CHAPTER 47

Respiration of *Crocodylus johnstoni* Embryos

Peter J. Whitehead[1]

WITH the possible exception of the earliest stages of development, metabolism in embryonic vertebrates is primarily aerobic (Bartels 1970; Kucera *et al.* 1984). The rate of oxygen uptake ($\dot{V}_{O_2}$) by avian and reptilian eggs provides an accurate indicator of the metabolic activity of embryos and their extra-embryonic membranes. It can be measured simply and non-invasively (Grodzinsky *et al.* 1975; McDonald 1976), and through regular measurements, total oxygen consumed during development (V_{O_2}[tot]) can be estimated. The rate at which eggs excrete carbon dioxide ($\dot{V}_{CO_2}$), and the total carbon dioxide production (V_{CO_2}[tot]), can be measured with similar techniques.

Gas exchange in avian eggs has been much studied. Embryonic metabolic rate and the energetics of development have been correlated with factors such as incubation time, egg mass and the structure of eggshells (Rahn *et al.* 1974, 1979; Hoyt and Rahn 1980; Rahn and Ar 1980; Rahn 1982). In comparison, there have been few comprehensive studies of metabolic rate in reptilian eggs, although data are available for six species of snake (Clark 1953; Dmi'el 1970) and nine species of turtle (Lynn and von Brand 1945; Ackerman 1981a; Thompson 1983; Gettinger *et al.* 1984; Webb *et al.* 1986a).

At a time when hatchling size and post-hatching survivorship and growth of crocodilians are being increasingly correlated with the environment in which eggs are incubated (Webb *et al.* Chapter 50; Joanen *et al.* Chapter 51), our understanding of the energetics of reptilian embryonic development, and of the interaction of gas exchange with development, remains rudimentary.

This chapter examines respiration of *C. johnstoni* eggs. The $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ throughout incubation are described and related to the growth and development of embryos. Gas conductance of egg-shells (see below) is quantified and gas tensions in a sample of natural nests are described. The results are combined to provide an overview of the gaseous conditions under which *C. johnstoni* embryos develop, and the association between gaseous conditions and embryonic growth and survival. Respiration of crocodilian eggs is compared with those of birds and the other reptiles for which there are data.

The measurement of $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ for *C. johnstoni* eggs provides guidance for choice of incubation regimes used to hatch crocodilian eggs for commercial and conservation purposes. When groups of eggs are incubated together, the potential exists for the gaseous environment of incubation to be altered by embryonic consumption of O_2 and excretion of CO_2. Reduced P_{O_2} (or elevated P_{CO_2} slows growth and increases mortality in embryonic marine turtles (Ackerman 1981b), and is likely to have similar effects in other reptiles. It was demonstrated more than 50 years ago that artificial incubation of chicken eggs was most successful when P_{O_2} and P_{CO_2} were contained within narrow limits (e.g. Barott 1937), and similar relationships may well exist with crocodilian eggs.

GAS EXCHANGE ACROSS EGGSHELLS

The recent resurgence of interest in the development of avian embryos depended largely on the emergence of concepts to describe how gases penetrate the eggshell (Wangensteen *et al.* 1969, 1970/71; Wangensteen and Rahn 1970/71; Wangensteen 1972; Rahn *et al.* 1971, 1974; Paganelli *et al.* 1978; Ar *et al.* 1974; Ar and Rahn 1978). Avian embryos are enclosed by an eggshell that comprises a brittle calcified outer mineral layer, and two thin underlying fibrous membranes (Romanoff and Romanoff 1949). Embryos do not possess functional lungs nor any other ventilatory pump able to generate a total pressure difference across the eggshell. Gas exchange occurs by diffusion through pores and spaces that are too small to permit significant convective flow in the absence of a total pressure difference. Diffusion of gases depends instead on their concentrations (partial pressures) being different on either side of the eggshell barrier (Paganelli 1980). The resistance that the eggshell offers to diffusion of a gas (g) is defined in terms of

[1]Conservation Commission of the Northern Territory, P.O. Box 38496, Winnellie, Northern Territory 5789.

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conductance (Gg) — the rate at which gas passes through the eggshell (Vg) under the influence of a given partial pressure gradient ($\triangle$Pg):

(Equation 1) $$Gg = \frac{Vg}{\triangle Pg}$$

Conductances of avian eggshells to respiratory gases (G_{O_2} and G_{CO_2}) and water vapour (G_{H_2O}) represent a compromise between the conflicting needs to limit water loss, and yet permit gas exchange sufficient to maintain oxidative metabolism. Because most birds incubate their eggs in open nests, neither external P_{O_2} nor P_{CO_2} fluctuates greatly nor deviates much from ambient (Walsberg 1980). One or both parents tend the nest throughout incubation, "buffering" embryos against environmental variability, particularly temperature (Drent 1975). The rate of development of the functionally ectothermic embryo, and hence the duration of incubation, are regulated by this parental attention.

The gas conductance characteristics of avian eggshells and shell membranes are matched to predictable incubation environments and embryonic metabolic rates. In combination they enhance the probability of successful development by promoting physiologically optimal gas tensions and water balance at hatching (Wangensteen *et al.* 1969, 1970/71; Wangensteen and Rahn 1970/71; Erasmus *et al.* 1970/71; Rahn *et al.* 1971, 1974, 1979; Rahn and Ar 1974, 1980; Ar and Rahn 1978, 1980; Hoyt and Rahn 1980; Paganelli *et al.* 1978; Paganelli and Rahn 1984; Rahn 1984).

The brittle-shelled eggs of many reptiles, including those of crocodilians, comprise a brittle outer mineral layer of calcium carbonate, closely attached to a thick fibrous organic membrane or layer of membranes (Ferguson 1982, 1985; Ewert 1985). They are thus superficially similar to avian eggs, and avian models have usually been employed to interpret metabolic rate and eggshell gas conductance data derived from reptilian eggs (e.g. Ackerman and Prange 1972; Ackerman 1977, 1981b; Packard *et al.* 1979a; Thompson 1983, 1987). However, reptilian eggs develop in much more variable environments than do avian eggs.

Among reptiles, species vary in their characteristic clutch sizes (from 1-2 eggs to more than 100), and eggs are separated from the atmosphere by nest substrates that may vary in depth, hydration, particle size, organic content, temperature and the presence of other respiring organisms. The gaseous environment of reptile nests can be expected to show considerable interspecific variation. If the eggshell and nest environment are to combine to generate a similar, physiologically tolerable gaseous environment within the egg, then gas conductance characteristics of eggshells should also vary between species. The optimum internal environment may not only be similar in different reptiles, but may be remarkably close to that experienced by embryonic birds (Erasmus *et al.* 1970/71; Ackerman 1977, 1980, 1981b; Seymour and Ackerman 1980; Paganelli and Rahn 1984).

Gas conductances of most avian eggs vary little after the first few days of incubation (Kutchai and Steen 1971; Lomholt 1976). Rates of gas exchange can rise only if partial pressure gradients across the eggshell are elevated (if P_{O_2} falls and P_{CO_2} rises within the eggshell). In contrast G_{O_2} and G_{CO_2} of the brittle-shelled eggs of reptiles may increase for a much larger proportion of the total incubation period (Ferguson 1982, 1985; Thompson 1985). Many brittle-shelled reptilian eggs, including those of crocodilians, develop opaque bands during incubation ("chalking"). The shells change in appearance from translucent at laying to completely opaque during the latter half of the incubation period (Webb *et al.* 1977, 1983, 1986b, Chapter 43; Ewert 1979, 1985; Ferguson 1982, 1985; Thompson 1985), and the opacity may be associated with increased gas conductance (Ferguson 1982, 1985; Thompson 1985). In some reptiles the mineral layer appears to degrade during incubation, which may enhance gas exchange (Feder *et al.* 1982; Ferguson 1982; Woodall 1984). Degradation can occur extrinsically, by the action of micro-organisms in the nesting medium (Ferguson 1982), or intrinsically by the withdrawal of solids from the eggshell (Jenkins 1975). Thus, in reptilian eggs, variation in the structure and function of the eggshell during incubation may moderate the extent of hypoxia and hypercapnia that would otherwise accompany increasing metabolic demands.

METHODS

1. LABORATORY

Egg incubation

Eggs were incubated at constant temperatures between 28 and 33°C (± 0.1°C; SE) in water-jacketed heating cabinets within an air-conditioned room maintained at 18-20°C. Eggs were placed in single layers suspended above water within plastic boxes (Method A of Webb *et al.* Chapter 50), and each box was enclosed within a plastic bag to maintain high humidity. The bags were opened several times daily, and P_{CO_2} and P_{O_2} did not fluctuate markedly (see later).

Oxygen Consumption

Oxygen consumption ($\dot{V}_{O_2}$) was measured in constant pressure double-chambered respirometers using standard techniques (McDonald 1976). Respirometers were immersed in a temperature controlled (± 0.1°C) water bath calibrated against a certified thermometer (Australian Standards Association), and were adjusted to the incubation temperature of the eggs being measured. The CO_2 absorbent

was 15% potassium hydroxide (KOH). Initial measurements demonstrated that respirometers reached thermal equilibrium within 1.25 hours of immersion in water baths, and an equilibration time of 1.5 hours or greater was applied. Chamber stopcocks were open to the atmosphere during equilibration.

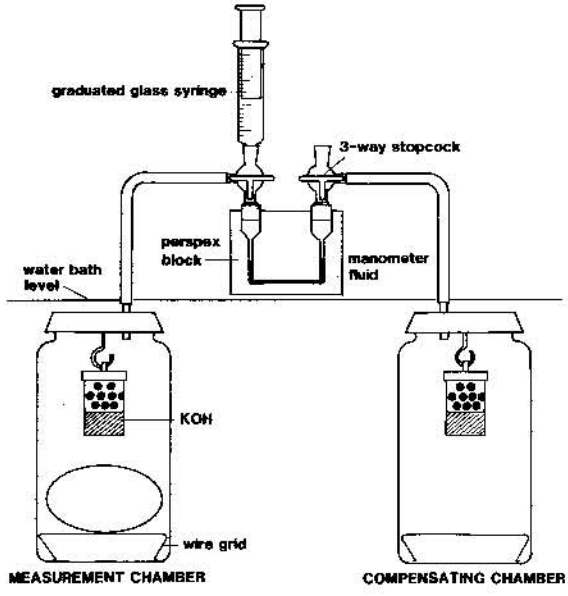

Fig. 1. Constant pressure respirometers used to measure $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ of crocodile eggs. The equipment is immersed in a constant temperature water bath during measurements.

At the commencement of measurements stopcocks of both chambers were closed and fluid in a manometer connecting the measurement and compensating chambers (Fig. 1) was displaced by injection of humidified O_2 from a graduated syringe. Temperature and volume of O_2 injected were recorded together with the time taken for the displaced manometer fluid to return to its original position. Measurements on each egg were repeated until at least three consecutive values (within approximately 5% of each other) were obtained showing no consistent increase or decrease. Results were converted to standard (STPD) values using atmospheric pressure records taken approximately 4 km from the laboratory (Darwin Bureau of Meteorology) and conversion factors provided by Dejours (1981). Measurements were made throughout incubation at 29 and 31°C, and sufficient measurements were made at other temperatures (28, 30, 32 and 33°C) to allow G_{O_2} to be calculated for eggs incubated at these temperatures (see below).

$\dot{V}_{O_2}$ of hatchlings from eggs incubated at 31°C (N=11) was measured similarly. Observations were discarded if hatchling movement was observed in the respirometers in the 30 min preceding, or during measurements. Between observations hatchlings were unfed and maintained in the dark at 31°C. Hatchlings were sacrificed progressively over a period of 27 days and yolk-free hatchling mass and residual yolk mass were determined.

After about 60% of incubation embryo size increases with increasing egg size, and hatchlings from large eggs have a significantly higher yolk-free mass (Webb *et al.* Chapter 50). Thus the potential exists for $\dot{V}_{O_2}$ to be increasingly affected by egg mass as incubation proceeds. To derive predictive equations that took account of this effect, $\dot{V}_{O_2}$ data were normalized by dividing by the fresh mass of the egg from which the observation was taken.

Carbon Dioxide Production

Rates of CO_2 excretion were determined by consecutive measurements on the same egg with and without CO_2 absorbent present in the respirometry chambers. $\dot{V}_{CO_2}$ was calculated as the difference between $\dot{V}_{O_2}$ and the rate of volume change without absorbent (McDonald 1976). All pairs of measurements were made within 2.5 hours of each other.

Respiratory Exchange Ratio

The respiratory exchange ratio (RE) was calculated for individual eggs by the equation:

(Equation 2) $$RE = \frac{\dot{V}_{CO_2}}{\dot{V}_{O_2}}$$

Gas Conductance

Samples

Eggs that had lost 1.5 g or more during incubation developed air spaces large enough to permit sampling. Samples of gas from these spaces were obtained from eggs being sacrificed for other studies (28 to 32°C; from 40 to 93% of incubation). Sometimes these eggs came directly from the incubators and at other times they were sacrificed immediately after $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ measurements in the respirometers.

In some eggs the air space was clearly apparent between the mineral layer and shell membrane, and samples were taken from this area. In many eggs spaces beneath the shell membrane were not apparent from external examination. These eggs were "floated" in slightly acidified water and a small hole was made in the mineral layer with sharp scissors prior to passing a syringe needle through the shell, and (if necessary) through the underlying shell membrane. Samples were drawn into greased glass syringes with the egg held beneath the surface of acidified water, as described by Wangensteen and Rahn (1970/71). All samples were stored under water in the glass syringes until analysis. The O_2 and CO_2 fractions of the gas samples were determined using a Scholander gas analyser and standard techniques (Scholander 1947). Gas tensions (P_{O_2} and P_{CO_2}) were calculated assuming that samples were saturated with water vapour at the time of sampling.

Mass lost (WL in g) from eggs between times of collection and sacrifice was regarded as water loss (Rahn 1984). An average daily rate of water loss (DWL in g d^{-1}) was calculated by dividing the total loss by the age of the egg when sacrificed.

Gas samples taken from incubator bags through permanently sited fine polyethylene tubes indicated that P_{O_2} was 144 ± 5.7 torr (SD; N = 6) and the mean P_{CO_2} was 7 ± 4.5 torr (SD; N = 6). These mean values were used as ambient gas tensions for eggs sampled directly from incubators. Samples were also taken from respirometers by introducing fine tubes through the stopcock bores. P_{O_2} adjacent to the eggshells of eggs in respirometers in which KOH was present could be predicted from the formula:

(Equation 3)

$$P_{O_2} = 154.5 - 2.08\dot{V}_{O_2}$$

$$[r^2 = 0.551, F_{1,9} = 11.0, p<0.05, SEE = 2.9]$$

This equation was used to calculate ambient P_{O_2} for eggs which came from respirometers prior to sampling of air space gases, when respirometer P_{O_2} had not been measured directly. P_{CO_2} near the outer surface of eggshells did not vary significantly with egg $\dot{V}_{CO_2}$ ($r^2 = 0.340$, $p>0.05$). Mean P_{CO_2} was 3 ± 0.8 torr (SD; N = 11).

