

# Lingual Salt Glands in *Crocodylus acutus* and *C. johnstoni* and their absence from *Alligator mississippiensis* and *Caiman crocodilus*

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**Summary.** 1. Lingual salt glands, secreting hyperosmotic Na/K solutions in response to methacholine, are present in *Crocodylus acutus* and *C. johnstoni* but apparently absent from the alligatorids, *Alligator mississippiensis* and *Caiman crocodilus*.

2. Both secretory rates (6-20 [ $\mu\text{mol}/100\text{ g-h}$ ]) and concentrations (450-600 mM Na) of glandular secretions are essentially identical in the marine/estuarine *C. acutus* and *C. porosus* and significantly higher than in the freshwater *C. johnstoni* (1-2  $\mu\text{mol}/100\text{ g-h}$ ; 320-420 mM Na).

3. Lingual glands in *Alligator* secrete isosmotic Na/K at low rates (1-2  $\mu\text{mol}/100\text{ g-h}$ ) while those of *Caiman* show no response to methacholine.

4. The physiological contrast between alligatorids and crocodylids is reflected in distinct differences in the superficial appearance of the tongue and lingual pores.

5. It is postulated that the alligatorid condition of low secretory capacity and isosmotic secretion reflects the primitive salivary function of lingual glands from which the salt-secreting capability in crocodylids was derived.

## Introduction

It has been shown recently that the lingual glands of *Crocodylus porosus* are functional salt glands which play an essential role in osmoregulation in salt water (Taplin and Grigg 1981). Lingual glands are known to occur in several other Crocodylidae and Alligatoridae and descriptions of their gross and microscopic structure show some distinct interspecific differences (Owen 1866; Ferdinand 1884; Reese 1915, 1925; Taguchi 1920). In all crocodylians examined the lingual glands are compound, branched and tubular. However, the glands of *Alligator* and *Caiman* consist of relatively small numbers of quite loosely aggregated tubules with very wide lumina (Reese 1915, 1925). In contrast, *Crocodylus porosus* has multi-lobular glands each lobule containing scores of tightly packed tubules with minute lumina (Taplin and Grigg 1981). Distinct differences in cellular structure, particularly the presence of large secretory droplets in *Alligator*, have also been described (Taguchi 1920) and confirmed in light microscope and EM sections (Taplin, 1982).

The demonstration of a salt-excreting capability in the lingual glands of *Crocodylus porosus* suggests, however, that similar glands in some other crocodylians may also have an osmoregulatory role. This is particularly likely in the American crocodile *Crocodylus acutus*, the only other crocodylian found principally in saline habitats. The demonstration of lingual salt-secreting glands in *Crocodylus acutus* would be of great interest because previous authors have proposed that intolerance of high salinities adversely affects hatchling survivorship in Florida populations (Dunson 1970, 1982; Evans and Ellis 1977). Low survival rates in hatchlings have in turn been seen as a major impediment to recovery of the severely depleted American population of this crocodile.

In contrast to *Crocodylus porosus* and *C. acutus*, most crocodylians are essentially restricted to freshwater (FW) though at least four species (*Alligator mississippiensis*, *Crocodylus niloticus*, *C. palustris* and *C. johnstoni*) are known to occur occasionally in saline waters (Neill 1971; Messel et al. 1979). Our present understanding of salt and water balance strategies of freshwater reptiles suggests that freshwater crocodylians should not require functional salt glands, relying instead on the renal/ cloacal complex for salt excretion. Incursions of primarily FW crocodylians into saline habitats may be facilitated by a resistance to (or tolerance of) dehydration and hypernatraemia, low skin water and electrolyte permeability, a relatively low surface area:mass ratio, a diet of fish or other organisms low in electrolytes, and periodic access to fresh or brackish drinking water (Dunson 1976). Such adaptations alone may allow survival of FW crocodylians in saline habitats but the potential for salt secretion via the lingual glands cannot be overlooked.

This paper presents the results of a preliminary study of the secretory characteristics of lingual glands in both seawater (SW) and FW crocodylians. Marked differences between crocodylids and alligatorids, in both secretory rates and concentrating abilities of the lingual glands, were found.

