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Some Physiological Consequences of Estivation by Freshwater Crocodiles, *Crocodylus johnstoni*

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ABSTRACT.—**Estivating crocodiles were studied at their dry season refuge in northern Australia over six years. The crocodiles spent three or four months a year underground without access to water. Doubly labeled water was used to measure field metabolic rates and water flux, and plasma and cloacal fluid samples were taken at approximately monthly intervals during some years to monitor the effects of estivation with respect to the accumulation of nitrogenous wastes and electrolyte concentrations. Estivating crocodiles expended a mean of 26.1 kJ kg^{-1} day^{-1}. Although environmental and, hence, body temperatures increased with increasing time in estivation, water efflux decreased with increasing time in estivation to 9.3 mL kg^{-1} day^{-1}, which was only 23% of the flux rate of crocodiles in water before estivation (40.2 mL kg^{-1} day^{-1}). The absolute size of body water pools declined proportionately with body mass and body solids; thus the crocodiles were not dehydrated even after three months without access to water. With increasing time in estivation, the following parameters increased in the cloacal fluid: osmolality, potassium, and magnesium; and the following increased in the blood plasma: osmolality, and protein concentration. Electrolytes in plasma were highest when the crocodiles were in the water and late in the estivation period. Freshwater crocodiles do not appear to have any specific adaptations for estivation, but given an adequate refuge, they can survive many months without access to water.**

Estivation, or dormancy in response to dry conditions (Gregory, 1982), poses physiological problems that are parallel to winter dormancy but, despite the large number of reptile species that inhabit areas subject to periodic drought, few studies have investigated the physiological consequences of estivation in reptiles (Gregory, 1982). Unlike winter dormancy, estivation often occurs during warm or hot seasons and, although estivating animals presumably select refugia that are cooler than external conditions, the ambient temperatures will nevertheless be higher than those experienced by animals in winter dormancy. Relatively high temperatures will result in dormant animals with higher metabolic rates, the potential for higher rates of water loss, and the production of significant amounts of waste products. Thus, estivation may pose some physiological challenges that are different and more severe than those experienced by animals over winter.

Physiological characteristics of reptiles that allow them to survive periods of prolonged drought include the ability to tolerate elevated osmotic concentrations in the body fluids and to maintain plasma volume while dehydrated (Minnich, 1982; Grigg et al., 1986). One adaptation that would minimize energy loss, water loss, and the production of wastes by estivating animals is metabolic depression (Hochachka and Guppy, 1987; Storey and Storey, 1990). Despite the apparent advantages, metabolic depression in estivating reptiles has been demonstrated only in the turtles *Kinosternon flavescens* (Seidel, 1978) and *Chelodina rugosa* (Kennett and Christian, 1994).

Estivation has been observed in at least 10 species of crocodilians, but no published studies examine the physiological consequences of estivation by crocodilians (Taplin, 1988). However, a number of studies have investigated osmoregulation by crocodilians in various habitats, including desiccating conditions (reviewed by Taplin, 1988; Mazzotti and Dunson, 1989).

Freshwater crocodiles were observed estivating at one site in northern Australia over a period of six years (Kennett and Christian, 1993). Here we report the results of measurements taken from the estivating crocodiles during their annual three to four month period of inactivity in underground refugia. Isotopic techniques were used to measure rates of water flux and CO_2 production, and cloacal fluid and blood plasma were examined with respect to electrolyte concentrations, nitrogenous wastes, osmolality, and plasma proteins. The physiological consequences of estivation are discussed in relation to the gradual seasonal increase in ambient temperature inside refugia and compared to the physiological consequences of other extreme conditions.

MATERIALS AND METHODS

Freshwater crocodiles *Crocodylus johnstoni* were studied at a site on Saunders Creek, a trib-

utary of the Margaret River, approximately 200 km south of Darwin, Northern Territory, Australia. Crocodiles were found in an undercut cavern at the level of the creek bed and approximately 2 m below the creek bank (described in detail by Walsh, 1989; Kennett and Christian, 1993). Samples for physiological analysis were taken from crocodiles at this site during the period 1987–1992. In every year except 1991 the creek dried during the second half of August and the crocodiles remained in the underground refuge from the last week of August until early or mid December when rains filled the creek. During 1991 late rains resulted in water remaining in the creek until late September. Samples were collected from active crocodiles netted in the water during early August, 1988, and estivating crocodiles were sampled at intervals that included late August, September, October, and mid November.