The surface area of eggs (A) was calculated from measurements of length and breadth using the formula for a prolate spheroid (see Ackerman *et al.* 1985).

Calculation of Gas Conductance

Gas conductance of the eggshell was calculated as defined by Ar *et al.* (1974) and Paganelli (1980).

(Equation 4) $$G_{O_2} = \frac{\dot{V}_{O_2}}{\Delta P_{O_2}}$$; and

(Equation 5) $$G_{CO_2} = \frac{\dot{V}_{CO_2}}{\Delta P_{CO_2}}$$

where $\dot{V}_{O_2}$ = rate of O_2 uptake by the egg;

$\dot{V}_{CO_2}$ = rate of CO_2 excretion from the egg;

ΔP_{O_2} = partial pressure gradient across the eggshell [P_{O_2} (ambient) — P_{O_2} (egg air space)];

ΔP_{CO_2} = partial pressure gradient across the shell [P_{CO_2} (ambient) — P_{CO_2} (egg air space)].

$\dot{V}_{O_2}$ was measured immediately before sampling (N = 10) or calculated (N = 20) from equations relating $\dot{V}_{O_2}$ to percentage incubation time (I) and temperature (Whitehead 1987). Ambient gas tensions were sometimes estimated from means or regression equations. In all cases P_{O_2} within eggs was significantly lower than the estimated ambient P_{O_2} (1-tailed t-test, $p<0.05$) and G_{O_2} was therefore calculated for all eggs. In five eggs P_{CO_2} was not significantly greater than the estimated ambient P_{CO_2} (1-tailed t-test, $p>0.05$), and hence calculations of ΔP_{CO_2} and G_{CO_2} were not attempted. Conductances measured in eggs at temperatures other than 30°C were converted to 30°C equivalents as described by Paganelli *et al.* (1978).

2. FIELD

Gas Tensions in Natural Nests

This study was carried out in conjunction with Anthony Smith's broader study of *C. johnstoni* nesting, and some of the data discussed here were kindly supplied by him.

Nesting areas were searched by probing substrates with a 5 mm diameter stainless steel rod during the brief nesting period (Webb *et al.* 1983). As soon as possible after location, nests were excavated to the level of the top one or two eggs, and the following details recorded:

1. *Initial nest temperature.* Temperature was taken at the level of the top egg, using a calibrated mercury thermometer (± 0.1°C). Temperatures were corrected to a 12 noon equivalent using data on diurnal trends in nest temperatures (A. Smith, unpublished data).
2. *Depth to the top egg* (± 1.0 cm).
3. *Substrate type.* Assigned to one of five categories: coarse sand and gravel; medium grain sand; fine sand; fine sand and humus; and, clay and humus.
4. *Extent of opaque band development* and presence/absence of mucous (to estimate time of laying; Webb *et al.* 1983).
5. *Egg mass.* Because variation in the mass of eggs within a clutch is small compared with variation between clutches, the mass of one egg provides a satisfactory estimate of mean egg mass (G. Webb, unpublished data).

A copper/constantan thermocouple was situated among the eggs together with a gas sampling tube of fine polyethylene (external diameter 1.0 mm, wall thickness approximately 0.2 mm). The buried end of each tube was covered by a small square of loosely woven cheese-cloth, and the exposed end, fitted with a 24 gauge syringe needle, was plugged with a plastic golf tee between sampling. Thermocouples and gas sampling tubes were usually placed beneath the level of the top egg, but were not necessarily centrally situated in each clutch.

Efforts were made to protect nests from predation (chiefly by *Varanus gouldii*) by staking small squares (40 × 40 cm) of light wire netting (1.5 cm mesh) over the nest opening.

At the completion of incubation additional information was recorded from each nest.

1. *Clutch size.*
2. *Number of potentially viable eggs.* Eggs were classified as infertile if there was no development of the opaque band when other eggs showed banding and no sign of an embryo. If banding had begun but not completed and/or if a dead embryo was present they were classified as dead. The number of potentially viable eggs was calculated by subtraction.
3. *Mass of unpipped eggs* (± 0.1 g).

An estimate of egg mass lost (or gained) during incubation was made for each clutch, by subtracting the mass of the egg(s) measured at initial excavation from the mean mass of unpipped eggs in that clutch at the completion of incubation. The change was expressed as a percentage of initial egg mass, and this figure converted to an index of water exchange (IWE) for each clutch by multiplying by the mean initial egg mass (mean over all clutches). When hatching was complete the mass of hatchlings and of their enclosed yolks were measured (0.01 g) following sacrifice by an overdose of "Nembutal".

For controls, holes were excavated in substrate of similar appearance between 40 and 200 cm from natural nests, and they were fitted with thermocouples and gas sampling tubes at depths similar to those in the nests. Many of the controls placed early in the incubation period were destroyed by nesting crocodiles. Because the remaining controls were not disturbed, they may have been sited in substrates that were unattractive to nesting crocodiles, and hence may have differed from nest sites.

Gas samples of 1.0-1.5 ml were taken from nests and controls in 2 ml greased glass syringes fitted with 3-way Luer-lock stopcocks, after a volume of gas at least three times the estimated syringe and sampling tube "dead space" had been withdrawn and expelled. Nest (or control) temperature was recorded when each sample was taken.

Delays of up to 18 hours occurred between collection and analysis of gas samples, but they did not appear to affect results. In a series of duplicate samples (N = 7) examined as soon as practical after sampling and after a further delay, P_{O_2} difference was 0.5 ± 1.6 torr (SD; N = 7) and P_{CO_2} difference was 0.4 ± 1.4 torr (SD; N = 7); variation between duplicates did not increase with time between analyses (range 0.25 to 14.0 h; P_{O_2}, $r^2 = 0.024$, $p>0.50$; P_{CO_2}, $r^2 = 0.05$, $p>0.50$).

Gas tension observations (P_{O_2} and P_{CO_2}) for each nest were assigned to one of nine incubation intervals based on the percentage of the total nest incubation period completed at the time of sampling: 0-20%; 21-40%; 41-50%; 51-60%; 61-70%; 71-80%; 81-90%; 91-99%; and, 100% (hatching). When more than one observation was available for an individual nest during a given period, the mean was used.

Statistics and Terms

Statistical analyses follow Zar (1984) and SPSS Inc. (1983). Significance level was regarded as 0.05 throughout. Terms, symbols and units relating to gas exchange follow Dejours (1981). The term eggshell is used to refer to the combined mineral layer-shell membrane unit (Fig. 4). The mineral layer is the brittle, calcified, outermost layer, and the shell membrane the fibrous layer beneath.

RESULTS

Oxygen Consumption of Eggs

Rate of Oxygen Consumption

The pattern of change in $\dot{V}_{O_2}$ with time (I; percentage of total incubation period) is similar at 29°C and 31°C (Figs 2 and 3). $\dot{V}_{O_2}$ increases exponentially to about 60% incubation when the relative rate of increase begins to decline. A peak is reached at approximately 89% incubation and is followed by an absolute decline to the time of hatching. A similar pattern occurs in *C. porosus* at 30°C (unpublished data).

Equations to predict $\dot{V}_{O_2}$ from incubation time were derived by iterative least squares regression on data linearized by polynomial, logarithmic and logistic transformations. The equations generating the highest F values were chosen. Only polynomial transformation provided adequate models of the whole incubation pattern.

29°C incubation:

(Equation 6)

$$\dot{V}_{O_2} = -5.17 + 0.862I - 0.0149(I^2) - 5.111\times10^{-4}(I^3) + 2.0192\times10^{-5}(I^4) - 1.3755\times10^{-7}(I^5)$$

$[r^2 = 0.972, F_{5,186} = 6030.6, p<0.0001,$ SEE = 5.84]

31°C incubation:

(Equation 7)

$$\dot{V}_{O_2} = -36.52 + 4.486(I) - 0.182(I^2) + 3.164\times10^{-3}(I^3) - 1.6761\times10^{-5}(I^4)$$

$[r^2 = 0.963, F_{4,116} = 3034.3, p<0.0001,$ SEE = 6.97]

where $\dot{V}_{O_2}$ is in $\mu l\ h^{-1}\ g^{-1}_{stpd}$

Limited observations at other temperatures showed the same general trends (Whitehead 1987). The principal effect of temperature on the ontogeny of embryonic metabolic rate is to extend the time over which changes occur, without altering the pattern. Mean length of incubation at 29°C was 100.9 ± 1.8 days (SD; N = 8) and at 31°C was 81.6 ± 2.2 days (SD; N = 12).

Maximum and Pre-hatch Oxygen Consumption

The maximum mass-specific rates of O_2 consumption by *C. johnstoni* eggs are within the range reported for other non-squamate reptiles (Table 1).

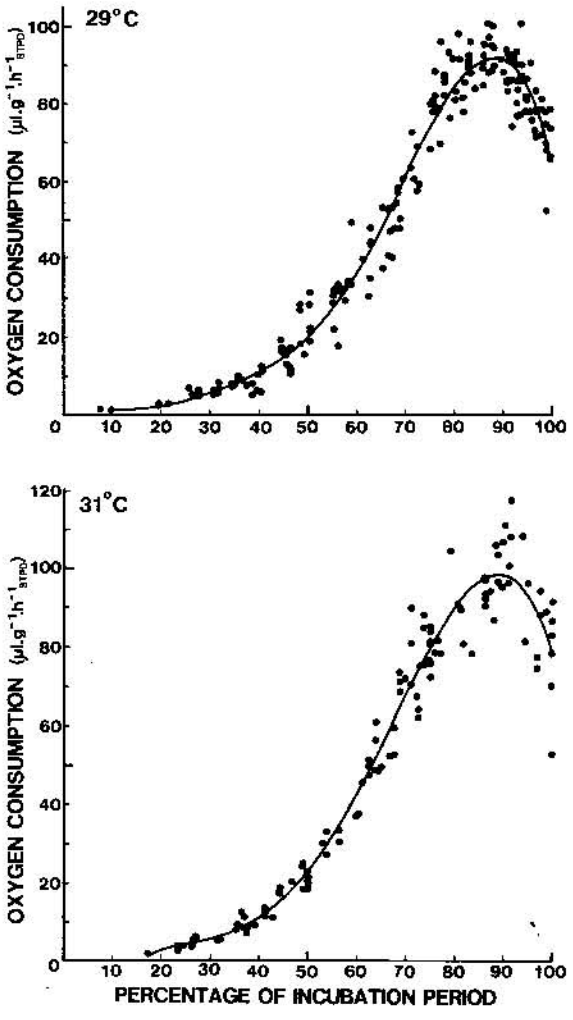

Fig. 2. Oxygen consumption of *C. johnstoni* eggs incubated at 29 and 31°C. Data were normalized by dividing $\dot{V}_{O_2}$ (ml h^{-1}_{stpd}) by the fresh mass of the egg from which the measurement was taken, to give a mass-specific $\dot{V}_{O_2}$ (μl h^{-1} g^{-1}_{stpd}).

Total Oxygen Consumption

Total oxygen consumption (V_{O_2}[tot]) was calculated by integration of the predictive equations. $\dot{V}_{O_2}$ was immeasurably low early in incubation and the

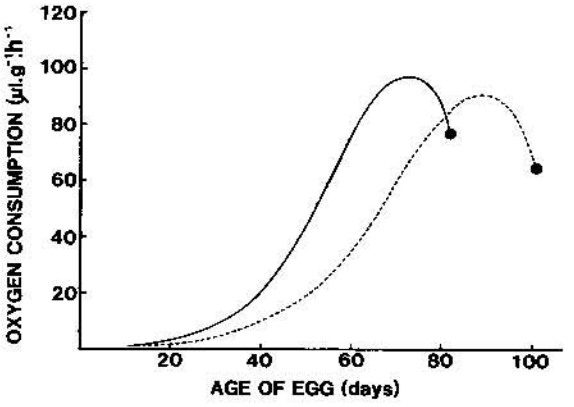

Fig. 3. Curves relating $\dot{V}_{O_2}$ to days of incubation for *C. johnstoni* eggs incubated at 29 and 31°C. The pattern is similar at both temperatures, but incubation time is extended at the lower temperature.

polynomial equations generate unrealistic predictions in this range (0-20% incubation). An estimate was derived from logistic equations fitted to the data up to the peak of $\dot{V}_{O_2}$ (Whitehead 1987). (The contribution to total estimates is minor and potential errors less than 10 ml_{stpd}).

The longer incubation period at 29°C is associated with increased V_{O_2}[tot]; 75.9 ml g^{-1}_{stpd} at 31°C and 85.6 ml g^{-1}_{stpd} at 29°C, or 5675 and 6402 ml_{stpd} respectively for an egg of the mean mass used in this study (74.81 g). The mass-specific V_{O_2}[tot] of crocodilian eggs appears to lie at the upper end of V_{O_2}[tot] for other non-squamate reptiles (Table 1), but is lower than the mean of 102 ml g^{-1}_{stpd} reported for 34 species of birds (Hoyt and Rahn 1980).

To determine whether the temperature dependent differences in V_{O_2}[tot] were significant, a random sample (N = 5) of 20 $\dot{V}_{O_2}$ observations from individual eggs was plotted against incubation time for each incubation temperature. Points were joined by straight lines and the area under each plot was estimated by counting squares. The mean index at 29°C [6850 ± 148 (SD)] and at 31°C [5841 ± 212 (SD)] differ significantly ($t = 8.72$, $df = 9$, $p<0.001$). V_{O_2}[tot] of eggs incubated at 29°C is greater than

Table 1. Maximum and pre-hatch $\dot{V}_{O_2}$ and whole incubation O_2 consumption (Total) of non-squamate reptile eggs. Pre-hatch and maximum rates are the same in the marine turtles that have a sigmoid pattern of $\dot{V}_{O_2}$. Mass specific rates are given in μl h^{-1} g^{-1}_{stpd} and absolute rates (in parentheses) in ml h^{-1}_{stpd}. Whole incubation consumption is in ml_{stpd} and (ml g^{-1}_{stpd}). Data are from: 1. Whitehead 1987; 2. Webb *et al.* 1986a; 3. Thompson 1983; 4. Ackerman 1981b.