## Materials and Methods

Two alligatorids (*Alligator mississippiensis*, 4 specimens, and *Caiman crocodilus*, 3 specimens) were collected from natural and feral populations, respectively, in southern Florida. Examples of a crocodylid (*Crocodylus acutus*, 3 specimens) were obtained from a captive stock originally caught in Jamaica. These animals were studied at the University of Miami. Two further crocodylids, *C. porosus* and *C. johnstoni* (4 specimens of each) were collected from the Liverpool/Tomkinson Rivers in Arnhem Land, northern Australia and studied at the University of Sydney's Crocodile Research Facility at Maningrida.

Any comparison of lingual glands must take into account the salinity history of the animals used in the study. Fortunately, both *C. porosus* and *C. acutus*, though frequently marine and estuarine, occur naturally in FW so a valid comparison between all species is possible in freshwater. All animals used in this study were either newly captured from FW or had been maintained in FW periodically for several months. Body masses ranged from 0.3 to 32 kg, but most weighed between 3 and 10 kg.

A standard technique to induce secretion by salt glands in birds and in other reptiles is salt loading, usually by intraperitoneal injection (Peaker and Linzell 1975). This method has been unsuccessful in *C. porosus* but secretion can be stimulated by IP injection of methacholine chloride (Taplin and Grigg 1981). In other work, Taplin (1982) has shown that injection of 0.5-4 mg/kg methacholine into *C. porosus* from 100 g to 50 kg body mass initiates a tri-phasic pattern of response: (a) an initial period of rapid secretion; (b) a subsequent period of reduced secretion, associated with respiratory distress; and (c) a second period of rapid secretion followed by a decline.

These three phases, lasting 1-5 h in total, depending upon the size of the animal, are thought to indicate a period of rising drug concentration, glandular inhibition above some threshold and a period of further stimulation as the drug concentration falls again below the threshold. The appearance of the second phase in any particular trial, with respiratory depression and glandular inhibition, was used to assess whether or not a maximum safe dose of methacholine had been given and, hence, the presumed maximum secretory capability approached. No animal died from an overdose of methacholine and no differences in susceptibility to methacholine were apparent between species.

In each experiment, an animal was restrained with its mouth propped open and injected with an appropriate dose of methacholine. All experiments were performed at ambient temperature of 24-28 °C. Lingual gland secretions were either collected continuously into a syringe or absorbed onto filter paper pads placed on the tongue for measured periods of 5-10 min. Secretions collected by syringe were used for analysis of electrolyte concentrations and filter paper collections for determinations of secretory rate. The two treatments were applied alternately for 5-10 min periods throughout each experiment. Rates of electrolyte secretion in  $\mu\text{mol Na}/100 \text{ g-h}$  were determined by immersing the dried filter papers in 5-10 ml of distilled water to dissolve the accumulated electrolytes and analyzing for Na. The highest rate of secretion achieved over a 10 min collecting period was taken as the maximum secretory rate for the glands. Cation concentrations were determined by flame photometry.

## Results

### Lingual Gland Secretions

In all three crocodylids, lingual gland secretions were rich in Na and K and clearly hyperosmotic to plasma (Table 1). Secretions from *Crocodylus porosus* and *C. acutus*, the two SW crocodylids, showed a marked similarity in both concentration and secretory rates. However, the FW *C. johnstoni* showed secretory rates about one sixth those in *C. porosus* and *C. acutus*, with concentrations of Na and K 25-35% lower.

Of the alligatorids, *Caiman* produced no observable secretions while in *Alligator* the secretory rate was so low that only a single animal produced enough secretion for concentration analysis. The secretion was roughly isosmotic with plasma and the secretory rate ( $1 \mu\text{mol}/100 \text{ g-h}$ ) only half that in *Crocodylus johnstoni*, its FW equivalent in the Crocodylidae (Table 1). It is notable that the volume rate of fluid secretion was essentially identical in *Alligator* and *C. johnstoni*, as doubling the Na concentration of the secretion in *C. johnstoni* doubled the secretory rate for Na relative to *Alligator*. The volume of secretion was about five times as great in the SW crocodylians.

