



**RESPIRATION AND ENERGY UTILISATION IN THE EGGS  
OF THE AUSTRALIAN FRESHWATER CROCODILE,  
*CROCODYLUS JOHNSTONI* KREFFT, 1873**

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## SUMMARY

Understanding of the respiratory physiology of embryonic vertebrates has advanced greatly in recent years, but knowledge of reptilian eggs is limited. There are few published reports of metabolic rate, mechanisms of gas exchange, or gaseous conditions of incubation. Despite the limited data, it has been argued that development of embryonic reptiles is energetically inefficient, because incubation periods are prolonged compared with birds. Prolonged incubation is thought to increase total energetic costs by increasing the time for which embryonic tissue must be maintained.

The studies reported here were designed to examine energy costs of embryonic development in eggs of the Australian Freshwater Crocodile, *Crocodylus johnstoni*, incubating at temperatures of 29 and 31°C. Duration of incubation at 29°C is about 101 days, 19 days longer than at 31°C. Metabolic rates were measured throughout incubation at both temperatures, together with changes in the quantity and distribution of chemical potential energy in the egg. In order to examine the possibility that energetic processes are influenced by gas exchange between eggs and their incubation environment, gas conductances of egg-shells and gaseous conditions in nests were also measured.

*Crocodylus johnstoni* inhabits fresh waters in tropical northern Australia. It produces clutch of about 13 eggs, each weighing around 75 g. The clutch is deposited in a hole nest in friable substrate during a brief nesting period late in the dry season (August-September). Eggs are brittle-shelled and superficially similar to cleidoic avian eggs.

The pattern of  $\dot{V}_{O_2}$  in embryonic *C. johnstoni* is clearly peaked,  $\dot{V}_{O_2[\max]}$  (6.90 ml.h<sup>-1</sup><sub>STPD</sub> at 29°C and 7.38 ml.h<sup>-1</sup><sub>STPD</sub> at 31°C) occurring when incubation is about 90% complete. Similar patterns occur in other reptilian embryos developing in brittle-shelled (cleidoic) eggs. The pre-hatch peak in embryonic metabolic rate may reflect a development mode evolved in reptiles nesting in highly variable environments. Rapid early growth, followed by a

period of very limited growth and declining  $\dot{V}_{O_2}$  combine to provide a flexible hatching schedule that can be matched to environmental conditions. External pipping and hatching of *C. johnstoni* eggs are not associated with an increase in embryonic  $\dot{V}_{O_2}$ . Hatchling  $\dot{V}_{O_2}$  (resting) falls steadily for several weeks after hatching, and appears to continue the pre-hatch decline.

Whole incubation  $O_2$  consumption of *C. johnstoni* eggs incubated at 29°C (6402 ml<sub>STPD</sub>) is significantly higher than at 31 °C (5675 ml<sub>STPD</sub>). Whole incubation  $O_2$  consumption of reptilian eggs in general increases with both egg mass and incubation time.

The pattern of growth of *C. johnstoni* embryos (wet mass) is sigmoid, and can be satisfactorily modelled with a logistic equation. Absolute growth rate peaks between 77 and 82% of the incubation period, and then declines to around 50% of the peak by the time of hatching. Under artificial incubation slowed late term growth is unlikely to be caused by constraints of space within the egg, nutrient availability, or gas exchange. Growth rate increases with incubation temperature but yolk-free hatchling mass is lower at higher temperatures. Growth constants from logistic equations increase with incubation temperature, the increase being equivalent to a  $Q_{10}$  of 2.5 for rate of growth.

Temperature effects on embryonic growth are most pronounced in early incubation, during the period of organogenesis. Relative and absolute growth rates of embryonic reptiles may approach those of some slow-growing birds. The extended incubation times of reptilian embryos may be associated with low incubation temperatures during early development, rather than an intrinsically lower capacity for rapid growth.

The energetic cost of embryonic growth, and hence of maintenance, have not been successfully measured. Allometric equations relating embryonic metabolic rate to embryonic mass generate highly variable exponents, which do not provide a useful index of the metabolic correlates of rapid growth. Models reliant on multiple regression techniques to separate the maintenance and growth components of energy budgets confront statistical difficulties (intercorrelation of variables) that may cause the model to generate spurious

results. Similarly, growth rates of *C. johnstoni* embryos at a given mass are so closely correlated with their incubation temperature, that growth rate and temperature each explains variation in embryonic metabolic rate as well as the other. Comparisons among species reveal no consistent relationship between embryonic metabolic rates and growth rates.

The water content of *C. johnstoni* eggs is similar to eggs of other reptiles and precocial birds. Evaporative water losses up to 12.7% of fresh egg mass do not appreciably affect embryonic development. Rate of water loss accelerates during incubation, probably due to the combined effects of metabolic heating and increased  $G_{H_2O}$  of egg-shells. Relative water content of hatchling crocodiles is influenced by the volume of enclosed yolk, and this factor may also contribute to the distinct differences in water content between altricial and precocial avian hatchlings.

The rate of depletion of egg solids with embryonic growth does not vary markedly between incubation temperatures. In particular, there is no evidence of increased usage of egg solids during the longer incubation period at lower temperatures. Conversion ratios (of egg solids to tissue) are similar in reptilian and avian embryos.