Species	Egg mass	Maximum $\dot{V}_{O_2}$		Pre-hatch $\dot{V}_{O_2}$		Total $\dot{V}_{O_2}$		Temp (°C)
Crocodylus johnstoni	74.8	92.3	(6.90)	65.0	(4.86)	6402	(85.6)	29
	74.8	98.7	(7.38)	78.3	(5.86)	5675	(75.9)	31
Crocodylus porosus[1]	112.1	112.9	(12.66)	81.3	(9.12)	10,442	(93.1)	30
Carettochelys insculpta[2]	33.7	81.3	(2.74)	34.1	(1.15)	2816	(83.6)	30
Emydura macquarii[3]	10.4	123.1	(1.28)	86.5	(0.90)	580	(55.6)	30
Chelonia mydas[4]	61.6	92.6	(5.71)	92.6	(5.71)	2739	(44.4)	30
Chelonia mydas[4]	48.2	78.3	(3.76)	78.3	(3.76)	3142	(65.2)	30
Caretta caretta[4]	42.6	105.7	(4.50)	105.7	(4.50)	1939	(45.5)	30

that of eggs incubated at 31°C. However, the volume of O_2 consumed to produce a unit mass of hatchling (wet and yolk-free) varied little between incubation temperatures, being 154 ml g^{-1}_{stpd} at 29°C and 170 ml g^{-1}_{stpd} at 31°C (Whitehead 1987).

Carbon Dioxide Production and Respiratory Exchange Ratio (RE)

RE did not differ significantly between eggs incubated at 29°C [0.69 ± 0.03 (SD; N = 12) and 31°C 0.66 ± 0.03 (SD; N = 25)] eggs (t = 1.92, df =36, p = 0.063). The mean for pooled data was 0.67 ± 0.03 (N = 37). *Crocodylus johnstoni* eggs excrete CO_2 at 0.67 times the rate at which they consume O_2.

Oxygen Consumption of Pipped Eggs

Data on the $\dot{V}_{O_2}$ of pipped eggs are summarized in Table 2. The post-peak decline in $\dot{V}_{O_2}$ continued to the time of pipping. In two cases where pipping occurred during $\dot{V}_{O_2}$ measurements, and activity was monitored, $\dot{V}_{O_2}$ remained low and in fact appeared to decline as the embryos attempted to penetrate the shell and membrane. When $\dot{V}_{O_2}$ was measured on the same eggs (i) during the two days prior to pipping (ii) at pipping and (iii) in the day following hatching, differences were not significant (paired t-tests: Pre-pipping-Pipping, t = 0.182, df = 5, p>0.50; Pipping-Hatching, t = 0.03, df = 3, p>0.50).

Table 2. Oxygen consumption ($\dot{V}_{O_2}$) in ml h^{-1}_{stpd} of eggs before pipping, when pipped, and of hatchlings within one day of hatching.

Egg No.	Prepipping	Pipped	Hatchling
1	5.14	4.98	4.34
2	3.88	5.46	6.61
3	6.37	6.10	–
4	6.29	5.57	–
5	6.97	5.28	–
6	–	5.90	6.71
7	7.48	7.57	6.99
8	–	4.00	–
Mean	6.02	5.61	6.16
SD	1.31	1.02	1.23

Thus pipping and the initiation of pulmonary respiration do not appear to be associated with a major discontinuity in metabolic patterns.

Oxygen Consumption of Hatchlings

Hatchling $\dot{V}_{O_2}$ at 31°C [mean weight at hatching including residual yolk = 50.4 ± 2.9 g (SD); range 44.8-54.6; N = 11] declined significantly in the 27 days over which measurements were taken. The equation relating $\dot{V}_{O_2}$ (ml h^{-1}_{stpd}) and hatchling age (D_h) in days is:

(Equation 8)

$$\dot{V}_{O_2} = 6.48 - 0.075D_h$$

$$[r^2 = 0.606, F_{1,19} = 31.7, p<0.001, SEE = 0.53]$$

The regression was not significantly improved by including hatchling total mass or yolk-free hatchling mass (maximum addition to r^2 = 0.008, p>0.50). Regression of $\dot{V}_{O_2}$ on hatchling mass revealed no significant relationship using either log-transformed or untransformed data (p>0.50). The decline in $\dot{V}_{O_2}$ was not due to starvation as each hatchling retained some residual yolk (>0.5 g) when sacrificed.

Gas Conductance of Eggshells

Air Spaces

Three types of air space were found in *C. johnstoni* eggs (Fig. 4): *Sub-shell spaces* formed between the mineral layer and shell membrane; *sub-membrane spaces* formed between the chorioallantois and the inner surface of the shell membrane; and, in one egg that had lost a large amount of water (15.09 g), an air space formed beneath the chorioallantois. Air spaces did not form between layers of the shell membrane. Both sub-shell and sub-membrane spaces have been reported in viable chelonian eggs (Ewert 1985).

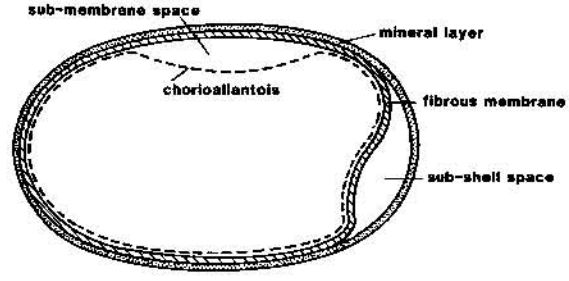

Fig. 4. Air spaces found in artificially incubated *C. johnstoni* eggs. Sub-shell spaces occur between the mineral layer and the deformable shell membrane. Sub-membrane spaces lie between the shell membrane and the chorioallantois. Only one air space was found beneath the chorioallantois.

Gas samples were taken from each type of space [sub- shell (N = 3); sub-membrane (N = 26); and, sub-chorioallantoic (N = 1)] but the sampling was non-random and does not reflect the relative frequency of the different types of spaces (see Manolis *et al.* Chapter 46). Sub-shell spaces were the most common but were often associated with cracks or dents in the mineral layer, and gas samples were not extracted. Unless otherwise indicated, the analyses below relate to sub-membrane spaces.

Gas Tensions in Air Spaces

Oxygen (P_{O_2})

Oxygen tensions fell during incubation (Fig. 5). Up to about 65% incubation, P_{O_2} was relatively stable at a mean of 121 ± 8.4 torr (SD; N = 13), but after 65% incubation P_{O_2} fell significantly to levels as low as 53 torr. Rate of water loss did not contribute significantly to explained variance, and the linear regression equation of best fit after 65% incubation was:

(Equation 9)

$$P_{O_2} = 222 - 1.66(1)$$

$$[r^2 = 0.322, F_{1,11} = 5.2, p = 0.043, SEE = 20.9]$$

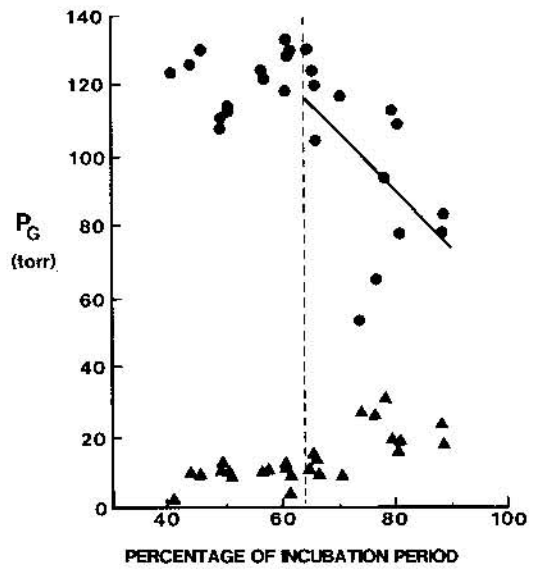

Fig. 5. Oxygen (circles) and CO_2 (triangles) tensions in spaces between the shell membrane and chorioallantois of *C. johnstoni* eggs. The dotted line indicates the time at which opaque band expansion was complete. The solid line is the regression line relating P_{O_2} to percentage incubation time after the egg is completely opaque.

The stability of P_{O_2} up to 65% incubation was maintained despite a 7-fold increase in $\dot{V}_{O_2}$ from about 0.5 to 3.5 ml h^{-1}_{stpd} (Fig. 2). In fact P_{O_2} appeared to rise slightly with increasing $\dot{V}_{O_2}$ during this stage of incubation, which is consistent with Thompson's (1985) observation that rising eggshell conductance anticipates the increasing metabolic demands of the embryo. After 65% incubation P_{O_2} fell sharply under the influence of rising $\dot{V}_{O_2}$:

(Equation 10)

$$P_{O_2} = 156 - 10.37\dot{V}_{O_2}$$
$$[r^2 = 0.511, F_{1,11} = 11.5, p = 0.006, SEE = 17.8]$$

The change from relatively constant P_{O_2} to a substantial decline coincided with the completion of chorioallantoic expansion (Webb *et al.* Chapter 43).

Carbon Dioxide (P_{CO_2})

As expected, the general pattern of change in P_{CO_2} was the inverse of that in p_{O_2} (Fig. 5). A period of relative stability was followed by a period of increase and greater variability. Over the whole of the period for which data were available the rise was significant (r^2 =0.500, $F_{1,24}$ = 26.0, p<0.001), and the rate of water loss from the egg accounted for a significant proportion of variance in P_{CO_2} (r^2 addition = 0.108, F change = 6.3, p = 0.019):

(Equation 11)

$$P_{CO_2} = -4.4 + 0.35(I) - 75.5DWL$$
$$[r^2 = 0.595, F_{2,23} = 19.4, p<0.001, SEE = 4.5]$$

Before 65% incubation p_{CO_2} changed little with incubation time (r^2 = 0.067, $F_{1,11}$ = 0.79, p = 0.393) and had a mean value of 9.3 ± 2.9 torr (SD; N= 13). Stable P_{CO_2} was maintained despite a very significant increase in $\dot{V}_{CO_2}$ from about 0.3 to 2.3 ml h^{-1}_{stpd}. After 65% incubation, P_{CO_2} rose with incubation time (I), but the trend was just outside the 5% significance level (r^2 = 0.278, p = 0.060). The mean P_{CO_2} was 18.2 ± 7.1 torr (SD; N = 13; range 9.1 to 30.8) or double that found up to 65% incubation.

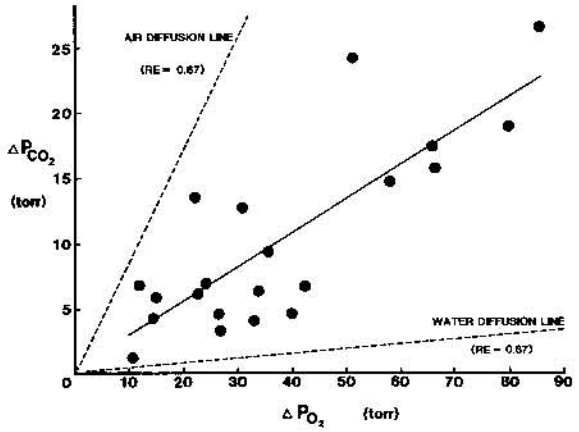

Fig. 6. The relationship between P_{O_2} and P_{CO_2} gradients across the eggshell of *C. johnstoni* eggs. The solid line represents the regression equation of best fit, while the dotted lines indicate the expected position of the line if the eggshell was completely dry (upper) or if a continuous film of water was present (lower). RE = respiratory exchange ratio (see text).

Relationship Between P_{O_2} and P_{CO_2}

P_{CO_2} within an egg rises as O_2 is consumed and CO_2 is produced by oxidative metabolism. The extent of the increase in P_{CO_2} relative to the decline in P_{O_2} depends on the respiratory exchange ratio (RE) (Rahn *et al.* 1971), characteristics of the eggshell barrier through which these gases move, and also on the partial pressures of the gases outside the egg. The relationship of P_{O_2} gradients to P_{CO_2} gradients (ΔP_{O_2} and ΔP_{CO_2} respectively) between the air spaces within eggs and their incubation environment, is summarized in Figure 6. There was a significant rise in ΔP_{CO_2} with increasing ΔP_{O_2}:

(Equation 12)

$$\Delta P_{CO_2} = 0.6 + 0.25\ \Delta P_{O_2}$$
$$[r^2 = 0.642, F_{1,18} = 32.3, p<0.001, SEE = 4.3]$$

The intercept (0.6) did not differ significantly from zero (t = 0.32, df = 18, p = 0.751). At an RE of 0.67, CO_2 is excreted from the egg at 0.67 times the rate that O_2 enters it. The ratio of the rates of diffusion of CO_2 and O_2 in air at 30°C is 0.78 (Paganelli *et al.* 1978). Thus if partial pressure gradients are determined by diffusion of both O_2 and CO_2 through gas-filled pores in the mineral layer and gas-filled spaces in the shell membrane, then gradients should exist in the ratio 0.67/0.78 = 0.86. The slope of the regression line (0.25; Equation 12) relating elevation of P_{CO_2} to depression of P_{O_2} is significantly lower than 0.86 (t = 13.87, df = 24, p<0.001).

Because CO_2 is much more soluble in water than is O_2, it can enter, attain greater concentrations, and diffuse through an aqueous barrier much more rapidly (Dejours 1981). If these gases were exchanging between the egg and its incubation environment through a continuous aqueous layer, then the ratio of partial pressure gradients fixed by their rates of diffusion in air (0.86) would be modified according to their relative solubilities in water (Dejours 1981). That is, the ratio is expected to be:

$$0.86 \times \frac{\beta_{O_2}}{\beta_{CO_2}} = \frac{0.86 \times 1.544}{39.238} = 0.034$$

where β_{O_2} = solubility coefficient of O_2 in water at 30°C

β_{CO_2} = solubility coefficient of CO_2 in water at 30°C

and values are taken from Dejours (1981).

Thus P_{CO_2} should never be more than a few torr, regardless of the extent of the depression of P_{O_2}. The observed ratio (0.25) also differed significantly from 0.034 ($t = 4.91$, $df = 24$, $P<0.001$). The observed slope fell between that expected from a water-filled diffusion pathway and an entirely dry gas-filled one. It suggests that diffusion pathways were partially hydrated. Variations in ΔP_{CO_2} relative to ΔP_{O_2} were likely to have been related to changes in the water content of the eggshell.

Sub-shell Spaces

The mean sub-shell P_{O_2} was 145 ± 4.7 torr (SD; N = 3) and P_{CO_2} was 7 ± 8.4 torr (SD; N = 3). As these gas tensions were indistinguishable from ambient tensions it was not possible to calculate gas conductance of the mineral layer. In one egg samples were obtained from both sub-shell and sub-membrane spaces: the sub-shell P_{O_2} was indistinguishable from ambient P_{O_2} (149 torr) while the sub-membrane P_{O_2} was 107 torr.