For most species, the range of body masses involved was small (Table 1) and the variability of observed secretory rates was low. However, the four *C. porosus* available ranged from 3.6-32 kg and these showed a consistent inverse relationship between Na secretory rate ( $\mu\text{mol}/100 \text{ g-h}$ ) and body mass. That this is a real effect has been confirmed in similar studies of 14 SW-acclimatized *C. porosus* in which the

Na secretion rate ( $\mu\text{mol/h}$ ) was proportional to  $\text{BM}^{0.7}$  (Taplin 1982). However, in this study, there was little difference in secretory rate between *C. porosus* and *C. acutus* of similar size.

Table 1. Comparison of lingual gland secretions and plasma in crocodylians from freshwater, expressed as  $\bar{x}\pm\text{SE}$  (N) where N is the number of animals. Range in parentheses

	<i>Crocodylus porosus</i>	<i>Crocodylus acutus</i>	<i>Crocodylus johnstoni</i>	<i>Alligator mississippiensis</i>	<i>Caiman crocodilus</i>
Body mass (kg)	12.7 $\pm$ 6.59(4) (3.6-32)	5.1 $\pm$ 0.78(3) (3.6-6.2)	6.1 $\pm$ 0.96(4) (3.6-8.1)	3.8 $\pm$ 0.25(5) (3.0-4.5)	1.0 $\pm$ 0.44(3) (0.3-1.8)
Na secretion rate ( $\mu\text{mol}/100\text{ g}\cdot\text{h}$ )	12.6 $\pm$ 3.17(4) (6.3-20.0)	13.5 $\pm$ 1.58(3) (11.1-16.5)	2.0 $\pm$ 0.56(4) (0.41-2.86)	1.0 $\pm$ 0.15(4) (0.83-1.47)	None
<i>Secretion</i>					
[Na] <sup>a</sup> (mmol/l)	510 $\pm$ 31.3(4) (458-601)	498 $\pm$ 10.0(3) (479-502)	386 $\pm$ 20.5(4) (327-422)	186	-
Maximum					
Average	477 $\pm$ 35.5(4) (408-574)	455 $\pm$ 37.0(3) (383-506)	365 $\pm$ 13.1(4) (327-385)	183 $\pm$ 3.0(2) (180-186)	-
[K] <sup>a</sup> (mmol/l)	15.5 $\pm$ 0.57(4) (14.0-16.7)	12.4 $\pm$ 0.44(4) (11.6-13.5)	10.3 $\pm$ 0.81(3) (8.8-11.6)	-	-
Maximum					
Average	14.0 $\pm$ 0.57(4) (13.1-15.6)	11.7 $\pm$ 0.49(4) (10.4-12.8)	9.1 $\pm$ 0.82(3) (8.1-10.7)	-	-
<i>Plasma</i>					
[Na] (mmol/l)	145 $\pm$ 4.8(4)	155 $\pm$ 7.3(4)	158 $\pm$ 5.9(4)	147 $\pm$ 4.1(5)	148 $\pm$ 4.0(3)
[K] (mmol/l)	4.3 $\pm$ 0.35(4)	3.4 $\pm$ 0.18(3)	3.3 $\pm$ 0.24(4)	3.3 $\pm$ 0.40(5)	2.9 $\pm$ 0.45(3)

<sup>a</sup> Na and K concentrations are expressed as means of both the maximum concentration observed in each crocodile and of the concentration averaged over the collection period

#### *External Appearance of the Tongue*

Superficially, marked differences were apparent between the tongues of crocodylids, on the one hand, and alligatorids on the other. In all three crocodylids the tongue has a relatively smooth surface punctuated by 20-40 prominent pores (Fig. 1 a, b). The pores carry secretions from the lingual glands to the tongue surface and are interconnected by a network of shallow grooves. In contrast, the tongues of both *Alligator* and *Caiman* have large numbers of minute pores (200 or more in *Alligator*) which are scarcely visible to the unaided eye. In many alligators, however, they are marked by quite intense red pigmentation of the tongue epithelium surrounding the pores (Fig. 1 c). A further marked contrast with the Crocodylidae is afforded by the presence of a very glutinous, mucous layer over the proximal region of the tongue and extending onto the palatal epithelia surrounding the buccal valve. This mucous zone is distinct from the distal portion of the tongue which, though it also has secretory pores, is coarse to the touch and lacks a mucous coating (Fig. 1 c, d).