At the time of each capture we measured deep cloacal temperature with a calibrated thermocouple thermometer as soon as the animals were removed from the refuge. The crocodiles were weighed to the nearest 20 g with spring scales that had been calibrated against standards.

In August, 1988, crocodiles were caught in nets in the water, but at all other sampling periods crocodiles were captured inside the refuge by noosing. After capturing the animals in nets in August, 1988, a 1 ml blood sample was taken from the caudal vein, and the crocodiles were then injected intraperitoneally with 1.0 ml of tritiated water (20 MBq) and with 1.0 ml of 95% atoms excess $H_2{}^{18}O$. After an eight hour equilibration period a second 1 ml blood sample was taken, and the animals were returned to the creek. Subsequent analysis of the samples revealed that the initial period in the water resulted in a rapid loss of isotopes and, although the levels of tritiated water were still sufficient for measurements of water flux rates, the levels of $H_2{}^{18}O$ were insufficient for measurements of rates of carbon dioxide production. In November, 1988, the animals were reinjected with tritiated water in order to determine whether or not the pool of body water, as a percentage of body mass, had changed over the period of estivation.

In 1990 the crocodiles were injected again with doubly labeled water, but only after they had entered their estivation refuge in late August. During both 1988 and 1990 the animals were recaptured at approximately monthly intervals and 1 ml blood samples were collected. Blood samples were subsampled and the fractions used for isotopic analysis were stored whole, but samples used for other assays were centrifuged in the field and the supernatant was drawn off and transported to the laboratory for analysis.

In addition to blood samples, we also collected cloacal fluid samples by inserting a plastic pipette into the cloaca and allowing a sample of fluid to flow down the pipette into a vial. These were sealed and transported to the laboratory for analysis. In order to disrupt the water pool as little as possible, we collected only a sub-sample of cloacal fluid. For that reason we could not investigate the relative proportions of liquid and solid urates. The chemical analyses of cloacal fluid (below) were performed on liquids.

In the laboratory the cloacal fluid samples were centrifuged and the supernatant was analyzed for osmolality, electrolyte concentrations (Na, K, Mg, Ca, and Cl), ammonia, urea, and dissolved uric acid. Osmolality was measured with a Wescor vapor pressure osmometer (model 5500XR), and electrolyte concentrations were measured with a Perkin-Elmer ICP. Ammonia and urea concentrations were determined by the Berthelot colorimetric procedure (Sigma diagnostic test No. 640, Sigma Chemical Co.), and soluble uric acid was measured by Sigma diagnostic test No. 680.

Plasma samples were analyzed for osmolality, electrolytes, and urea by the methods described above. Plasma protein concentrations were determined by the Biuret method (Sigma diagnostic test No. 541-2).

Blood samples for isotopic analysis were subjected to vacuum distillation in the laboratory. Subsamples of extracted water were analyzed for tritium by liquid scintillation counting (Beckman 2002 L.S.C.). Other subsamples of extracted water were placed in Urey exchange tubes and subjected to equilibration with standard CO_2 charges at 80 C and then the $^{16}O{:}^{18}O$ ratios were determined by isotopic mass spectrometry (V. G. Isogas Model 903).

Body water pool sizes were calculated from the dilution of both isotopes in the equilibrated blood samples. Changes in the concentrations of the isotopes were used to calculate rates of water influx, water efflux and CO_2 production (Lifson and McClintock, 1966; Nagy, 1980; Nagy and Costa, 1980). It was assumed that any changes in body mass and water pools were linear and that mass-specific water pools were stable during the experimental period. Assuming that the metabolic substrate was a mixture of fat and protein (Garnett, 1986), we used a thermal equivalent of 26 kJ L^{-1} CO_2 to convert CO_2 production to units of energy.