Sub-chorioallantoic Space

The single sample gave a P_{O_2} of 119 torr and a P_{CO_2} of 4 torr.

Variation in Gas Conductance of Eggshells

Oxygen Conductance (G_{O_2})

G_{O_2} of the eggshell rose with increasing incubation time (Fig. 7a), the increase being most significant during the first 65% of the incubation period (Equation 13). Entry of daily water loss did not significantly improve explained variance (addition to $r^2 = 0.124$, $p = 0.077$).

(Equation 13)

$$G_{O_2} = -10.89 + 0.25(1)$$

$[r^2 = 0.558, F_{1,10} = 13.90, p = 0.003, SEE = 1.70]$

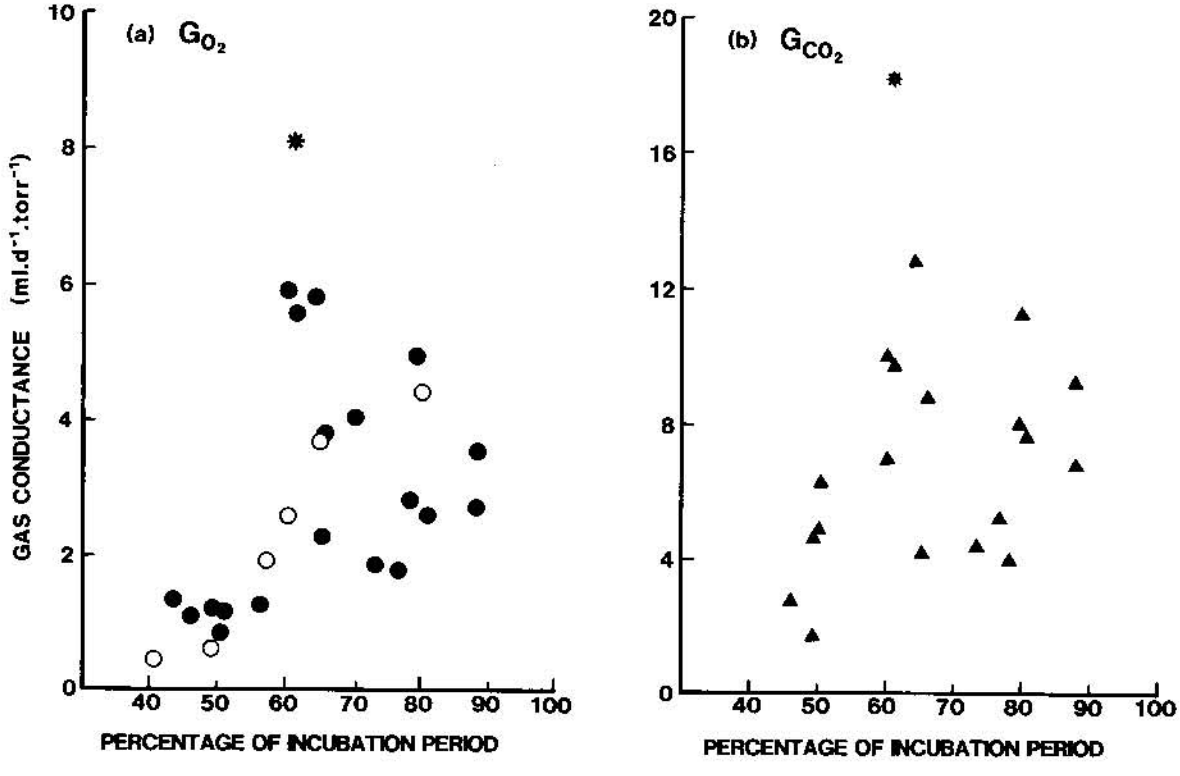

Fig. 7. Variation in O_2 (a) and CO_2 (b) conductances of the eggshells of *C. johnstoni* eggs with incubation time. The asterisks indicate observations from a particularly porous egg that lost unusually large quantities of water. This observation was excluded from the regression analyses. The open circles are G_{O_2} observations from eggs of the same clutch.

After 65% incubation, mean G_{O_2} was 3.41 ± 1.20 ml d^{-1} $torr^{-1}$ (SD; N = 13), and it did not vary significantly with percentage of incubation time (I) (r^2 = 0.051, $F_{1,11}$ = 0.590, p = 0.459), although it did vary with increasing duration of incubation in days (D):

(Equation 14)

$$G_{O_2} = -3.13 + 0.092D$$
$$[r^2 = 0.470, F_{1,11} = 9.8, p = 0.010, SEE = 0.91]$$

Thus the longer incubation period associated with lower incubation temperatures results in eggs having a higher G_{O_2} after completing a similar proportion of the incubation period.

Carbon Dioxide Conductance (G_{CO_2})

G_{CO_2} also rises significantly as incubation progresses (Fig. 7b), especially while the opaque band is expanding. Up to 65% incubation it could be predicted from:

(Equation 15)

$$G_{CO_2} = -26.7 + 0.62(I)$$
$$[r^2 = 0.626, F_{1,7} = 11.7, p = 0.011, SEE = 3.26]$$

After 65% incubation G_{CO_2} was highly variable but was not related to percentage of the incubation period completed (r^2 = 0.001, $F_{1,9}$ = 0.011, p = 0.917) nor the variables DWL, or D (p>0.25 in each case).

Surface Area of Eggshells

Variation in G_{O_2} appeared to be unrelated to the surface area of eggshells, whether considered over the whole of the period for which data were available (r_2 = 0.014, $F_{1,24}$ = 0.342, p = 0.564), during the period of opaque band expansion (r^2 = 0.055, $F_{1,11}$ = 0.643, p = 0.440), or after the egg-shell was entirely opaque (r^2 = 0.007, $F_{1,11}$ = 0.074, p = 0.790). Similar results were obtained with G_{CO_2}.

Gaseous Environment of Nests

Physical Characteristics of Nests

Nests were found in friable substrate within 100 m of permanent water. There was substantial variation in the texture of the substrate, which ranged from very coarse sand or gravel to fine talc-like sand or clay.

Depth from the substrate surface to the level of the top egg varied from 0 to 36 cm. Eggs were partly exposed in one extremely shallow nest on a clay bank. Excluding this nest, mean depth was 16.6 ± 5.7 cm (SD; N = 36). Nest depth varied between substrates, with nests tending to be shallower in the fine substrates (ANOVA, $F_{4,30}$ = 2.74, p = 0.046).

Nest Temperatures

The mean corrected nest temperature on the day of excavation (T_i) was 28.7 ± 1.87°C (SD; N = 37). The mean final nest temperature (T_f) at the time of excavation of the nests was 31.9 ± 2.48°C (SD; N = 21) or 3.2°C higher than at the beginning of incubation. Nest temperature rose significantly during incubation (paired t-test; t = 5.50, df = 20, p<0.001) despite the final temperatures of some nests being reduced by rainfall. When four nests in which temperature was affected by heavy rainfall immediately prior to sampling were excluded, the mean final nest temperature was 32.8 ± 1.57°C (SD; N = 17) and the mean rise was 4.1°C.

Initial Gas Tensions

During the initial 20% of incubation, nest P_{O_2} ranged from 118 to 151 torr [mean = 145.3 ± 6.8 (SD; N = 30)] and P_{CO_2} from 1 to 28 torr [mean = 5.7 ± 5.7 (SD; N = 30)]. Nest gas tensions did not differ significantly from those in the associated controls (paired t-tests: P_{O_2}, t = 0.31, df = 6, p = 0.769; P_{CO_2}, t = 1.30, df = 6, p = 0.242).

Variation in P_{O_2} between nests in the 0-20% incubation interval was related to nest depth (Fig. 8). P_{O_2} was lower in deeper nests:

(Equation 16)

$$P_{O_2} = 151.8 - 0.34\,\text{Depth}$$
$$[r^2 = 0.191, F_{1,27} = 6.38, p = 0.018, SEE = 4.13]$$

The relationship between depth and P_{O_2} varied significantly between coarse and fine substrates (observations from coarse and medium grain sands were pooled for comparison with pooled observations from finer sand and clay substrates). Finer substrates were associated with lower P_{O_2}, (ANCOVA, F = 4.68, p = 0.040). (An extreme observation of 118 torr in a shallow clay nest was excluded from these analyses). Trends in P_{CO_2} mirrored those in P_{O_2}; P_{CO_2} rose with increasing nest depth, and at a given depth was higher in finer sands or clay than in coarser sands or gravel (Fig. 8).

Unexplained variation in gas tensions between nests may be associated with "patchiness" in the distribution of other respiring organisms in the soil. For example, at one site where nests were made in fine sand and leaf litter, P_{O_2} in two control samples, taken on the same day at the same depth but 40 cm apart, differed by as much as 11 torr.

Gas Tensions During Incubation

Nest P_{O_2} fell throughout incubation and P_{CO_2} rose correspondingly (Fig. 9). The mean fall in P_{O_2} from 20% incubation to the time of hatching was 17.9 ± 12.0 torr (SD; N = 9), and the mean P_{CO_2} rose by 16.3 ± 13.5 torr (SD; N = 9). Both changes were highly significant (paired t-tests: P_{O_2}, t = 4.46, df = 8, p = 0.002; P_{CO_2}, t = 3.64, df = 8, p = 0.007). The very depressed P_{O_2} of a number of nests at the time of hatching appears to have been influenced by preceding heavy rainfall (see below). Consideration

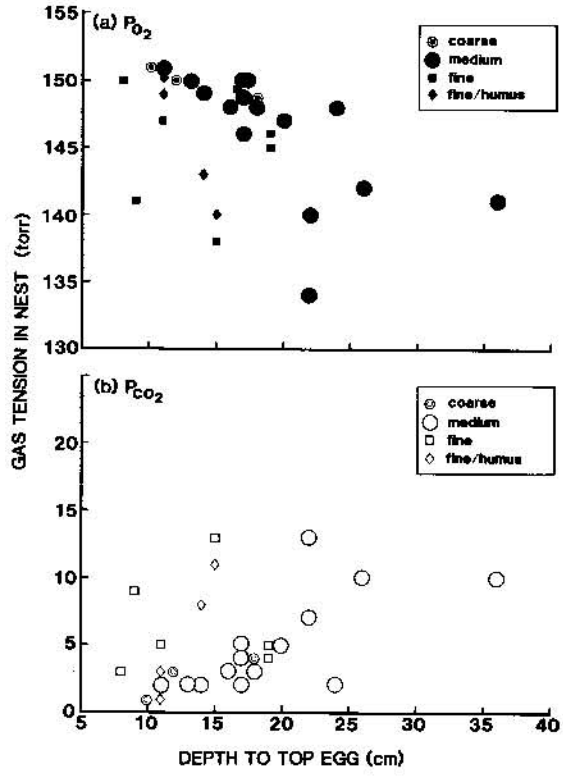

Fig. 8. Variation in nest P_{O_2} and P_{CO_2} during the first 20% of incubation as a function of nest depth. At this stage of incubation the V_{O_2} of eggs is negligible. The effect of depth is most clearly seen in the nests made in sand of medium grain size (large solid circles). An extreme observation from a nest with P_{O_2} of 118 torr and P_{CO_2} of 28 torr has not been included.

of the effect of nest depth and substrate type on the decline in P_{O_2} was therefore based on the difference between P_{O_2} in the 0-20% and the 91-99% incubation intervals. Falls in P_{O_2} between these intervals were not significantly influenced by substrate type [ANOVA, substrate in two categories (medium-coarse and fine-clay), $F_{1,15} = 1.66$, $p = 0.217$] or nest depth ($r^2 = 0.059$, $F_{1,15} = 0.94$, $p = 0.347$).

Gas tensions in controls changed significantly during incubation (paired t-tests; P_{O_2}, $t = 6.7$, $df = 2$, $P = 0.021$; P_{CO_2} $t = 6.1$, $df = 2$, $P=0.026$), but not as much as in nests. From the 21-40% incubation interval onwards, nests showed significantly lower P_{O_2} and higher P_{CO_2} than controls. The mean difference in P_{O_2} between nests and associated controls increased with incubation time, from about 2 torr in the 21-40% incubation interval to 11 torr at hatching.

Relationship Between Nest P_{O_2} and P_{CO_2}

Partial pressures of O_2 and CO_2 were closely correlated throughout incubation. Carbon dioxide tensions rose in tandem with declining P_{O_2}:

(Equation 17)

$$P_{CO_2} = 141.4 - 0.934\,P_{O_2}$$

$[r^2 = 0.965, F_{1,175} = 4858.4, p<0.001, SEE = 1.9]$

The slope (-0.934) is equivalent to an RE of 0.73, which indicates that CO_2 was produced in and around the nest at 0.73 times the rate that O_2 was consumed. The close correlation between P_{O_2} and P_{CO_2} removes the requirement to analyse the relationship of both gases with other nest and clutch variables, and thus analyses were confined to P_{O_2}. However, in all cases where a significant relationship between P_{O_2} and a variable was demonstrated, P_{CO_2} and that variable were also significantly related.

Effects of Rain on Substrate Gas Tensions

Declining P_{O_2} and rising P_{CO_2} in controls over the study period indicated that substrate gas tensions were subject to seasonal variation independent of the presence of eggs. These changes appear to be associated with rainfall.

Table 3. P_{O_2} and P_{CO_2} in nests three days before, immediately following, and three days after rain. All nests were on the same well-drained nesting area.

	Before		During (banks wet)		After	
Nest	P_{O_2}	P_{CO_2}	P_{O_2}	P_{CO_2}	P_{O_2}	P_{CO_2}
N14	127	26	102	42	115	36
N30	134	18	117	30	123	28
N36	140	12	118	29	132	19
N37	138	13	130	18	135	16
Mean	134.8	17.3	116.8	29.8	126.3	24.8
SD	5.7	6.4	11.5	9.8	9.1	9.1

The short-term impact of rain on nest P_{O_2} was examined by comparing observations taken three days before a heavy rainstorm with those made on the day of the storm while the nesting bank was wet, and with further observations taken three days later when the surface of the substrate had dried (Table 4). P_{O_2} was significantly lower when banks were wet than either before or after rain (paired t-test: before-during, $t = 4.84$, $df = 3$, $p< 0.01$; during-after, $t = 4.08$, $df = 3$, $p< 0.05$). The effect is also illustrated in Figure 10 where arrows indicate the time at which rain fell. The impact of rain appears to be greater in finer substrates.