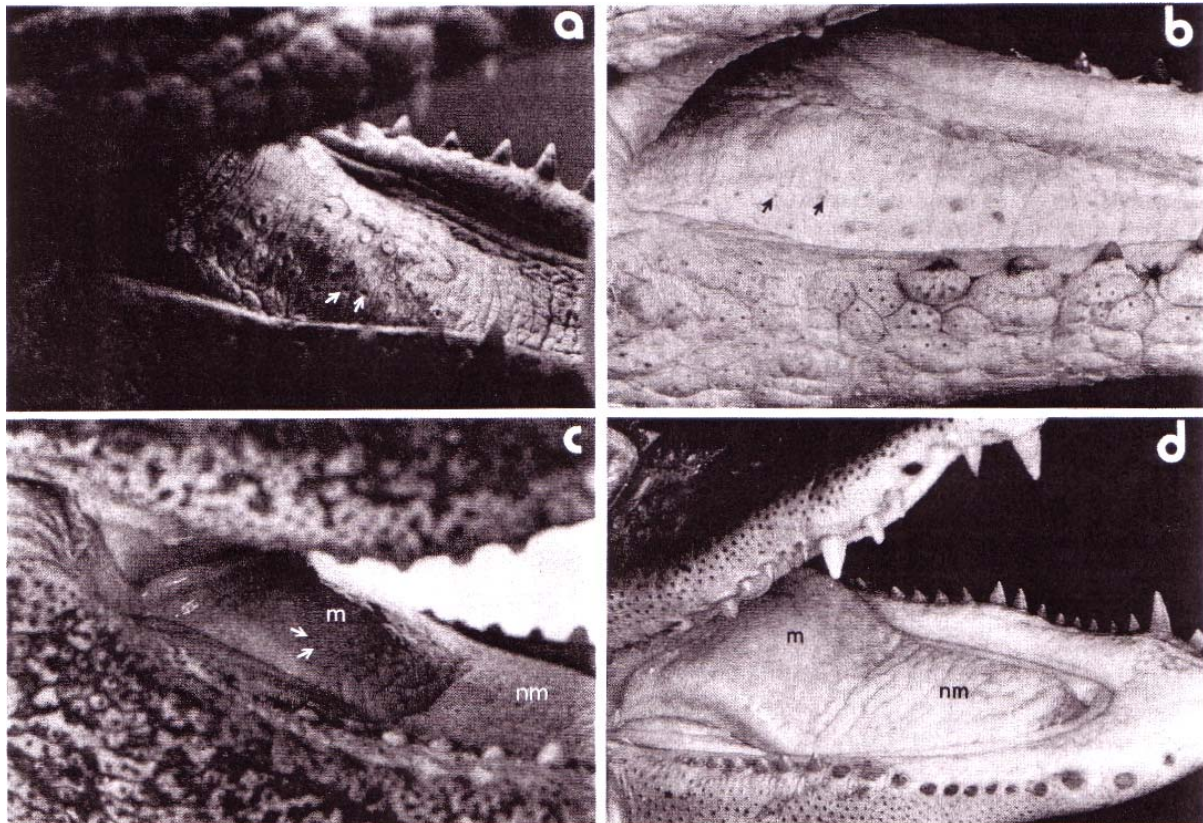


Fig. 1 a-d. Tongues of crocodylids (a, b) and alligatorids (c, d) showing lingual gland pores (arrowed) and the division on the tongue epithelium in alligatorids into mucus-coated (m) and nonmucous (nm) regions. Lingual gland secretions are clearly visible on the tongue of *Crocodylus acutus*. a *Crocodylus acutus*; b *Crocodylus johnstoni*; c *Alligator mississippiensis*; d *Caiman crocodilus*

## Discussion

Salt glands secrete electrolyte solutions considerably hyperosmotic to plasma, usually by at least two-fold (Dunson 1976). Clearly, *Crocodylus acutus* possesses lingual salt glands. The lingual glands in *Crocodylus johnstoni* appear less specialized for salt excretion but still possess some characteristics of salt glands. Very little is known at present regarding the contribution of the glands to overall salt and water balance in either species.

