment.

Figures 3 and 4 illustrate also that crocodiles from saline waters did not have substantially higher or more variable plasma electrolyte levels than crocodiles from permanent fresh waters, even though the difference was statistically significant. Mean plasma osmolarity in tidal waters was 303 + 1.7 (n = 36) compared to 288 + 1.2 (n=33) in fresh waters, a difference of 5%. Similarly small differences in Na^+, K^+, and Cl^- were present also (Fig. 4). Coefficients of variation in the four measured quantities (osmolarity, Na+, K+, and Cl^-) were 3.4, 3.2, 1.5 and 0.8 %, respectively, in tidal waters and 2.5, 3.2, 1.3 and 0.5%, respectively, in fresh waters. Again, the data confirm the view that *C. johnstoni* has no difficulty adjusting to the late dry season influx of saline water.

That *C. johnstoni* does not achieve osmoregulation by allowing its body fluid volume to vary substantially is demonstrated convincingly by CF and HF (Figs. 5a, b and Table 2). Not only were CF and HF essentially constant across the salinity spectrum, but there were no significant differences in mean CF or HF between crocodiles from tidal or permanent freshwater habitats. Nor were C. *johnstoni* from tidal habitats significantly more variable in CF, HF or weight-specific TBW than crocodiles from permanent fresh water (Table 2).

ExNa data from crocodiles captured in 1982 showed similar constancy. No statistically significant trend of weight-specific ExNa with salinity was present in crocodiles from tidal waters. Mean ExNa was marginally higher in tidal water than in permanent fresh water whether expressed in weight-standardised or length-standardised terms, but the difference was not statistically significant (Table 2).

The only element of the various measures of osmoregulatory homeostasis in *C. johnstoni* which might point to some significant perturbation lies in the 1984 ExNa data. The absolute values of ExNa in these crocodiles were compromised (see Materials and methods) but their relativities remain relevant. A reasonable starting point is to assume that mean ExNa remained constant in *C. johnstoni* from permanent fresh water between 1982 and 1984. That this assumption is sound is supported by the constancy of TBW, CF, and plasma composition in all crocodiles from these habitats between years. Adjustment of the 1984 data from this assumption gives a mean value of *40.1 ± 0.84 (n=8)* mmol.kg$^{-0.904}$ for weight-standardised ExNa and *4.36±0.156 (n=8)* mmol.mm$^{-2.69}$ for length standardised ExNa in C. *johnstoni* from tidal waters, 30% lower than in crocodiles captured in the same waters in 1982 (refer to Table 2). ExNa values for these 1984 crocodiles were less variable than those from 1982. Coefficients of variation for weight-specific ExNa in fresh and saline waters were 10.1% and 7.9%, respectively, lower than in their 1982 counterparts (Table 2). This internal consistency implies that the difference between 1982 and 1984 results is unlikely to reflect some unidentified experimental artifact.

Table 2. Water and sodium balance variables and condition factors for C. *johnstoni* captured from permanent fresh waters and tidal waters of the Limmen Bight River in 1982 and 1984.

Year	Total body water (ml · kg$^{-0.98}$)	Hydration factor (ml · mm$^{-2.91}$ · 10^6)	Condition factor (kg · mm$^{-2.98}$ · 10^6)	Exchangeable sodium (mmol · kg$^{-0.90}$)	Sodium factor (mmol · mm$^{-2.69}$ · 10^6)
Permanent fresh water					
1982	737± 20.8(8) (689−848) 8.0%	22.1± 0.84(8) (18.4−26.4) 10.7%	18.7± 0.56(8) (15.9−21.0) 8.4%	53.5± 1.34(8) (46.8−59.1) 7.1%	5.78±0.205(8) (4.893−6.81) 10.0%
1984 −	743± 13.2(14) (666−837) 6.6%	23.5± 0.48(14) (20.7−26.2) 7.7%	19.3± 0.37(27) (14.0−24.0) 9.8%	−	−
Tidal water					
1982	724± 11.8(20) (639−843) 7.3%	23.1± 0.58(20) (18.7−29.6) 11.2%	19.9± 0.37(20) (17.2−24.3) 8.2%	57.4± 1.24(20) (49.8−72.2) 9.7%	6.56±0.172(20) (5.48−8.22) 11.8%
1984	821± 22.1(8) (733−924) 7.6%	24.8± 0.93(8) (21.7−29.9) 10.5%	18.6± 0.36(16) (16.3−22.2) 7.8%	−	−

Condition factors, hydration factors, and sodium factors are calculated as functions of the snout-vent length of crocodiles, using scaling exponents calculated by analysis of covariance. These measures allow comparison of condition, hydration status and whole body

sodium content independently of body weight, which itself fluctuates with body water content. Scaling exponents for exchange able sodium and body water with weight are derived empirically by analysis of covariance. Data are given as mean±SE(n), range in parentheses, coefficient of variation given as %. Raw values for sodium factor and hydration factor have been multiplied by 10^6 and for condition factor by 10^9 for convenience of presentation

Discussion

The findings of this study are significant in demonstrating a physiological capacity for colonisation of estuarine waters in at least one population of a characteristically stenohaline freshwater crocodile. The data extend the findings of Taplin et al. (1985) and demonstrate that quite remarkable osmotic and ionic homeostasis can be achieved by C. *johnstoni* of a wide variety of sizes across a range of habitats. Clearly, C. *johnstoni* has the physiological ability to survive and thrive in periodically hyperosmotic environments at least.