In the study area, the incubation period of *C. johnstoni* corresponds with the end of the dry season and start of the wet season (Taylor and Tulloch 1985; Fig. 11). Rainfall could well influence longer term (seasonal) trends in substrate gas tensions, by reducing diffusivity of O_2 and CO_2 through soil layers as water accumulates in them, and by enhancing biological activity in the substrate as moisture content rises.

Clutch Characteristics

Of the 37 nests located, eggs from 23 were examined at the actual or predicted time of hatching (A. Smith, unpublished data). Mean clutch size was

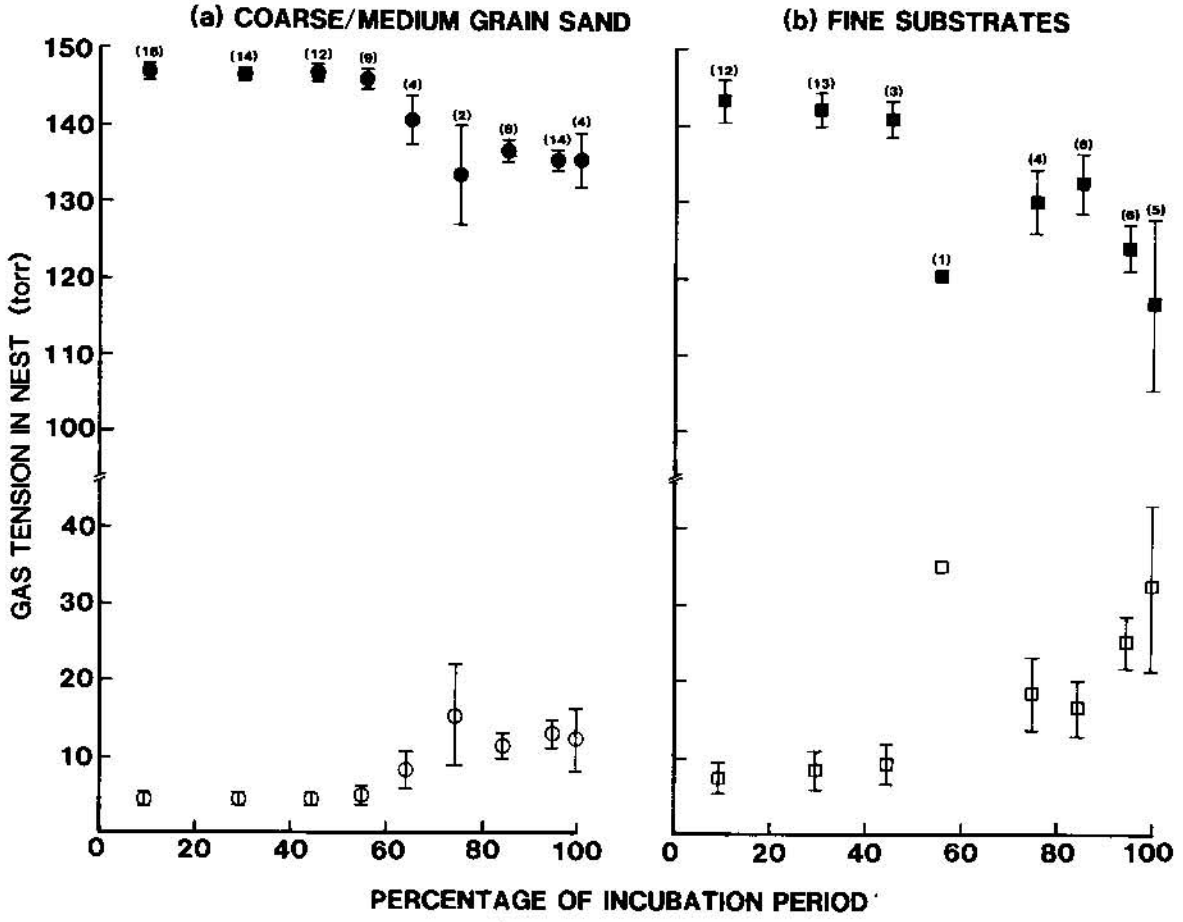

Fig. 9. Gas tensions in *C. johnstoni* nests during incubation. P_{O_2} fell and P_{CO_2} rose significantly. Points are means for the number of observations shown in parentheses, and horizontal bars are ± one standard error.

11.7 ± 3.4 (SD; N= 23; range 7 to 18) and mean initial egg mass was 71.2 ± 5.84 g (SD; N = 37; range 57.1 to 82.9). The mean mass of unpipped eggs at the time nests were excavated was 68.1 ± 6.16 g (SD; mean of nest means; N = 21; range = 56.8 to 80.2) which was significantly lower than the initial mass (paired t-test, t = 4.05, df = 20, p = 0.001). The mean index of water loss (IWL) was −1.75 (N = 21; SD = 2.00; range −5.77 to 1.62). This is equivalent to a mean loss of 2.5% of initial egg mass.

Embryo Survival

The 23 protected nests contained a total of 270 eggs. Of these 37 were infertile, 14 were damaged by the probing technique, and five contained embryos that appeared to have died very early in incubation. Of the total of 214 potentially viable eggs, 183 (85.5%) produced hatchlings. Nine of the "hatchlings" died when a nest was flooded on the day of hatching. They were found dead in the nest with their heads protuding from eggs, but had clearly been unable to escape from the nest unaided. In the remaining nests, embryo survival to hatching ranged from 50 to 100%. In addition to embryonic mortalities, 10 hatchlings (from four nests) were grossly deformed. Thus 80.8% of potentially viable eggs

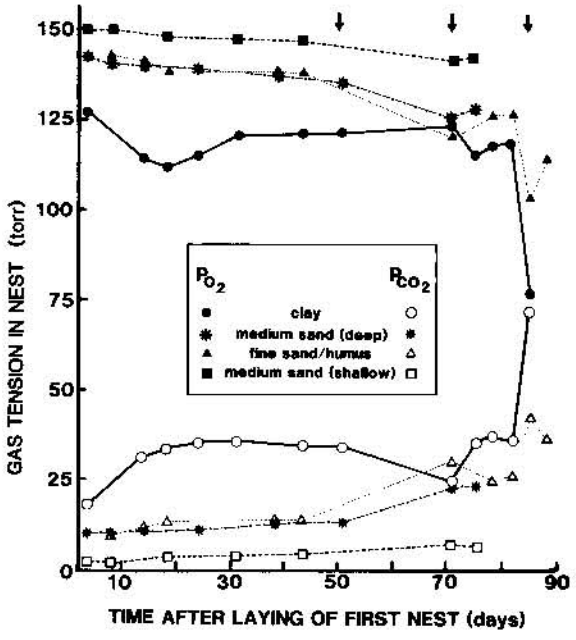

Fig. 10. Gas tensions in *C. johnstoni* nests in a range of substrate types. All except the clay nest were located within 20 m of each other on the same nesting bank. The arrows indicate observations made following rain.

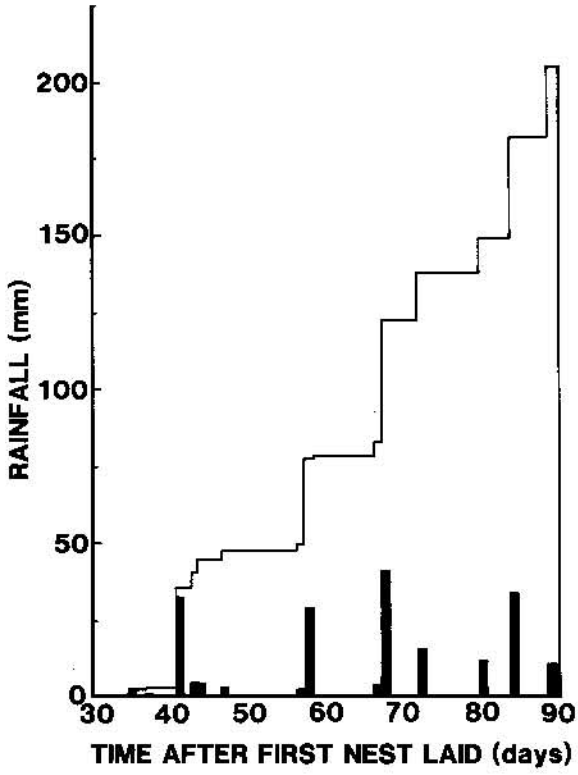

Fig. 11. Daily (bars) and cumulative (line) rainfall measured at the Bureau of Meteorology recording centre nearest the study site (Beatrice Hill; approx. 50 km to the north-west). There had been no rain for 20 weeks preceding the first record shown here.

produced apparently viable hatchlings. Total incubation time varied from 69 to 82 days [mean 75.2 ± 4.2 (SD; N = 20)] which is within the range (65-98 days) reported previously for the same area (Webb and Smith 1984).

Relationship Between Clutch and Nest Characteristics

Depth to the top egg was positively correlated with clutch size ($r = 0.411$, $p = 0.026$). Although a relationship between clutch size and the size of the female parent has not been demonstrated in *C. johnstoni*, larger female crocodilians commonly produce larger clutches (Ferguson 1985). Large female *C. johnstoni* may deposit their eggs at greater depths.

Water loss from eggs (as indicated by the water loss index) was not significantly correlated with nest depth, initial nest temperature, final nest temperature, duration of incubation or nest gas tensions ($p>0.20$ in all cases).

Clutch size was significantly and negatively correlated with P_{O_2} during a number of the incubation intervals [0-20% ($r^2 = 0.48$, $F_{1,18} = 5.95$, $p = 0.025$); 21-40% ($r^2 = 0.208$, $F_{1,19} = 4.99$, $p = 0.038$); and 81-90% ($r^2 = 0.536$, $F_{1,10} = 11.56$, $p = 0.007$). The existence of a significant correlation of P_{O_2} with clutch size early in incubation — before clutch $\dot{V}_{O_2}$ is likely to be significant (Fig. 2) — suggests that the lower P_{O_2} of nests containing larger clutches is related to nest depth rather than to the metabolic activity of the clutches themselves.

A comparison of P_{O_2} in nests and associated controls showed that differences between them were significantly and negatively correlated ($r^2 = 0.561$, $F_{1,7} = 8.95$, $p = 0.020$) with clutch size only in the 80-90% incubation interval, when embryonic $\dot{V}_{O_2}$ is at its peak. In addition, the extent to which nest P_{O_2} fell during incubation [P_{O_2} (0-20%) — P_{O_2} (91-99%)] was not significantly related to clutch size ($r^2 = 0.071$, $F_{1,12} = 0.91$, $p = 0.358$). Repetition of these analyses using the number of potentially viable eggs and the number of eggs that hatched as independent variables produced similar results.

Thus, although P_{O_2} tends to be lower in nests containing a larger number of developing eggs, the results do not necessarily indicate that nest P_{O_2} is influenced by the clutch's metabolic activity. Trends associated with clutch size are likely to be obscured by the heterogeneity of underlying substrate P_{O_2}, or variation in the shape of the nest and hence the position of the gas sampling tube relative to its centre. It is also possible that controls do not adequately replicate the type of sites chosen by nesting crocodiles.

Embryonic Development, Survivorship, and Nest Characteristics

The nest characteristics most likely to influence embryonic development and survival (temperature, water loss, and gas tensions) are highly intercorrelated. They also vary significantly diurnally, seasonally and, less predictably, in response to environmental factors such as rain. It is therefore difficult to quantify separately their effect on the success of field nests. The analyses below are intended to identify broad trends that justify further examination, rather than demonstrate causal relationships.

The duration of incubation (D in days) decreases with increasing nest temperature, and is most precisely predicted from final nest temperature (T_f).

(Equation 18)

$$D = 122.7 - 1.47T_f$$

[$r^2 = 0.584$, $F_{1,16} = 22.49$, $p<0.001$, SEE = 3.03]

Entry of nest P_{O_2} (during any incubation interval) into the regression equation did not add significantly to explained variance (maximum r^2 addition = 0.039, $p = 0.280$).

The mean yolk-free hatchling mass (H) in individual nests ranged from 30.36 to 43.04 g [mean of means =38.68 ± 2.69 (SD; N = 20)]. Yolk-free hatchling mass varied with fresh egg mass (r^2 = 0.201, $F_{1,18}$ = 4.36, $p<0.05$). Larger eggs produced significantly larger hatchlings. Entry of nest temperatures (T_i and T_f), water loss, duration of incubation, and mean nest P_{O_2} during the final 20% of incubation, showed that only P_{O_2} contributed significantly to explained variance (addition to r^2 = 0.371, $p<0.005$). Hatchling mass was lower in nests with low P_{O_2} after egg mass was taken into account. The trend is illustrated in Figure 12 in which hatchling mass has been adjusted for the effects of egg size. The statistical significance of the relationship between hatchling mass and P_{O_2} is heavily dependent on observations from one nest (circled in Fig. 12) that had chronically low P_{O_2} and P_{CO_2} throughout incubation (clay nest in Fig. 10). While the trend is sufficiently clear to lend limited support to the hypothesis that growth may be influenced by gaseous conditions in natural nests, the effect appears minor except under extreme conditions.

There was no clear relationship between embryonic survival and nest temperature (Fig. 13). However, a linear relationship would not be expected. A "plateau" of relatively high embryonic survivorship over a favourable temperature range is more likely, with sharply declining survivorship at temperatures lying significantly outside the plateau (Webb *et al.* 1983; Webb and Smith 1984). There is no evidence of a significant decline in embryonic survival at either end of the distribution of nest temperatures recorded in this study, suggesting that they fell within the range of embryonic tolerances.

Embryonic survival (S; proportion of potentially viable eggs that hatched) appears to have been affected by water exchange between eggs and nests (Fig. 14a). There was a significant rise in survivorship with increased water loss [r^2 (arcsine transformed S) = 0.356, $F_{1,13}$ = 7.20, $p<0.025$]. The small number of nests with eggs that lost little water or may have absorbed water (IWE >-1) suffered particularly high mortality. Water exchange and nest P_{O_2} were not significantly correlated (r = 0.20, $p>0.20$). Nevertheless combinations of eggshell conductance and nest environment that prevent evaporative water loss may also inhibit exchange of O_2 and CO_2.