The fresh *C. johnstoni* egg contains about 440 kJ of potential energy. The egg's energy density ( $5.88 \text{ kJ.g}^{-1}_{FEM}$ ) is higher than other reptiles and altricial birds but lower than precocial birds. The energy density of dry contents ( $27.36 \text{ kJ.g}^{-1}_{FEM}$ ) is greater than in the eggs of other reptiles, but lower than all birds. Variation in energy densities among species of reptiles is related to differences in lipid levels, which may be associated with a wide range of developmental strategies, and the variable energy demands these strategies present.

Energy densities of *C. johnstoni* embryos rise during incubation from about  $20.6 \text{ kJ.g}^{-1}_{FEM}$  about half way through incubation, to  $23.4 \text{ kJ.g}^{-1}_{FEM}$  at hatching, probably due to increasing incorporation of fats in embryonic tissues. Energy densities of yolks in *C. johnstoni* eggs also rise substantially (from 28.1 to around  $30.0 \text{ kJ.g}^{-1}_{FEM}$ ), as protein stores are incorporated in embryonic and

extra-embryonic tissues. The total energetic cost of embryonic growth in *C. johnstoni* eggs is about 0.54 kJ for each kJ of energy incorporated in embryonic tissue, equivalent to a total production efficiency of 65%. There is no evidence of increased energetic demands during prolonged, low temperature incubation. Indeed, energetic efficiencies in embryonic reptiles are higher than in embryonic birds. Energy costs of development in avian embryos are probably increased by important physiological differences between them and reptilian embryos, which increase late term energy demands. There is no evidence that maintenance (in the sense of basal or standard metabolic rate) is a significant component of the embryonic energy budget in either birds or reptiles.

The term maintenance has been used as a convenient "catch-all" to explain variation in the energy cost of embryonic development, and the artificial separation of energy budgets into growth and maintenance components has tended to obscure rather than clarify energetic processes. Improved understanding of embryonic energetics may require greater concentration on sources of variation between species. The notion of embryonic energy allocations could provide an improved conceptual framework to replace the growth/maintenance dichotomy. The energy allocations of greatest interest are embryonic growth, extra-embryonic growth, excretion, physiological maturation (transition to pulmonary respiration, development of thermogenic capacity), and hatching (pipping, escape from egg and/or nest, late term embryonic movement).

Oxygen conductance of *C. johnstoni* eggs rises while the opaque band is expanding, and the water content of the egg-shell is falling. The mean  $G_{O_2}$  of completely opaque *C. johnstoni* eggs is 3.41 ml.d<sup>-1</sup>.torr<sup>-1</sup>. The  $G_{O_2}$  of the reptilian egg is depressed relative to avian eggs of similar mass by its thick hydrated membrane which, despite the early decline in water content, contains substantial quantities of water throughout incubation. The presence of water in the membrane elevates  $G_{CO_2}$  relative to  $G_{O_2}$ , leading to relatively lower  $P_{CO_2}$  in reptilian eggs than in avian. Degradation of the mineral layer does not significantly alter  $G_{O_2}$ . Models of diffusive gas exchange in avian eggs are not

directly transferable to reptilian eggs because there are significant structural and physiological differences.

The mean  $P_{O_2}$  gradient between *C. johnstoni* nests and the atmosphere is 6 torr at laying, increasing to 26 torr at hatching. Carbon dioxide gradients rise from 6 to 24 torr. These mean changes fall within the range reported for other reptiles. However, there is great variation between individual *C. johnstoni* nests, due to differences in substrate type and nest depth. The effects of clutch metabolism on nest gases are obscured by the heterogeneity of background gas tensions, which change during the nesting season in controls as well as nests. Thus a large part of the decline in nest  $P_{O_2}$  and increase in  $P_{CO_2}$  is due to seasonal change in substrate gas tensions, unrelated to the presence of eggs. Rainfall, which becomes more frequent as the wet season approaches, causes substantial short term declines in nest  $P_{O_2}$  and increases in  $P_{CO_2}$ , and may have longer term effects.

The nest environment, egg-shell conductance, and embryonic metabolism combine to produce a calculated mean  $P_{O_2}$  of 88 torr and  $P_{CO_2}$  of 32 torr at the chorioallantois of eggs before they hatch. However, these calculations are based on means of highly variable parameters, and obscure the potential for extreme variation between individual eggs and clutches. The close match of egg conductance and nest environment needed to generate particular "optimum" gas tensions for embryos are unlikely to be reliably achieved. Extreme hypoxic or hypercapnic conditions may compromise growth and even survival of *C. johnstoni* embryos, but embryonic tolerances appear to be broad. Low  $G_{O_2}$  resulting from the hydration of the membrane may be a side effect of a mechanism to limit excursions in  $P_{CO_2}$  and pH within the egg, that may otherwise be associated with incubation in a hypercapnic subterranean environment.

**DECLARATION**

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference is made in the text.

I consent to the thesis being made available for photocopying and loan if accepted for the award of the degree.

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**PETER JOHN WHITEHEAD**

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