Whether the osmoregulatory competence of C. *johnstoni* extends to continuously hyperosmotic environments is not demonstrated by the data. However, the distributional data suggest that C. *johnstoni* may require periodic access to hypoosmotic salt water. Several observations from other studies lend weight to this interpretation. Firstly, Messel et al. (1980) found C. *johnstoni* as far downstream as km 20.8 in the Limmen Bight mainstream and km 6.5 in one of its side creeks during surveys in the early dry season (May) of 1979. Messel's surveys were conducted when hypoosmotic conditions extended to km 20 in the mainstream. Five C. *johnstoni* were found downstream of the Nathan River junction (km 30), the downstream limit of their distribution in our late dry season surveys. Three of the five were clearly in, or close to, areas exposed periodically to hypoosmotic salt water. Another was in 22 mg.ml^{-1} salinity in a tributary near the river mouth, but in an area where freshwater runoff would certainly have made hypoosmotic water available within a kilometre or two upstream [see Table 12.6 and Fig. 12.9 of Messel et al. (1980)]. Only one C. *johnstoni* was located in hyperosmotic salt water (22 mg.ml^{-1}) and substantially removed from access to hypoosmotic water.

Secondly, Webb et al. (1983) described distributional shifts in a population of C. *johnstoni* on the Adelaide River in the Northern Territory. They found C. *johnstoni* concentrated in upstream, low salinity reaches during both wet and dry seasons. However, individual crocodiles were usually sighted farther downstream immediately after the wet season than at any later stage in the dry season. Their summarised data suggest a shift upstream of about 15 km from the early to the late dry season in both 1979 and 1980. The upstream shifts of C. *johnstoni* suggested by Webb et al. (1983), Messel et al. (1980) and our observations may well reflect downstream dispersal of individual animals during wet season floods and their movement upstream as salt water intrudes during the winter. If these distributional shifts do reflect behavioural avoidance of hyperosmotic salt water, then they would parallel reports of behavioural osmoregulation in C. *acutus* (Dunson 1982; Mazotti and Dunson 1984; Thorbjarnarson 1989).

A third line of evidence also supports the view that C. *johnstoni* has a limited capacity for colonisation of hyperosmotic salt waters. Webb et al. (1983) noted that the presence of the larger estuarine crocodile, C. *porosus*, in northern Australian rivers might influence the distribution of C. *johnstoni* through competition or predation. While this appears likely for rivers such as the Adelaide where C. *porosus* is abundant, surveys of rivers in the southern Gulf of Carpentaria [Taplin (1988) and unpublished observations] suggest that other factors are also important. In the Limmen Bight and many other southern Gulf rivers, C. *porosus* is present at extremely low densities (Messel et al. 1979-1986; Taplin 1990). Sighting indices in the Leichhardt, Bynoe and Albert Rivers in Queensland were as low as 0.1 non-hatchlings per kilometre in 1985, 20-30 times lower than in the Adelaide River (Taplin 1990). However, very few C. *johnstoni* have been sighted in the saline reaches of these rivers, despite their abundance in adjacent tidal and non-tidal fresh waters [Taplin (1990) and unpublished observations]. Isolated instances have been recorded, such as a 4-kg individual captured in hypersaline water (44 mg.ml^{-1}) on a tributary of the Albert River (17.8$^°$ S, 139.4$^°$ E) in 1985 (Taplin, unpublished observations) and sightings of three individuals in waters of 17-26.5 mg - ml^{-1} in Duck Creek in the late wet season of 1979 (Messel et al. 1981). Detailed surveys of virtually every significant northern Australian river system over the past 20 years have failed to identify a single substantial population of C. *johnstoni* inhabiting permanently hyperosmotic salt water. If competitive exclusion or predation by C. *porosus* was a predominating influence on the estuarine distribution of C. *johnstoni,* then some instances of permanent colonisation of downstream estuaries could be expected.

The availability of nesting habitat in mangrove- and salt-pan-dominated estuaries of northern Australia may also influence their colonisation by C. *johnstoni,* a hole nester which typically uses sand banks as nest sites. In the absence of any osmoregulatory constraints, the influence of nest site availability might be reflected in the distributions of sexes and size classes. This is clearly the case in *C. porosus*, in which hatchling and immature crocodiles in hyperosmotic salt waters are commonly concentrated around discrete nesting sites (Taplin and Grigg, unpublished observations). No such distributional differences are apparent among C. *johnstoni* in the Limmen Bight River (Fig. 2).