The data were grouped into the following four periods: while the animals were active in water, early estivation (after about one month in estivation), mid estivation (after two months), and late estivation (after three months in estivation). Water flux and cloacal fluid electrolyte data were collected at approximately monthly intervals from the same individuals, and these data were analyzed by repeated measures one-way ANOVA (Zar, 1984) using StatView (Abacus Concepts, Inc., Berkeley, CA) statistical package. Measurements of the osmolality of cloacal fluid and plasma, electrolyte composition of plasma, concentrations of nitrogenous wastes, and body temperatures were taken over a larger number of years and individuals, and these data were analyzed by factorial ANOVA. No repeated measurements were included in the factorial ANOVA analyses. Fisher's protected least significant difference was used to separate means (at the 5% level) in the ANOVA analyses.

TABLE 1. The body mass changes and flux rates of *Crocodylus johnstoni*. Data are given for animals active in water and for three times during estivation: early (about 1 month in estivation), mid (about 2 months), and late (about 3 months). Mass changes are expressed as percent of initial mass, which was measured while the animals were active in the water. Mean values are shown with N and SD in parentheses below the means. Means followed by the same letter are not significantly different at the 5% level.

	Active in water	Estivation			Statistic	P
		Early	Mid	Late		
Percent initial body mass (%)	100 (14, —)	96.1 (5, 2.9)	91.9 (5, 2.4)	87.3 (14, 5.1)	—	—
Total body water (% body mass)	76.1[a] (12, 3.1)			75.7[a] (12, 2.8)	$t = 0.5$	0.61
Water influx rate (ml kg^{-1} day^{-1})	39.0[a] (5, 7.1)	24.3[b] (5, 3.6)	16.8[c] (5, 3.4)	7.7[d] (5, 4.0)	$F = 38.3$	<0.0001
Water efflux rate (ml kg^{-1} day^{-1})	40.2[a] (5, 7.6)	25.0[b] (5, 3.9)	17.3[c] (5, 3.4)	9.3[d] (5, 4.3)	$F = 32.4$	<0.0001

RESULTS

Ambient temperatures in the wet-dry tropics of northern Australia are lowest in the dry season (June through August) and highest during the transition between dry and wet (September through November). The body temperatures (T_b) reflect this seasonal pattern with a significant ($P < 0.0001$) increase during the period of estivation. The mean T_bs were as follows: 22.7 C during August ($N = 11$, $SD = 1.1$), 26.3 C during September ($N = 5$, $SD = 0.3$), 25.8 C during October ($N = 10$, $SD = 1.0$), and 28.3 C during November ($N = 34$, $SD = 1.2$). The mean T_bs of September and October were not significantly different from each other at the 5% level, but the August mean was significantly lower and the November mean was significantly higher than September-October.

The mean initial mass of crocodiles used for isotopic studies was 6.5 kg (range = 3.3–10.3 kg). Over approximately 80 d of estivation, the body masses of crocodiles declined to 83.7 ± 5.1% of the initial body masses measured before the animals moved from the water into their refuge (Table 1); a mean daily loss of 0.16% of body mass.

The mean mass-specific body water pool of crocodiles immediately prior to the onset of estivation was 76.1 ± 3.1% of body mass (Table 1). At the end of estivation, the mean mass-specific body water pool was 75.7 ± 2.8%, and there was no significant difference between these two pool estimates (paired t test after arcsine transformation: $t_{11} = 0.52$, $P = 0.61$). Thus, the absolute size of body water pools declined proportionately with body mass and body solids.

Water flux rates, both influx and efflux, changed markedly throughout estivation; they were highest for active animals in water and decreased significantly ($P < 0.0001$) with increasing time in the refuge (Table 1). Water fluxes in late estivation were about 25% of those for active animals in water. Water efflux rates were slightly higher than influx rates in all cases (Table 1), again reflecting the decline in absolute body water pools.

The mean CO_2 production rate during the estivation period from 5 October to 5 November was 0.042 ± 0.014 ml CO_2 g^{-1} h^{-1} ($n = 8$), which is equivalent to an energy expenditure of 26.1 ± 8.5 kJ kg^{-1} day^{-1}.

The osmolality of both cloacal fluid and plasma increased significantly with increasing time in the refuge (Tables 2 and 3). Similarly, the cloacal fluid to plasma (U/P) ratio increased significantly with time in the refuge ($P = 0.0001$: Table 2).

The results of the other chemical analyses of the cloacal fluid are given in Table 2. None of the three forms of nitrogenous wastes measured in cloacal fluid changed significantly over time in estivation. Of the electrolytes analyzed, Mg and K showed a significant increase with time in estivation (Table 2). The other electrolytes analyzed did not change significantly.