Embryonic survival also appeared to decline with falling nest P_{O_2} (Fig. 14b). The proportion of potentially viable eggs hatching successfully was significantly lower in nests with low P_{O_2} during the final 20% of incubation [r^2 (arcsine transformed S) = 0.340, $F_{1,15}$ = 7.74, $p<0.025$]. The statistical significance of the relationships between embryonic survivorship, P_{O_2} and water loss were again heavily dependent on

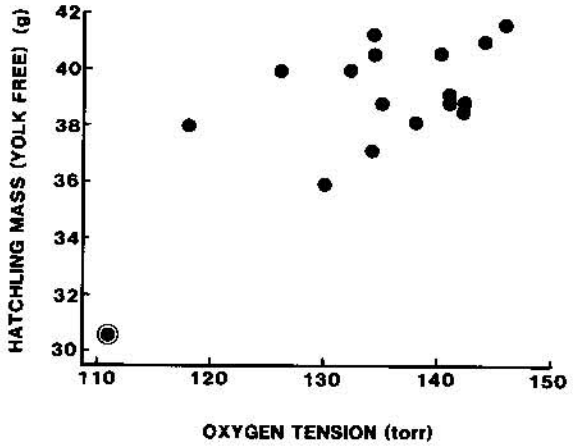

Fig. 12. Variation in hatchling mass with P_{O_2} in nests during the final 20% of the incubation period. Nests with a low P_{O_2} and elevated P_{CO_2} produced relatively smaller hatchlings. The circled observation was from a nest with an extremely low P_{O_2} throughout incubation (see text). Hatchling mass has been adjusted to take account of the effects of egg mass (see text).

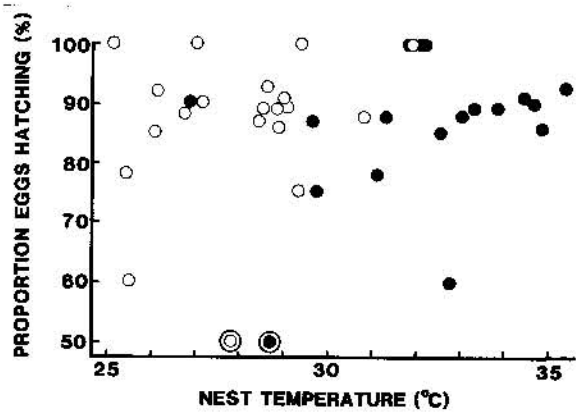

Fig. 13. The relationship between initial (open circles) and final (closed circles) nest temperature and the proportion of potentially viable eggs that produced hatchlings. There is no evidence of a relationship between temperature and survivorship within this temperature range. The highlighted observation is from a nest with an unusually low P_{O_2} (see text).

observations from one nest (circled in Fig. 14). Removal of this nest from the analyses generated non-significant regressions in each case. The trends to reduced embryonic survival and growth in the remainder of the sample are weak, and suggest quite broad tolerances of variation in gaseous conditions.

DISCUSSION

Patterns in Embryonic Metabolic Rate

The pattern of O_2 consumption in developing embryonic *C. johnstoni* is clearly peaked, similar to ratite birds (D. Vleck *et al.* 1980) and a number of other reptiles (Gettinger *et al.* 1984; Hoyt and Albers unpublished manuscript; Thompson 1983, 1987; Webb *et al.* 1986a), *Crocodylus porosus* shows a similar peak (Whitehead 1987). Reduced absolute or mass-specific $\dot{V}_{O_2}$ in late incubation probably reflects

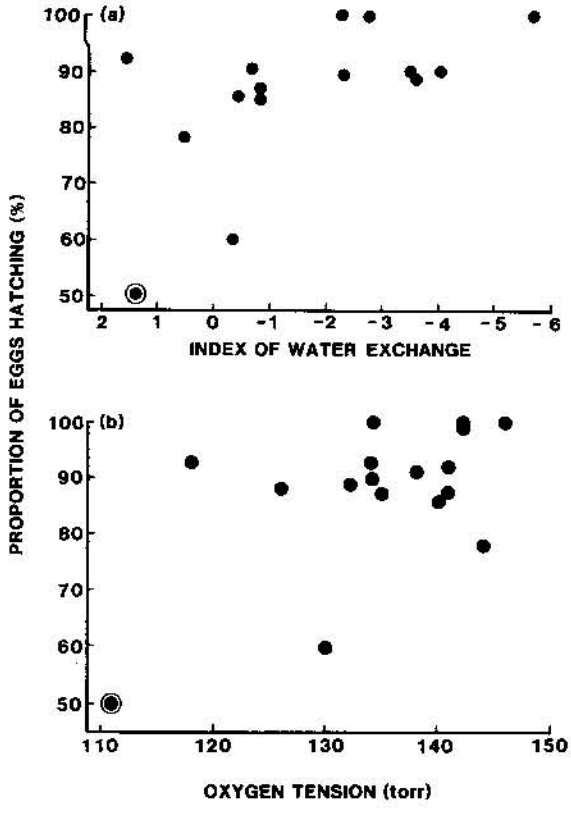

Fig. 14. The relationship of (a) water exchange and (b) nest P_{O_2} during the final 20% of incubation, to the proportion of potentially viable eggs producing hatchlings. Reduced embryonic survivorship in nests with eggs that had lost little water or taken up water may be related to the gas exchange characteristics of nests and eggs that inhibit water loss. The highlighted observation is from a nest with unusually low P_{O_2} (see text).

a reduced demand for energy as embryonic growth declines. Slowed growth of late-term embryos has been clearly demonstrated in a number of avian and reptilian species with peaked or sigmoid patterns of O_2 consumption (C. Vleck *et al.* 1979, 1980; Ackerman 1981a, b; Gettinger *et al.* 1984; Thompson 1983, 1987; Webb *et al.* 1986a). In *C. johnstoni* the absolute rate of growth in embryonic wet mass is highest when about 80% of the incubation period has been completed, and it then declines until hatching (Whitehead 1987).

Thompson (1983, 1987) has provided a detailed analysis of patterns of metabolic rate in embryonic chelonians, and it is likely that many of his conclusions apply to other embryonic reptiles.

Reduced growth rates late in incubation may facilitate synchronous hatching by allowing slower developing embryos in a clutch to "catch up" with more advanced ones. Divergent embryonic development rates are likely within the clutches of many hole-nesting reptiles, including *C. johnstoni*, because temperature gradients develop within their nests (Thompson 1983; Webb *et al.* 1983; Packard *et al.* 1985; A. Smith, unpublished data). Embryos near the top of the nest develop at higher mean temperatures than those near the bottom. Even slight differences in mean incubation temperatures may cause significant variation in the development times of individual embryos within the one clutch (Webb *et al.* Chapter 50). The slower growth and reduced O_2 consumption of advanced embryos conserves energy during the "catch up" period (Webb *et al.* 1986a).

Advantages of synchronous hatching may include an improved ability to escape the nest (Carr and Hirth 1961); saturation of predators (Carr 1967); reduced probability of nest predation (Congdon *et al.* 1983); and, the associated hatching delay may permit timing of emergence to coincide with favourable environmental conditions (Webb *et al.* 1986a). Among crocodilians, which excavate nests and may open eggs (Lang Chapter 28), hatchling survival is likely to be enhanced if all embryos are at a similar, advanced stage of development when the female opens the nest.

Initiation of Pulmonary Respiration

The metabolism of late term avian embryos appears to be constrained by the fixed gas conductance of the shell and reliance on chorioallantoic gas exchange (Metcalfe *et al.* 1984; Paganelli and Rahn 1984). Internal pipping and breathing of the gases in the air cell (i.e. pulmonary gas exchange) are accompanied by an increase in $\dot{V}_{O_2}$. Further increases follow external pipping and hatching (Paganelli and Rahn 1984). In contrast, transition to pulmonary respiration in reptiles appears to occur with little change in O_2 consumption, although a minor and temporary increase associated with the exertion needed to escape from eggs has been reported in the turtle *Emydura macquarii* (Thompson 1983). Pipping in *C. johnstoni* results in no $\dot{V}_{O_2}$ increase, and once out of the egg, the O_2 consumption of hatchlings continues to fall during the first weeks of post-embryonic life.

Thus the maximum O_2 demand of crocodilian embryos occurs well before they are due to hatch. At 31°C the metabolic rate of *C. johnstoni* embryos peaks about seven days prior to hatching. That peak is also higher than predicted from allometric equations relating $\dot{V}_{O_2}$ [max] to the mass of reptilian eggs (Seymour 1979; Ackerman 1981a). These equations predict $\dot{V}_{O_2}$ [max] for *C. johnstoni* eggs of either 5.86 (Seymour 1979) or 6.06 ml h^{-1}_{stpd} (Ackerman 1981a), some 20% lower than the actual rate, and in the larger *C. porosus* eggs the prediction is about 35% lower than the measured rate.

Although more O_2 is consumed during the longer incubation period at 29°C than at 31°C, the resultant hatchlings are larger, and the energy used to

produce a unit mass of hatchling is similar. The main energetic effect of lower incubation temperature is to reduce the reserve of energy available to the hatchling in residual yolk (*Webb et al.* Chapter 50).

AIR SPACES

Most avian eggs lose water equivalent to about 18% of their fresh mass between laying and hatching (Ar and Rahn 1980; Rahn 1984). The consequent development of an air space during incubation is an important feature of development, that facilitates the embryo's transition from chorioallantoic to pulmonary respiration (Visschedijk 1968a, b) and perhaps provides an optimum water balance at hatching (Rahn 1984). Several days prior to hatching, the embryo penetrates the chorioallantois and inner shell membrane and begins to breathe the air in the space, which invariably forms at the blunt end of the egg between the inner and outer shell membranes. The role of the chorioallantois as the embryo's principal respiratory exchange organ is gradually transferred to the lungs. At hatching the chorioallantois has ceased to function as a respiratory organ making the hatchling entirely dependent on pulmonary respiration.

Air spaces in the eggs of crocodilians have been described as abnormal (Ferguson 1982, 1985). However, water losses from brittle-shelled reptilian eggs are common under artificial incubation and in natural nests (Moore 1953; Pooley 1969a, b; Bustard 1971; Packard *et al.* 1979b; Webb *et al.* 1983; Lutz and Dunbar-Cooper 1984) and as pointed out by Ewert (1985), the volume of water lost must be replaced unless the shell deforms. In heavily calcified eggs like those of crocodilians, collapse of the calcite mineral layer may occur following extreme dehydration (G. Webb, pers. comm.), but in most cases air spaces form within the brittle shell. Air spaces were found in artificially incubated *C. johnstoni* eggs that had lost as little as 1.4 g of water (<2% of fresh egg mass). In all cases the development of the embryo, and where examined its metabolic rate, showed no association with the extent of water loss up to 12.7% of fresh egg mass. In natural nests embryonic survival was highest in eggs that lost some water. It is incorrect to regard the presence of air spaces in eggs of crocodilians as evidence of abnormal development.

Sub-shell air spaces were found in *C. johnstoni* eggs at various positions along the major axis, including close to the middle of eggs, at their poles, and on the upper and lower surfaces. Submembrane spaces were most often found close to the median upper surface of eggs (20 of 24) or were displaced slightly to one end or to one side of the eggs' uppermost surface. It is probable that air moves to the upper portion of the egg between the membrane and chorioallantois under the influence of hydrostatic pressure. Ewert (1985) describes such "bubbles" as moving freely in some chelonian eggs if they are tilted.

Spaces did not occur between layers in the shell membrane although one such space has been reported in a turtle egg (Ewert 1985). The shell membrane of crocodilian eggs does not appear to separate into structurally or functionally distinct subunits, and is most appropriately regarded as a single structure (Ferguson 1985; Webb *et al.* 1986b).

Functional Significance of Air Spaces

Crocodilian embryos do not use air spaces in a manner analagous with the internal pipping of birds. Once through the shell membrane, the embryo invariably penetrates the mineral layer (personal observation). The variable location of such spaces also suggests that internal pipping is improbable. Nor do embryos appear to breathe air within submembrane spaces. Although these spaces tend to occur in a relatively predictable position near the minor axis of the egg, embryos usually pip close to one pole.

The closed nest environment of reptilian eggs may duplicate the role of the air space in avian eggs. Crocodilian embryos may remain in the egg for up to two days after pipping (personal observation), and probably longer if the parent delays excavation of the nest. The delay between pipping and emergence may provide a period of transition from chorioallantoic to pulmonary respiration, in the humid and protected nest chamber. Chicks of mound-nesting megapode birds, whose eggs do not form functional air spaces, do not pip internally. They also remain in the nest mound for about two days, much of this time being spent at rest (Seymour and Ackerman 1980; Seymour 1984; Vleck *et al.* 1984).

Air spaces in brittle-shelled reptilian eggs may have no functional role, but are an inevitable consequence of water loss (Ewert 1985).

Gas Tensions in Air Spaces

Oxygen tensions have been directly measured in the eggs of one other reptile, the Burmese python, *Python molurus*, which lays large (200 g) parchment-shelled eggs. When incubation is about 75% complete P_{O_2} ranges from around 120 to 130 torr (Black *et al.* 1984). Shortly before hatching, P_{O_2} falls to around 105 torr in eggs incubated in dry substrate (water potential −360 kPa) and to 70 torr in wetter substrate (−80 kPa).

In *C. johnstoni* eggs the mean P_{O_2} before eggshells became completely opaque was 121 torr, and two eggs that were near the peak $\dot{V}_{O_2}$ had sub-shell

P_{O_2}'s of 77 and 83 torr. The lowest P_{O_2} was 54 torr. Thus oxygen tensions in *P. molurus* and *C. johnstoni* eggs are generally comparable. During late incubation at least, P_{O_2} may fall to lower levels than in the eggs of most birds (Paganelli and Rahn 1984).

Carbon dioxide tensions have not previously been measured in reptilian eggs. The mean P_{CO_2} in completely opaque *C. johnstoni* eggs was 18 torr, much lower than the 40 torr in the air cells of avian eggs immediately prior to internal pipping (Paganelli and Rahn 1984). Depressed P_{CO_2} in combination with a low P_{O_2} (Equation 11) may be related to the greater water content of the eggshells of reptilian eggs compared with those of avian eggs (see below).

Changes in Gas Conductance During Incubation

Oxygen conductance (G_{O_2}) of avian eggs increases during the first few days of development, as the outer shell membrane dries (Kutchai and Steen 1971; Tullet and Board 1976). Thompson (1985) found that G_{O_2} of *Emydura macquarii* eggs increased for about the first 50% of incubation, as the area of the opaque band expanded. Both G_{O_2} and G_{CO_2} of *C. johnstoni* eggs also increased for more than half of the total incubation period (Fig. 7), while the opaque band was expanding. These results support the hypothesis that opaque band development enhances conductance to meet the respiratory needs of the embryo (Ferguson 1982, 1985; Thompson 1983, 1985; Webb *et al.* 1986b).