Despite the distributional evidence suggesting limited salinity tolerance in C. *johnstoni,* there is little physiological evidence pointing in this direction. The only evidence to suggest that C. *johnstoni* might suffer significant perturbations of its salt and water balance in salt water lies in the 1984 ExNa data. These remain unexplained, but may reflect some short-term disturbance brought on by the initial flush of salt water across upstream barriers in the late dry season. The question will need to be addressed in future studies. A less likely alternative is that the assumed constancy of ExNa in crocodiles from permanent freshwater habitats between 1982 and 1984 is incorrect. None of the other physiological measurements on the freshwater groups supports this proposition. No significant differences were found between groups of crocodiles from various permanent freshwater habitats in their plasma composition, total body water, condition factor, or 1982 values for weight-specific or length-specific ExNa. It would be extraordinary for this degree of constancy to be combined with a systematic difference of some 30% in weight-specific ExNa.

This study has not established whether C. *johnstoni* has the capacity to colonise continuously hyperosmotic waters. It has, however, established that C. *johnstoni* has some of the prerequisite osmoregulatory capabilities which would facilitate colonisation in the absence of other constraining factors. The study has particular relevance to comparable studies of the many other crocodilians which are restricted to fresh waters throughout most of their range but occur in isolated pockets in saline waters (Taplin 1988). It also illustrates some of the problems encountered in trying to determine the osmoregulatory capabilities of animals which can be difficult to work with even under the best of conditions, and whose saltwater populations occur in remote areas where longitudinal studies of adaptation are not possible. It emphasises the importance of identifying the types of `natural experiments' which have enabled a sound appreciation of the remarkable euryhalinity shown by *C. porosus* in the wild. To extend our understanding of the capabilities, of *C. johnstoni* it will be necessary to undertake further studies under more controlled conditions in the laboratory.

References

Dunson WA (1982) Salinity relations of crocodiles in Florida Bay. Copeia 1982:374-385

Grigg GC (1981) Plasma homeostasis and cloacal urine composition in Crocodylus porosus caught along a natural salinity gradient. J Comp Physiol 144:261-270

Grigg GC, Taplin LE, Harlow P, Wright J (1980) Survival and growth of hatchling Crocodylus porosus in salt water without access to fresh drinking water. Oecologia (Berlin) 47:264-266 Grigg GC, Taplin LE, Green B, Harlow P (1986) Sodium and water fluxes in free-living Crocodylus porosus in marine and brackish conditions. Physiol Zool 59:240-253

Hall N, Wainwright RW, Wolf LJ (1981) Summary of meteorological data in Australia. CSIRO Division of Forest Research, Divisional Report No. 6, CSIRO, Canberra

Mazotti FJ, Dunson WA (1984) Adaptations of Crocodylus acutus and Alligator for life in saline water. Comp Biochem Physiol 79A: 641-646

Messel H et al (1979-1986) Surveys of tidal waterways in the Northern Territory of Australia and their crocodile populations (a series of 20 monographs). Pergamon, Sydney

Messel H et al (1980) Tidal waterways on the south-western coast of the Gulf of Carpentaria (Monograph 12). In: Messel H (ed) Surveys of tidal waterways in the Northern Territory of Australia and their crocodile populations (a series of 20 monographs). Pergamon, Sydney

Messel H et al (1981) South western Cape York Peninsula (Monograph 16). In: Messel H (ed) Surveys of tidal waterways in the Northern Territory of Australia and their crocodile populations (a series of 20 monographs). Pergamon, Sydney

Messel H et al (1986) Resurveys of the tidal waterways of Van Diemen Gulf and the Southern Gulf of Carpentaria, 1984 and 1985 (Monograph 19). In: Messel H (ed) Surveys of tidal waterways in the Northern Territory of Australia and their crocodile populations (a series of 20 monographs). Pergamon, Sydney Taplin LE (1984) Homeostasis of plasma electrolytes, water and sodium pools in the estuarine crocodile, Crocodylus porosus, from fresh, saline, and hypersaline waters. Oecologia (Berlin) 63:63-70

Taplin LE (1985) Sodium and water budgets of the unfed estuarine crocodile, Crocodylus porosus, in sea water. J Comp Physiol B 155:501-513

Taplin LE (1988) Osmoregulation in crocodilians. Biol Rev 63:333-377

Taplin LE (1990) The population status and management of estuarine crocodiles in Queensland - present situation and future prospects. In: Crocodiles: their ecology, management, and conservation. IUCN, Gland, Switzerland

Taplin LE, Grigg GC, Beard L (1985) Salt gland function in fresh water crocodiles: evidence for a marine phase in eusuchian evolution? In: Grigg GC, Shine R, Ehmann H (eds) The biology of Australasian frogs and reptiles. Surrey Beatty, Sydney

Taplin LE, Grigg GC (1981) Salt glands in the tongue of the estuarine crocodile, *Crocodylus porosus*. Science 212: 1045-1047 Taplin LE, Grigg GC (1989) Historical zoogeography of the eusuchian crocodilians: a physiological perspective. Am Zool 29:885-901 '

Thorbjarnarson JB (1989) Ecology of the American crocodile, *Crocodylus acutus*. In: Crocodiles: their ecology, management, and conservation. IUCN, Gland, Switzerland

Webb GJW, Manolis SC, Sack G (1983) Crocodylus johnstoni and C. porosus coexisting in a tidal river. Aust Wildl Res 10:639-50