Evans and Ellis (1977) measured Na effluxes in very young hatchling *Crocodylus acutus* (54-68 g) exposed to FW or 25% SW for 15 days. Na efflux in unrestrained animals increased from 2.5  $\mu\text{mol}/100\text{ g-h}$  in FW to 5.6  $\mu\text{mol}/100\text{ g-h}$  in 25% SW but the cephalic contribution to efflux in restrained animals fell from 69% of the total to 46% while cloacal Na efflux rose from 19-34%. However, the total Na efflux of the restrained animals in SW rose to 16.5 [ $\mu\text{mol}/100\text{ g-h}$ ]. Dunson (1982) exposed hatchling *C. acutus* to 100% SW and found very low Na efflux rates in unrestrained 84-413 g crocodiles (0.3-4.6  $\mu\text{mol}/100\text{ g-h}$ ), more typical of FW than marine reptiles and providing no evidence for significant extrarenal salt excretion. The very different rates of Na efflux in unrestrained and restrained animals are somewhat difficult to reconcile. The unrestrained values seem more likely to reflect the true effluxes. In contrast to the above, freshly-captured, SW-acclimatized *C. porosus* (100-300 g) had Na efflux rates of 10-30  $\mu\text{mol}/100\text{ g-h}$  of which roughly 80% occurred via the head (Taplin 1982). Similar measurements of efflux compartmentalization in SW acclimatized *C. acutus* and very young *C. porosus* may resolve the apparent differences in the degrees of extrarenal excretion in the two species and indicate the importance of the lingual salt glands in overall salt and water balance of *C. acutus*. It is notable, however, that in the present study the secretory rates and concentrations achieved by the lingual glands of juvenile *C. porosus* and *C. acutus* after methacholine stimulation were essentially identical (Table 1). *C. porosus* is able to survive in hyperosmotic saltwater without access to fresh drinking water (Grigg et al. 1980) while excreting little or no Na in the urine (Grigg 1981). *C. acutus* from saline habitats similarly excrete urine of low Na concentration (Dunson 1982). Taken together, the data suggest that the salt glands in juvenile *C. acutus* at least are likely to be important in osmoregulation in saline environments. The smallest functional salt gland previously known in reptiles was

the premaxillary of the estuarine snake, *Cerberus rhynchops* (Dunson and Dunson 1979) with an excretion rate of 16  $\mu\text{mol}/100 \text{ g-h}$ . In *Cerberus* the gland is inactive until activated by dehydration. A similar phenomenon may account for the low Na efflux from *C. acutus* compared with those from *C. porosus* and, perhaps, for the puzzling lack of sensitivity of crocodylian salt glands to salt loads.

The fact that *Crocodylus johnstoni* has salt glands is particularly intriguing because this species is rarely found in saline habitats. However, extensive surveys in northern Australia have revealed substantial numbers of *C. johnstoni* in water of up to 22‰ salinity in the Limmen Bight and Baines Rivers while individuals have been found occasionally in saltwater in several other rivers (Messel et al. 1979, 1980, 1981). The lingual salt glands may prove essential to salt and water balance under these circumstances. Nevertheless, the *C. johnstoni* studied here were collected from the upper reaches of the Liverpool River, Northern Territory, and the species has never been recorded from the saline section of the river despite intensive surveys over 8 years. Furthermore, *C. johnstoni* from the upper reaches of the Gilbert River System in Queensland, hundreds of kilometers from its mouth, produce secretions of similar concentration indicating that the phenomenon is not characteristic only of near-coastal populations (Taplin, unpublished observation). These upstream habitats are subject to prolonged dry spells during which lagoons may dry out entirely. *C. johnstoni* is reputed to be capable of surviving such dry periods by aestivating in burrows; a circumstance in which the ability of lingual salt glands to reduce plasma Na/K concentrations at the cost of little body water could be advantageous.

The low secretory rates and essentially isosmotic secretions seen in *Alligator* suggest that its lingual glands are salivary rather than salt glands. Together with the distinct differences between Alligatoridae and Crocodylidae in both the gross appearance of the tongue and the gross and microscopic structure of the lingual glands (see Introduction) the data point to a major physiological difference between the two families, probably of long standing.

The lingual glands of the Alligatoridae and Crocodylidae are likely to be homologous. The alligatorid condition probably reflects the primitive state in crocodylians generally, the salt-excreting function having arisen secondarily during invasion of saline habitats. Clearly, future investigation of a wider range of species can be expected to reveal differences between taxa which will reflect phylogenetic and environmental influences on lingual gland function and offer new insights into patterns of evolution among crocodylians.

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