The results of the chemical analyses of the plasma are given in Table 3. Of the plasma components analyzed, all of the electrolytes and plasma protein concentrations varied significantly with respect to the periods of estivation.

TABLE 2. Chemical analysis of cloacal fluid from estivating *Crocodylus johnstoni*, analyzed with respect to time in the refugium. Data are given for animals active in water (before estivation) and for three times during estivation: early (about 1 month in estivation), mid (about 2 months), and late (about 3 months). Mean values are shown with N and SD in parentheses below the means. Means followed by the same letter are not significantly different at the 5% level.

	Active in water	Estivation			F	P
		Early	Mid	Late		
Osmolality (mOsm kg^{-1})	100^a (13, 49.2)	157^b (9, 39.7)	224^c (13, 41.1)	226^c (23, 45.8)	26.8	<0.0001
U/P ratio	0.22^a (5, 0.06)	0.51^b (7, 0.14)	0.73^c (9, 0.16)	0.72^c (19, 0.13)	21.5	<0.0001
[K] (mmol L^{-1})	13.4^a (5, 7.5)	10.2^a (5, 2.7)	50.2^b (5, 24.6)	60.2^b (5, 25.6)	14.0	0.0003
[Na] (mmol L^{-1})	25.4^a (5, 21.6)	12.4^a (5, 13.0)	11.5^a (5, 8.7)	12.3^a (5, 11.4)	2.1	0.16
[Ca] (mmol L^{-1})	4.1^a (5, 5.5)	3.0^a (5, 4.8)	2.2^a (5, 2.7)	3.1^a (5, 3.4)	1.8	0.20
[Mg] (mmol L^{-1})	0.3^a (5, 0.4)	0.2^a (5, 0.4)	1.1^b (5, 0.6)	0.9^b (5, 0.6)	4.5	0.02
[Cl] (mmol L^{-1})	30.5^a (5, 28.2)	67.5^a (5, 43.0)	56.8^a (5, 24.7)	27.8^a (5, 10.8)	2.2	0.14
Ammonia (mg mL^{-1})	78.8^a (11, 47.8)	81.7^a (8, 57.6)	96.3^a (13, 37.3)	99.4^a (22, 51.1)	0.6	0.62
Urea (mg mL^{-1})	26.6^a (2, 18.0)	29.7^a (6, 57.2)	7.0^a (9, 20.2)	38.0^a (22, 58.5)	1.0	0.42
Uric acid (mg mL^{-1})	0.06^a (11, 0.05)	0.05^a (9, 0.08)	0.09^a (13, 0.10)	0.12^a (22, 0.12)	1.8	0.16

However, the temporal pattern for the electrolytes in plasma was bimodal rather than a simple linear increase with time. The concentrations of these components of the plasma were relatively high while the animals were in the water, the concentrations were low during early and mid estivation, but they increased late in estivation. Plasma protein concentration followed a similar pattern; however, the concentration late in estivation was significantly higher than other time periods (Table 3).

DISCUSSION

Although the crocodiles showed a net loss of water throughout the estivation period, the body water remained a constant proportion of body mass. The animals had lost only 13% of their initial mass after about 80 d in estivation. In a worst-case scenario, in which we assume that all of the mass loss is due to water loss, the crocodiles could presumably survive a further 80 d based on a vital limit (defined as percentage of the initial mass lost at death) of 27.4% (as in hatchling *C. porosus*; Garnett, 1986), and much longer if the vital limit is 43.5% (as in *C. niloticus*; Cloudsley-Thompson, 1969, cited in Mautz, 1982). Although this represents a crude estimate, it nevertheless suggests that water loss, in the conditions of the observed refuge, is unlikely to result in death during all but the most extreme dry seasons. In addition to water loss, other factors such as electrolyte balance and starvation could limit the amount of time animals could survive estivation (below).