Importance of Water in the Shell

The water content of *C. johnstoni* eggshells falls during development (Fig. 15). Most of the reduction occurs during the first 65% of incubation, as the chorioallantois and the opaque band are expanding (Webb *et al.* 1986b). In eggs of the turtle *E. macquarii* the water content of opaque shell is lower than translucent shell (Thompson 1985). Thus G_{O_2} increases in the brittle-shelled eggs of these reptiles are associated with eggshell dehydration. Measurements of O_2 diffusion through eggshells or eggshell fragments that have been dehydrated to varying degrees have consistently shown that dehydration greatly increases O_2 permeability (Lutz *et al.* 1980; Feder *et al.* 1982; Thompson 1983). These insights into the effect of water on gas conductance can be considerably extended by examining the relationship between the G_{O_2} and G_{CO_2} of intact eggs (Thompson 1985).

If gas exchange occurs entirely through gas filled pores or spaces in the shell membrane and mineral layer, the ratio $G_{CO_2}:G_{O_2}$ is equal to the ratio of the diffusivities of CO_2 and O_2 in air — about 0.78. This relationship has been shown to apply in avian eggs after the outer shell membrane has dried (Paganelli

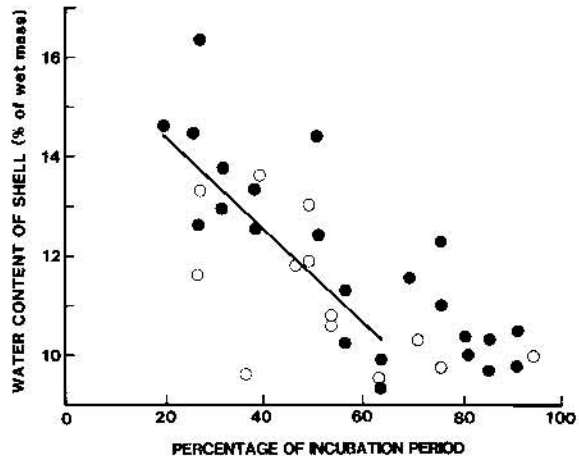

Fig. 15. Water content of *C. johnstoni* eggshells (mineral layer and shell membrane) throughout incubation. The solid line is the regression line of best fit up to 65% of the incubation period. Solid circles are observations from eggs incubated at 29°C, open from 31°C eggs.

et al. 1978). If diffusion pathways are entirely filled with water, the ratio of conductances is modified according to the relative solubilities of CO_2 and O_2 in water, and can be calculated to be about 19.8 (0.78 × 25.4; Dejours 1981). The mean G_{CO_2} of completely opaque *C. johnstoni* eggs is 7.46, or 2.2 times the mean G_{O_2}. Linear interpolation of the observed ratio between these theoretical extremes (all water — all air) indicates that about 7% of gas exchange occurs through water in opaque *C. johnstoni* eggshells, and the remainder through air-filled spaces. Thompson (1985) found a ratio of 1.8 in completely opaque *E. macquarii* eggs, indicating that about 5% of gas exchange occurred through water.

These calculations do not imply that 7% of pores or spaces in a *C. johnstoni* eggshell are filled with water, and the remaining 93% are dry. Such a distribution of water may have a relatively minor impact on the $G_{CO_2}:G_{O_2}$ ratio, as relatively little gas exchange would occur through the wet portions of the shell, and the relative rates of exchange would be close to the 0.78 ratio predicted from the diffusivities in air. Rather it appears that *portions* of many pathways are occluded by water, which has a most significant impact on movement of the relatively insoluble O_2.

The importance of the distribution as well as the quantity of water in the shell is illustrated by changes in gas conductance while the opaque band is forming. From 40% to 65% of the incubation period, water content of eggshells falls from 14% to 10% (Fig. 15), but G_{O_2} increases many times (Fig. 7). Over the same period the $G_{CO_2}:G_{O_2}$ ratio declines from about 4.0 to 2.2, indicating that the proportion of gas exchange through water falls from 17% to 7%. In *E. macquarii* eggs, the increases in G_{O_2} associated with opaque band formation follow a fall of water content from 20.4% (translucent eggshell) to 15.0% (opaque

eggshell) (Thompson 1983, 1985). Thus significant increases in G_{O_2} do not require complete dehydration of the eggshell. Kutchai and Steen (1971) suggested that dehydration of the membranes of hens' eggs may initially reduce the thickness of a continuous aqueous layer, but that further dehydration leads to a breakdown in continuity of the barrier to produce air filled gaps.

Changes in eggshell hydration provide a mechanism by which G_{O_2} and G_{CO_2} may be regulated to meet metabolic demands. It is probable that at least until the opaque band is complete, those changes in hydration are to a considerable extent regulated by the developing embryo and its extraembryonic membranes (Webb *et al.* 1986b), rather than depending on external processes such as evaporation.

Components of Diffusion Resistance

In reptilian eggs the shell membrane rather than the outer mineral layer appears to provide the greater part of the eggshell's resistance to gas exchange. The G_{O_2} of the mineral layer of *E. macquarii* eggs is 10 times higher than the shell membrane and mineral layer combined (Thompson 1983). Removal of the mineral layer from *Chelydra serpentina* eggshells may actually reduce G_{O_2}, rather than produce the increase that would be expected if the mineral layer contributed significantly to diffusion resistance (Feder *et al.* 1982). The calcified shell of *C. johnstoni* eggs also appears to contribute little to total eggshell resistance. In three eggs, P_{O_2} between the shell membrane and the mineral layer did not differ significantly from estimated ambient levels (i.e. ΔP_{O_2} was negligible). The clearest example of this effect came from one egg in which gas was sampled separately from spaces under the mineral layer and under the shell membrane: The sub-shell P_{O_2} was indistinguishable from ambient P_{O_2}, while the sub-membrane P_{O_2} was 77% of the ambient level.

Less direct evidence of the relative contribution of mineral layer and shell membrane to G_{O_2} was obtained by examining the relationship between rate of water loss from the egg, and G_{O_2}. Water losses are dependent in part on the porosity of the calcified portion of the eggshell (Packard *et al.* 1979a). Thus if G_{O_2} is also significantly influenced by the physical characteristics of the mineral layer, then a close correlation between G_{O_2} and rate of water loss would be anticipated. The weakness of the correlation is consistent with the hypothesis that the shell membrane is the principal barrier to O_2 exchange (Feder *et al.* 1982; Thompson 1983, 1985).

Changes in the Mineral Layer

Shell Degradation

Ferguson (1982) has described the extrinsic degradation of the mineral layer of *Alligator mississipiensis* eggs when they are incubated in natural nesting media. In addition, significant quantities of solids are known to be taken up from the shell and incorporated in the developing crocodilian embryo (Jenkins 1975; Ferguson 1982; Whitehead 1987). Ferguson (1982) argues that breakdown of the shell is essential to maintain adequate O_2 delivery to the developing *A. mississipiensis* embryo, and it has been suggested that exfoliation of turtle eggshells meets the same need (Feder *et al.* 1982; Woodall 1984). However, the relatively minor contribution of the mineral layer to total egg G_{O_2} is clearly inconsistent with this hypothesis.

Eggshell degradation is enhanced by incubation in moist substrates containing organic material (Ferguson 1982). But incubation in wet substrates significantly depresses the G_{O_2} of parchment-shelled eggs, probably by maintaining a higher water content in the eggshell (Black *et al.* 1984). A wet incubation substrate may also impede gas exchange between the egg mass and atmosphere and, if it contains organic material, the medium may itself consume O_2, depressing P_{O_2} around the eggs. Thus any potential increase in G_{O_2} associated with loss of all or a part of the mineral layer is likely to be minor, and more than offset by increased hydration of the shell membrane, and a lower ambient P_{O_2}. The increase in G_{O_2} observed in *C. johnstoni* eggs undergoing prolonged low temperature incubation, which showed no evidence of extrinsic degradation (Equation 14) is likely to be related to increased dehydration of shells. Both solids and water are lost from the shell, but the relative rate of loss of water is 10 times the rate of loss of solids (Whitehead 1987).

Although extrinsic degradation of the mineral layer is unlikely to have a significant impact on the delivery of O_2 to developing embryos, it may benefit them in other ways. It may facilitate hatching (Ferguson 1982; Grigg Chapter 48) and enhance CO_2 excretion. Because CO_2 crosses the hydrated shell membrane much more readily than O_2, the porosity and other physical characteristics of the mineral layer assume relatively greater importance in limiting G_{O_2} diffusion. Absolute P_{CO_2} is lower in eggs with porous shells that lose water more rapidly (Equation 11).

Interspecific Comparisons

Interspecific comparisons of eggshell conductance are complicated by the range of experimental treatments and materials that have been employed. Some reports (e.g. Feder *et al.* 1982) are based on pieces of shell, and the permeability figures given (equivalent to conductance/unit area) cannot be converted readily to whole shell conductances. Measurements for *Crocodylus acutus* were taken from infertile eggs following dehydration at 105°C (Lutz *et al.* 1980) and *Chelonia mydas* eggshells had part of the shell membrane stripped away

(Ackerman and Prange 1972). Measurements from intact shells containing developing embryos are summarized on Figure 16.

The limited data suggest that conductance may increase with egg mass, but much less steeply than occurs in birds' eggs (Fig. 16a). *Crocodylus johnstoni* and *P. molurus* eggs have G_{O_2}'s much lower than avian eggs of similar size, although the G_{O_2} of the smaller *E. macquarii* egg (10 g) is roughly equivalent to avian eggs of similar mass (Rahn *et al.* 1974; Ar and Rahn 1978; Hoyt *et al.* 1979; Thompson 1983, 1985). Differences between these reptilian eggs and avian eggs are reduced if incubation time is taken into consideration (Fig. 16b). The extended incubation times of reptilian embryos allow their whole incubation energy needs (Table 1) to be satisfied at a lower *rate* of O_2 uptake (Ackerman 1981b). Thus the lower G_{O_2} of the reptilian eggshell need not be associated with a correspondingly increased P_{O_2} gradient across the eggshell relative to avain eggs.

The low G_{O_2} of reptilian eggs compared with avian is likely to be related to the resistance to diffusion offered by a relatively thick and hydrated shell membrane (Lutz *et al.* 1980). In avian eggs the inner and outer shell membranes offer negligible resistance to gas exchange after the first few days of incubation (Piiper *et al.* 1980; Wangensteen and Weibel 1982).

Table 4. Oxygen conductances (G_{O_2}) and permeabilities (K_{O_2}) of eggshells of reptilian eggs and of the most studied avian egg (*Gallus gallus*). G_{O_2} is in ml d^{-1} $torr^{-1}$ and K_{O_2} in ml cm^{-2} sec^{-1} $torr^{-1}$. G_{O_2} for *C. johnstoni* and *E. macquarii* are take from completely opaque eggs. RH = relative humidity at which measurements were made. Data are from: 1. Black *et al.* 1984; 2. Thompson 1983; 3. Lutz *et al.* 1980; 4. Feder *et al.* 1982; 5. Ackerman and Prange 1972; 6. Wangensteen *et al.* 1970/71.

Species	G_{O_2}	K_{O_2} ($\times 10^{-6}$)
Crocodylus johnstoni	3.41	0.47
Python molurus[1]	4.5 to 7.0	–
Emydura macquarii[2]	2.64	1.36
Crocodylus acutus[3]	–	1.2 (70% RH)
	–	0.23 (100% RH)
Chelydra serpentina[4]	–	16.9 (dry)
	–	2.1 (moist)
Chelonia mydas[5]	–	6.6
Gallus gallus[6]	18.2	3.1

Gaseous Environment of Nests

Nest Gas Tensions

Considerable physiological significance has been attributed to the gas tensions, particularly the P_{CO_2} (about 40 torr), found in the air spaces of many avian eggs just before internal pipping (Wangensteen and Rahn 1970/71; Erasmus and Rahn 1970/71; Paganelli and Rahn 1984). The P_{CO_2} is similar to gas tensions in the lungs of adult birds, and this similarity may facilitate the transition from chorioallantoic to pulmonary respiration (Paganelli and Rahn 1984). Ackerman (1977) found that gas tensions in natural sea turtle nests prior to hatching were similar to those in the air spaces of avian eggs, and optimum embryonic survival and growth were obtained in artificial nests that mimicked the gas conductance characteristics of natural nests (Ackerman 1981b). If development of other embryonic reptiles is dependent on similar gaseous conditions, then natural selection should favour nesting strategies that produce these gas tensions within eggs at equivalent stages of development (Ackerman 1980; Seymour

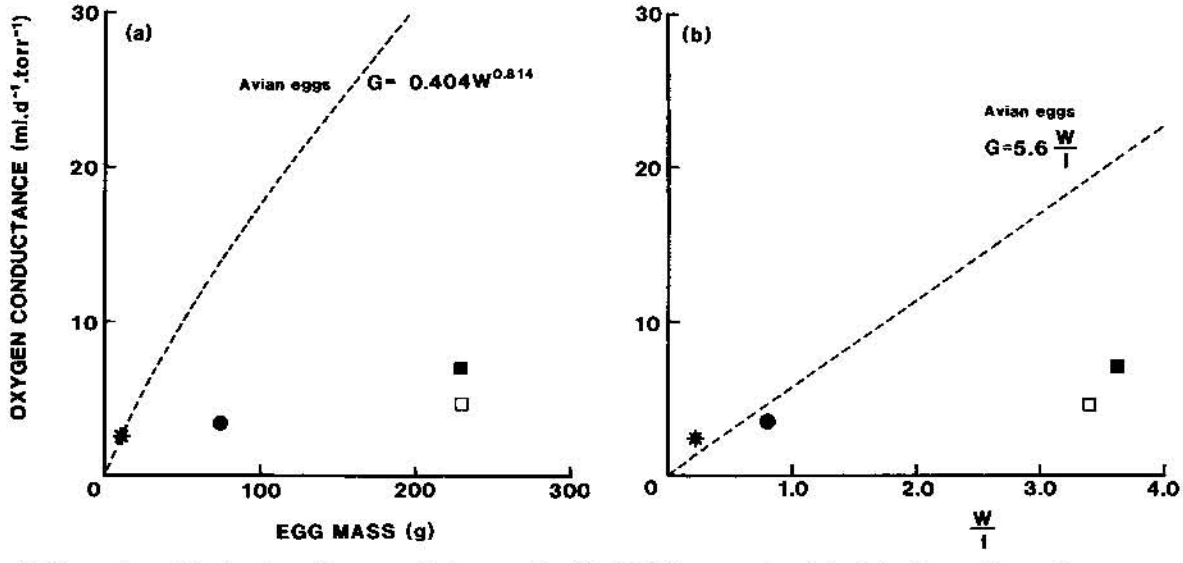

Fig. 16. Comparison of the G_{O_2} of reptilian eggs with those predicted for birds from equations (a) relating G_{H_2O} and hence G_{O_2} to egg mass (Ar and Rahn 1978; Hoyt *et al.* 1979); and (b) relating G_{O_2} to egg mass and incubation time (Rahn and Ar 1980). W = egg mass (g) and I = length of incubation (days). The G_{O_2}'s of larger reptilian eggs are lower than birds' eggs of equivalent mass. Asterisks are *Emydura macquarii*; circles are *Crocodylus johnstoni*; and squares are *Python molurus* (open = dry; closed = wet).

and Ackerman 1980). Clearly significant within-species or within-population variation in nest P_{O_2} and P_{CO_2} would not be predicted from this hypothesis.