It is difficult to compare the rates of water flux in *C. johnstoni* with previous studies involving isotopic measures of water flux in reptiles because the crocodiles are so much larger than most species studied. However, water fluxes have been measured in the saltwater crocodile *C. porosus* in hyperosmotic conditions (Grigg et al., 1986). A similarly sized *C. porosus* under these estuarine conditions had a water flux of approximately 31 ml kg^{-1} day^{-1} (calculated for a 6.5 kg animal from fig. 1 of Grigg et al., 1986). This value is higher than all the values for *C. johnstoni* except for those from animals in water (Table 1). Two other similarly sized reptiles, *Varanus giganteus* (mean mass: 7.7 kg) and *V. salvator* (mean mass: 7.3 kg) had water flux rates of 22.3 and 54.4 ml kg^{-1} day^{-1} respectively (Green et al., 1986; Dryden et al., 1992). The value for similarly sized *C. johnstoni* late in the third month of estivation was 7.7 ml kg^{-1} day^{-1}. From the limited data available it appears that the inactive crocodiles have considerably lower rates of water flux than active reptiles of similar size.

Because no food was ingested during the estivation period, energy can only have been derived from the catabolism of body tissues. Garnett (1986) found that starving hatchling *C. porosus* catabolized body protein and fat in the ratio of 2.4:1; thus, the catabolism of 1 g of dry body material would provide 24.1 kJ of metabolizable energy, 12.8 kJ from protein (0.71 g × 18 kJ g^{-1}) and 11.3 kJ from fat (0.29 g × 39 kJ g^{-1}). If estivating *C. johnstoni* catabolized body protein and fat in similar proportions, the daily energy expenditure of 26.1 kJ kg^{-1} would require 1.08 g kg^{-1} of substrate. Assuming that 0.50 g of metabolic water is generated for each g of protein catabolized in a uricotele and 1.07 g water accrue from each g fat (Schmidt-Nielsen, 1964), then the catabolism of 1.08 g of substrate would provide 0.71 g of metabolic water; 0.38 g from protein (0.77 g × 0.50 g g^{-1}) and 0.33 g from fat (0.31 g × 1.07 g g^{-1}). This contribution to water influx is only approximately 3% of the total water influx early in estivation and approximately 9% of the total water influx late in estivation. This analysis suggests that the bulk of water flux is due to pulmo-cutaneous exchange of water vapor.

The water flux rates during estivation decrease to approximately 25% of the initial rates (Table 1), despite a rise in body temperatures over the same period. The soil in the underground refuge became progressively drier during the estivation period (pers. obs.), and the drier environmental conditions may, in part, account for the decrease in water flux over the period (Table 1). Other factors that may have contributed to the decrease in water flux include the possibility of a decrease in activity over time inside the refuge, and the possibility of changes in the properties of the skin as occur in some lizards when water is scarce (Mautz, 1982).

The rate of energy expenditure should reveal whether or not metabolic depression is involved, but there are no data for the resting metabolic rate of non-estivating *C. johnstoni*, and most of the metabolic data from other crocodiles have been taken from very small individuals (reviewed by Bennett and Dawson, 1976). There are, however, some measurements of resting *C. porosus* (Grigg, 1978) and *Alligator mississippiensis* (Smith, 1975) of a size range that overlaps the size range of the *C. johnstoni* in this study. The resting metabolism of 11 juvenile *C. porosus* ranging in mass from 0.18 to 6.2 kg was measured over a range of temperatures (Grigg, 1978). At 27 C, the predicted energy expenditure of resting *C. porosus* is approximately 36 kJ kg^{-1} day^{-1} as calculated from fig. 2 of Grigg (1978). Although higher than the 26 kJ kg^{-1} day^{-1} measured for estivating *C. johnstoni*, this is probably not a significant difference. Although the data needed to calculate an error term for the *C. porosus* value were not available, the 95% confidence interval for estivating *C. johnstoni* extend from 19.0 to 33.2 kJ kg^{-1} day^{-1}. Even a small error term for resting *C. porosus* would almost certainly overlap this range. Alligators of a similar size had metabolic expenditures of 26.3 kJ kg^{-1} day^{-1} (calculated from fig. 4 of Smith, 1975), a value very close to that measured for estivating *C. johnstoni*. In another study of *C. porosus* (Garnett, 1988), the CO_2 production

TABLE 3. Chemical analysis of plasma from estivating *Crocodylus johnstoni* as analyzed with respect to time in the refugium. Data are given for animals active in water (before estivation) and for three times during estivation: early (about 1 month in estivation), mid (about 2 months), and late (about 3 months). Mean values are shown with N and SD in parentheses below the means. Means followed by the same letter are not significantly different at the 5% level.