A degree of hypoxia and hypercapnia is common in the hole nests of all reptiles for which data are available, and there is considerable interspecific variation (Table 5). In addition to these interspecific differences, however, there is significant variation between nests of the same species. For example, the range of P_{O_2} and P_{CO_2} recorded in *C. johnstoni* nests at hatching brackets the mean hatching gas tensions in nests of all other species (Table 5 and Fig. 10).

Table 5. Gas tensions in the hole nests of reptiles at the time of hatching. All data except those for *C. johnstoni* were obtained by estimating values from figures in the reports cited in the text.

	P_{O_2}				P_{CO_2}			
Species	N	Mean	SD	Range	N	Mean	SD	Range
C. johnstoni	9	126	21	71-144	9	24	21	5-71
C. acutus	6	133	7	120-143	6	15	5	11-18
E. macquarii	3	144	7	–	4	4	3	–
C. caretta	3	119	12	113-134	3	27	9	18-32

The nest parameters most likely to affect gas tensions in nests (Seymour and Ackerman 1980) are: egg mass and clutch size, and hence clutch $\dot{V}_{O_2}$; nest depth; substrate type including water and organic content; and, the extent of other biological activity in the substrate around the nest.

These factors are likely to be least variable in sea turtle nests. Large marine turtles produce a number of clutches of about 100 eggs rather than depositing the entire season's clutch in one nest. The substrate is beach sand above the high water mark, which typically contains little organic material and supports little biological activity (Ackerman 1977). In addition, excavation and construction of nests is a highly stereotyped behaviour that produces nests of consistent shape and depth (Carr 1967). In other species these potentially important influences show considerably greater variation.

Clutch Metabolic Rate

In a sea turtle nest the P_{O_2} and P_{CO_2} gradients between the atmosphere and the egg mass are created and maintained chiefly by the metabolic activity of the clutch itself (Ackerman 1977). Clutch metabolism may also influence gradients in *C. johnstoni* and *E. macquarii* nests. Gas tensions in nests diverge by a few torr from controls in the later stages of incubation, although part of this divergence may be attributable to different substrate characteristics in nests and controls (Thompson 1983; this study). In contrast to sea turtles, clutch size and mass of larger crocodilians may vary greatly. For example, the hole nesters *Crocodylus niloticus* and *C. acutus* may produce clutches containing 25-95, and 19-81 eggs respectively (Ferguson 1985). It would be surprising if the resultant variations in clutch $\dot{V}_{O_2}$ were not accompanied by significant differences in nest P_{O_2} and P_{CO_2}, at least when metabolic rate is at its peak (Seymour and Ackerman 1980).

Depth

The depths of *C. johnstoni* nests that produced hatchlings varied more than 4-fold, and similar variation occurs in other crocodilians (Ferguson 1985) and in non-marine turtles (e.g. Burger 1977). Nest depth affects gas tensions because soil P_{O_2} falls and P_{CO_2} rises with increasing depth. Nest depth also determines the thickness and hence the overall gas conductance of the diffusive barrier separating the clutch from the atmosphere.

Much of the variation in the gaseous environment of *C. johnstoni* nests can be attributed to differences in "background" gas tensions associated with nest depth, that are unrelated to the O_2 consumption of the clutch.

Substrate

Over much of the distributional range of *C. johnstoni*, there is considerable annual variation in the availability and composition of nesting substrates, much of which is dependent on the severity of wet season flooding and associated movements of silt and sand (Webb *et al.* 1983). The frequency of nesting in different substrate types, and thus the P_{O_2} and P_{CO_2} of nests, can be expected to change from year to year.

Nests of *C. acutus* are made in at least two distinct substrate types (sand/shell and marl) that vary markedly in their resistance to diffusion of respiratory gases, and probably in level of microbiological activity (Lutz and Dunbar-Cooper 1984). Constituents of the mound nests of *C. porosus* vary from chiefly vegetation, to mostly mud (Webb *et al.* 1977). Hutton (1984) found that *C. niloticus* nested in all three substrate types available to them (coarse river sand, dark silt and fine organic silt-loam), which had different moisture and organic contents and were likely to have different diffusivities and background gas tensions. Thompson (1983) found that *E. macquarii* did not select particular substrate types.

Substrate type and depth may also interact, so that P_{O_2} falls and P_{CO_2} rises more rapidly with increasing depth in clay soils than in sandy soils (Lyon *et al.* 1952).

Nest Site Selection

Cues for nest site selection used by most reptiles are unknown, although substrate temperature appears to be important in *Caretta caretta*

(Stoneburner and Richardson 1981). Substrate moisture levels may also be important (Webb *et al.* 1983). Female *C. johnstoni* frequently dig test holes before laying (Webb *et al.* 1983), and such searching behaviour, which involves probing or nuzzling of the substrate surface before digging, has been observed in other hole nesting reptiles (e.g. Burger 1977; Hutton 1984). Whatever the cue(s), nesting *C. johnstoni* and many other hole-nesting reptiles do not choose sites that produce a particular gaseous environment at the time of laying, nor later in development.

Effect of Gas Tensions on Development

Calculated gas tensions at the chorioallantois of eggs in different *C. johnstoni* nests are illustrated in Figure 17. However, the use of mean G_{O_2} and G_{CO_2} substantially underestimates the potential for extreme variation between eggs. For example if the *C. johnstoni* eggs with the lowest G_{O_2} had been deposited in nests with the lowest P_{O_2} (clay), then estimated P_{O_2} would be 6 torr at hatching. In contrast, high conductance eggs laid in a high conductance nest (shallow coarse sand), would have a P_{O_2} at the chorioallantois above 120 torr, even at the peak of embryonic O_2 consumption. Carbon dioxide tensions could range from 11 to 93 torr.

These estimates of in *C. johnstoni* eggs assume that the $\dot{V}_{O_2}$ of eggs in field nests is similar to that measured in the laboratory. It is perhaps more likely that $\dot{V}_{O_2}$ is constrained in nests with low P_{O_2} or elevated P_{CO_2}, reducing the potential for extreme hypoxia or hypercapnia that may threaten embryonic survival (Lutz and Dunbar-Cooper 1984).

Because energy used for biosynthesis makes up the major part of the overall embryonic energy budget (Whitehead 1987), reduced embryonic $\dot{V}_{O_2}$ is likely to be achieved at the expense of growth. However, there was no evidence that nest P_{O_2} or P_{CO_2} influenced the length of incubation of *C. johnstoni* eggs. In this context a distinction can be made between development and growth. Embryos undergo little obvious morphological change after 65% of the incubation period (Ferguson 1985), but increase in mass by more than 400% before they hatch (Whitehead 1987). Thus at least in late incubation, additions to embryonic mass may slow, without necessarily disrupting patterns or rates of morphological or physiological development. This appears to have occurred with *C. johnstoni* (Fig. 12).

This observation suggests an alternative to the synchronous hatching hypothesis to account for the peaked pattern of O_2 consumption in *C. johnstoni* and other embryonic reptiles. Reduced growth rate late in the incubation period, and a decline in embryonic $\dot{V}_{O_2}$, may help to limit excursions in P_{O_2} and P_{CO_2} within the egg. Mean gas tensions at the chorioallantois are relatively stable after about 70% of incubation (Fig. 17), despite deterioration in the nest environment. Selective advantages, in the form of improved probability of survival, may accrue to embryos that are able to grow slowly in late incubation.

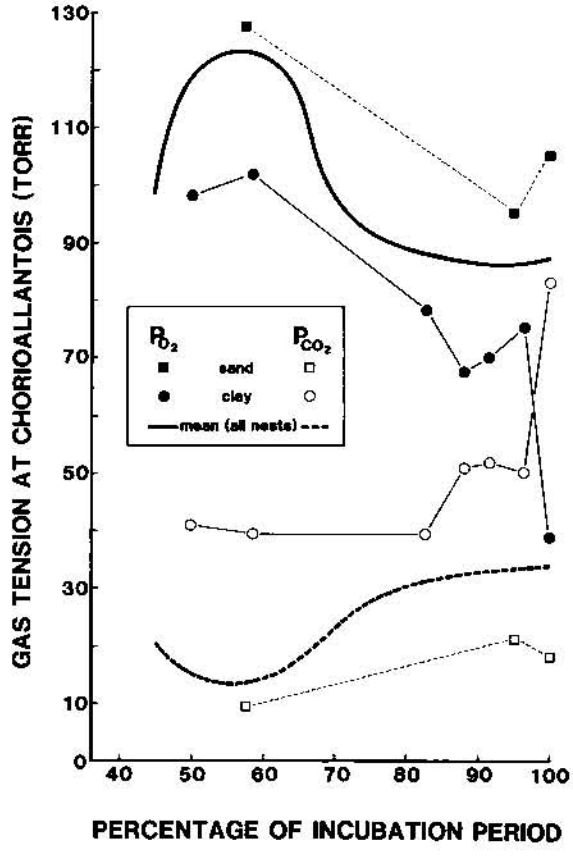

Fig. 17. Predicted gas tensions at the chorioallantois of *C. johnstoni* eggs in nests with the mean P_{O_2} and P_{CO_2} (heavy lines), and in two individual nests in different substrate types. The predictions were derived using equations relating G_{O_2} to incubation time up to 65% incubation and the mean G_{O_2} thereafter, together with observed nest gas tensions. Lines for the mean trend were fitted by eye.

The close correlation between P_{O_2} and P_{CO_2} in nests permitted analyses to be framed in terms of one of them (P_{O_2}). It was not possible to indicate whether variations in embryonic development were more likely to be related to hypoxia, or to hypercapnia. However, this statistical equivalence may not reflect their relative physiological importance. An eggshell structure that impedes uptake of O_2 while facilitating the excretion of CO_2, suggests that containment of P_{O_2} has greater physiological significance than access to O_2. The low G_{O_2} that results from the hydration of the shell membrane may be a "side-effect" of a mechanism to limit the physiological effects of high CO_2 concentrations. In avian eggs the embryo's capacity to compensate for elevated P_{CO_2} and buffer

pH change, breaks down when eggshell conductance is unusually low (Tazawa *et al.* 1983; Tullett and Burton 1985). Paganelli and Rahn (1984) have suggested that such disturbances may have greater physiological significance than reduced O_2 availability.

An Optimum Gaseous Environment?

Previous studies of gas conductance in reptilian eggs have demonstrated a high degree of variability between eggs (e.g. Feder *et al.* 1982; Thompson 1983). Variation between *C. johnstoni* eggs is also high. Gas tensions of individual *C. johnstoni* nests differed markedly even on the same nesting bank (Fig. 10), and seasonal and shorter term environmental changes added to variability. It is thus improbable that nest sites can be reliably matched with eggshell conductances to achieve particular gas tensions at the chorioallantois of most embryos. Not only is achievement of an optimum improbable, but embryonic survival may sometimes be threatened by unfavourable combinations of eggshell conductance and nest gases. Patterns of embryonic growth, metabolic rate, and eggshell function in *C. johnstoni* suggest selection to extend the capacity of individual embryos to survive a variable and often hostile gaseous environment, rather than to achieve a precise match of clutch and nest.

It does not follow, however, that hypotheses regarding optimum gaseous conditions for developing embryos are incorrect. Incubation environment is known to have a major impact on post-hatch survival and growth of captive crocodilians (Joanen *et al.* Chapter 51; G. Webb, unpublished data; A. Smith, unpublished data). Low survival rates of *C. johnstoni* hatchlings in nature [12% survive their first year (Smith and Webb 1985)] may result partly from unfavourable incubation conditions in wild nests. For example, while embryonic development under constant temperature artificial incubation appears to proceed normally over a broad range (around 5°C in *C. johnstoni* and *C. porosus*), the span that provides optimal post-hatch survival and growth may be very much narrower at 0.5 to 1.0°C (G. Webb, unpublished data). Gaseous conditions may act similarly. Broad tolerances enable most embryos to survive extreme combinations of eggshell conductance and nest gases, but their post-hatch development may be compromised.

Implications for Artificial Incubation

The described variability in eggshell conductance complicates choice of incubation regimes. However, the potential impact of gas conductance on both embryonic development and post-hatch survival and growth is likely to be minimized by maintaining P_{O_2} as near atmospheric levels as the simultaneous maintenance of high humidity will permit.

The data summarised here for *C. johnstoni* and *C. porosus* (Table 1) indicate that crocodile eggs at 30°C consume a maximum of 2.8 ml O_2 g^{-1} d^{-1}. Thus 400 eggs with a mass of 80 g each will consume 90 litre O_2 d^{-1}. The entire O_2 content of a large incubator (with a volume of around 300 litres) would be consumed in 16 hours. Clearly if P_{O_2} is to be maintained near ambient levels, then large volumes of (humidified) air need to be pumped into incubators containing large numbers of eggs, particularly when eggs are approaching the peak of metabolic rate. Standard electric aquarium pumps appear to be adequate for this purpose. A flow of humidified air through the incubator also inhibits the accumulation of CO_2. Failure to provide adequate ventilation has resulted in premature hatching of large numbers of *C. johnstoni* eggs at one of the Northern Territory farms.

Incubation conditions that permit moderate water loss (<10% of fresh egg mass) are unlikely to compromise development, and at least in the hole-nesting *C. johnstoni*, may actually improve hatching rates.

Attempts to replicate natural conditions by the use of nest material may be unnecessary. The use of nesting medium, although it may simplify maintenance of high humidity around the eggs, creates difficulties in the control of both temperature and gaseous conditions. It is likely to depress rather than increase gas conductance of eggshells and, in combination with the reduced ventilation caused by its presence, may affect embryonic growth. The frequency of bacterial or fungal infection of eggs is also likely to be increased.

Obviously, crocodile farmers and researchers are inclined to retain incubation systems that have achieved reasonable hatching success. But the increasing evidence of an association between incubation regimes and post-hatch growth and survival should encourage further research to identify the most significant variables, and refine artificial incubation techniques accordingly.

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