	Active in water	Estivation			F	P
		Early	Mid	Late		
Osmolality (mOsm kg^{-1})	297^a (5, 15.9)	291^a (7, 9.2)	296^a (14, 13.6)	310^b (19, 23.1)	3.2	0.03
[K] (mmol L^{-1})	3.4^a (4, 0.4)	1.3^b (7, 0.6)	1.8^b (9, 1.0)	3.9^a (13, 1.0)	19.3	<0.0001
[Na] (mmol L^{-1})	152.6^a (4, 10.3)	119.6^b (7, 6.3)	132.6^c (9, 16.0)	146.2^a (13, 11.2)	10.3	<0.0001
[Ca] (mmol L^{-1})	3.4^a (4, 0.3)	2.1^b (7, 0.1)	2.3^b (9, 0.3)	3.2^a (13, 0.4)	29.8	<0.0001
[Mg] (mmol L^{-1})	1.4^a (4, 0.2)	0.8^b (7, 0.1)	0.9^{b,c} (9, 0.2)	1.0^c (13, 0.2)	13.4	<0.0001
[Cl] (mmol L^{-1})	229^a (4, 18)	175^b (7, 33)	166^b (9, 16)	218^a (13, 27)	11.2	<0.0001
Urea (mmol L^{-1})	—	1.4^a (6, 1.3)	0.8^a (12, 1.1)	1.1^a (9, 1.4)	0.57	0.57
Total protein (g/100 mL)	6.3^a (12, 1.0)	5.0^b (4, 1.3)	6.2^{a,b} (12, 0.5)	7.2^c (26, 1.2)	7.2	0.0004

rates of juveniles ranging between 257 and 522 g were measured when fed a diet of lean pork (mean = 0.056 ml g^{-1} h^{-1}) and after fasting (mean = 0.040 ml g^{-1} h^{-1}). This indicated a 29% reduction in metabolic rate of the fasting crocodiles, and their CO_2 production rates were very similar to the mean rate for estivating *C. johnstoni* (mean = 0.042 ml g^{-1} h^{-1}). These interspecific comparisons suggests that, although estivating *C. johnstoni* may have a slight reduction in metabolism associated with fasting, there does not appear to be a large depression in metabolism. Reptiles and other ectothermic vertebrates that exhibit metabolic depression during estivation have metabolic rates reduced by 75 to 85% of the standard metabolic rate at the same temperature (Seidel, 1978; Storey and Storey, 1990; Withers, 1993; Kennett and Christian, 1994). Even allowing for differences in standard metabolism between *C. johnstoni*, *C. porosus* and *Alligator*, it is unlikely that *C. johnstoni* employs metabolic depression to the extent found in estivating lungfish (Smith, 1930; Fishman et al., 1988), amphibians (Withers, 1993), or turtles (Seidel, 1978; Kennett and Christian, 1994).

The effects of water deprivation on the nitrogenous waste products of reptiles have been reviewed extensively (Dantzler, 1976; Minnich, 1979, 1982). There were no changes in the nitrogenous products in the cloacal fluid of *C. johnstoni* over the period of estivation (Table 2). The amount of solid urate appeared to increase with increasing time in estivation, but this was not quantified. However, during the last month of estivation we were able to obtain only urate pastes and no cloacal fluid from many crocodiles. Dehydrated desert tortoises *Gopherus agassizi* also had increased urates in the urine (Minnich, 1979). The saltwater crocodile *C. porosus* produces more solid cloacal urine and less liquid when living in salt water as compared to the urine produced by crocodiles in fresh water (Grigg, 1981). Comparing the electrolyte concentrations in cloacal fluid (Table 2) to the data reviewed by Taplin (1988), the Cl and Na concentrations of the Saunders Creek *C. johnstoni* are higher than the values from this species from other sites (3.7–9.6 mmol L^{-1} and 4.4–4.6 mmol L^{-1} respectively). Although the mean values for estivating *C. johnstoni* are within the range of other crocodilians, the Cl concentration is at the high end of that range. The concentration of K in the cloacal fluid of *C. johnstoni* late in the estivation period (Table 2) is more than 10 times greater than that reported for this species from fresh water and is in the high end of the range for other crocodilians (table 6; Taplin, 1988).

The plasmas of reptiles generally have very low concentrations of nitrogenous wastes but, when dehydrated, some reptiles have elevated levels of plasma urea (Minnich, 1982). The saltwater crocodile *C. porosus* exhibits elevated plasma urea in salt water but not in brackish or fresh water (Grigg, 1981). *Crocodylus johnstoni*, however, did not show an increase in plasma urea with increasing time in estivation (Table 3).

Although the responses of specific components in plasma and urine vary among different reptile groups, the osmotic concentrations tend to increase with desiccation (Schmidt-Nielsen and Skadhauge, 1967; Dessauer, 1970; Bentley, 1976; Minnich, 1979, 1982; Grigg et al., 1986). The mean osmolality of *C. johnstoni* cloacal fluid late in the estivation period was 226 mOsm kg^{-1} (Table 2); this was higher than values reported previously from wild-caught individuals of this species from fresh water (175–191 mOsm kg^{-1}; Taplin, 1988), but within the range reported for other crocodilians (Taplin, 1988, table 6). The elevated osmolality of *C. johnstoni* cloacal fluid late in the estivation period is probably due in part to the increased levels of K. These changes are similar to those in the desert tortoise *Gopherus agassizi* after a period of prolonged drought (Minnich, 1979). When acutely exposed to the desiccating conditions of seawater, *C. niloticus* and *C. porosus* that have been reared in fresh water suffer dehydration, an increase in plasma Na and Cl, a marked increase in urine K, but no change in plasma K (Taplin, 1984, 1985; Taplin and Loveridge, 1988). The response of *C. johnstoni* to water deprivation is similar with respect to K in cloacal fluid, but neither Na nor K concentrations in plasma were different between animals in water compared to those late in the estivation period.

The mean plasma osmolality of *C. johnstoni* late in estivation was 310 mOsm kg^{-1} (Table 3), which is higher than values reported from wild caught individuals of this species from fresh water (292–294 mOsm kg^{-1}; Taplin, 1988, table 6) and at the high end of the range reported for other crocodilians (Taplin, 1988). Similarly, the U/P ratio for estivating *C. johnstoni* late in estivation was slightly higher than reported from wild caught individuals from fresh water (0.72 as compared to 0.60–0.65; Taplin, 1988), but is in the range of other crocodilians. The elevated osmolality of *C. johnstoni* plasma with increasing time in estivation is due, at least in part, to an increase in plasma proteins. The physiological significance of the bimodal pattern of plasma electrolytes in *C. johnstoni* is not known. Plasma electrolyte concentrations in *C. porosus* remain constant over a wide range of environmental salinities despite changes in urine electrolyte concentrations (Grigg, 1981; Taplin, 1984). By contrast, desert tortoises increased Na and Cl concentrations in plasma during drought (Minnich, 1979), and *Chelodina rugosa* increased plasma Na concentrations during estivation (Grigg et al., 1986). In comparison to other crocodilians from fresh water (Table 3, Taplin and Loveridge, 1988), the plasma concentrations of Na and K in estivating *C. johnstoni* were similar, but the Cl concentrations in this species were high.

Plasma protein concentrations increase with dehydration in mammals as a mechanism for maintaining plasma volume (Horowitz and Samueloff, 1979; Horowitz, 1984; Zurovsky et al., 1984; Genetzky et al., 1987), and a similar pattern has been described in two species of amphibians (Hillman et al., 1987). Although plasma proteins presumably perform a role in the distribution of water between blood and tissues in reptiles (Khalil and Abdel-Messeih, 1961; Dessauer, 1970, 1974), changes in plasma protein concentrations associated with hydration state have not been as clearly established as in mammals. Some turtles apparently have seasonal cycles of plasma protein concentration unrelated to hydration state (Hutton, 1960; Seidel, 1974). Although seasonal cycles of unknown function cannot be excluded as a possibility, the changes in plasma proteins in *C. johnstoni* more likely function to regulate colloid osmotic pressure in order to maintain plasma volume during prolonged periods of water deprivation.

The presence of lingual salt glands in some species of crocodilians, including *C. johnstoni* (Taplin et al., 1982), has been variously associated with a marine origin of eusuchians (Taplin et al., 1985) and with the conditions that freshwater species may experience during seasonal droughts (Taplin et al., 1982; Mazzotti and Dunson, 1989). Although we did not measure the activity of salt glands, our observations are nevertheless relevant to the significance of salt glands in a freshwater species. We know of no observations of *C. johnstoni* actively expelling the secretions from their salt glands into the air. Presumably the salt glands function in water by the secretions being washed out of the mouth into the surrounding water (Grigg, 1993). It is therefore not surprising that secretion by the salt glands of crocodiles tend to be inhibited when the animals are out of water (Grigg, 1993). In the absence of active expulsion of the secretions, the salts would presumably be reabsorbed by the epithelial lining of the tongue and mouth. Thus, it seems unlikely that salt glands inside the mouth would be of benefit in the conditions of complete drying of the habitat, as observed annually at Saunders Creek over a six year period. Although salt glands may be advantageous in conditions of less severe drought in which the water is concentrated but does not disappear, salt glands which secrete externally (such as lacrimal glands of turtles) or are easily evacuated (such as nasal glands of marine iguanas; Dunson, 1976) would be more advantageous than lingual glands in the event of complete drying of the environment. Without more knowledge of the environmental conditions experienced by crocodiles over their evolutionary past, it is difficult to relate the presence of salt glands to the selective pressures responsible for their evolution. Unfortunately, this would be the case even if the activities of salt glands during estivation were known. However, from a physiological perspective, it would be interesting to monitor the functions of the salt glands from conditions of abundant fresh water, through the period in which the water pool dries, and throughout the period of estivation.

Observations and measurements from recaptured individuals over a six year period did not reveal any long term detrimental effects of regular, and in some cases, annual estivation. The growth rates and body condition of the estivating crocodiles were not markedly different from crocodiles that inhabit the permanent water of the nearby McKinley River region (Kennett and Christian, 1993). Although we cannot discount the possibility that *C. johnstoni* has some specific physiological adaptations to survive estivation, most of the changes observed during estivation in this species probably reflect the general ability of reptiles to survive long periods without access to food or water. The field metabolic rates of estivating crocodiles indicate that, apart from the possibility of a small reduction in metabolism associated with fasting, there is not a large depression in metabolic rate as is found in some estivating ectothermic vertebrates. The decline in water fluxes during the period of estivation may include a behavioral component (decreased activity), and it may also include a physiological change in the properties of the skin that would be advantageous to estivating animals. However, much of the decrease in water flux may be due to the gradual drying of the refuge and the consequent decrease in pulmo-cutaneous exchange of isotopically labeled water vapor. These results indicate that estivation is a viable response to the extreme conditions of the dry season in tropical Australia, and given an adequate refuge, crocodiles can survive many months in estivation.

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Reproductive Biology of the Brahminy Blind Snake (*Ramphotyphlops braminus*) from the Ryukyu Archipelago, Japan

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ABSTRACT.—**Cyclicity and frequency of reproduction, fecundity variation, and correlations between body size and fecundity in the parthenogenetic blind snake *Ramphotyphlops braminus* were studied on the basis of captive observations of 49 individuals and dissection of 98 fresh dead specimens from the Ryukyu Archipelago. Ovulation commenced in mid May and oviposition occurred from mid June to mid July. At all adult snout-vent length's (SVL), the mean number of enlarged ovarian follicles was greater than the mean number of eggs laid. Some follicles may be retained in the ovaries or become atretic during ovulation. Clutch size inferred from the number of enlarged follicles therefore could be overestimated. Relative clutch mass (RCM) was somewhat greater than the mean RCM of henophidian and caenophidian species. In *R. braminus*, clutch size was significantly correlated with SVL, whereas RCM was not, as in most other snakes. This might suggest the presence of particular factors of selection operating on RCM of *R. braminus* as well as other snakes.**

Snake reproduction has been studied by many researchers, and several reviews have attempted to analyze available data for the elucidation of general reproductive patterns in this group of reptiles from ecological and evolutionary view points (see Seigel and Ford, 1987 for review). However, most original works on snake reproduction have dealt with henophidian and caenophidian species; very little attention has been paid to the reproductive biology of the

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