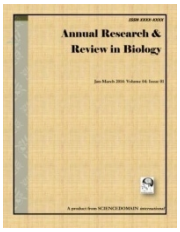

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Longer Incubation Periods are Energetically Costly for Turtle Embryos

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Author's contribution

This whole work was carried out by author DTB.

Original Research Article

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ABSTRACT

Aims: To test the hypothesis that similar sized turtle eggs with longer incubation periods have a greater energetic cost of producing a hatchling compared with eggs that have a shorter incubation period.
Study Design: Eggs of the Eastern snake-neck turtle (*Chelodina longicollis*) were incubated at 26ºC and their oxygen consumption measured throughout incubation and these data compared to that from eggs of the Brisbane river turtle (*Emydura macquarii*) incubated at 26ºC.
Place and Duration of Study: The University of Queensland St Lucia Campus, November 2009 - February 2010.
Methodology: Eggs were collected and incubated at 26ºC and their rate of oxygen measured at regular intervals throughout incubation. Total energy expended during incubation was calculated by integrating the area under the rate of oxygen consumption versus time curve.
Results: Incubation period of *C. longicollis* eggs (83.1±0.5 d, N=12) and hatchling production cost (8.94±0.52 kJ/g, N=12, dry yolk-free mass basis) were significantly greater ($P<0.001$ and $P=0.008$ respectively) than the incubation period (61.8±0.3 d, N=11) and hatchling production cost (7.33±0.11 kJ/g, N=11) of *E. macquarii* eggs. These findings are consistent with the hypothesis that longer incubation periods incur a greater energetic cost because embryo tissue has to be maintained for a longer period of time and results in a greater amount of energy spent on maintaining the embryo.

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1. INTRODUCTION

The energy available for reproduction in oviparous animals can be packaged in different ways. For example, the same amount of energy could produce a relatively large number of small sized eggs, or a relatively small number of large sized eggs. However, once an egg is formed the amount of energy available for embryonic development is constrained by the amount of energy deposited into the egg while being formed in the mother's reproductive tract. In turtles, the majority of this energy is contained in the yolk which is manufactured in the ovary before ovulation, but some is added in the form of albumen as the egg passes down through the oviduct after ovulation on its way to be shelled in the uterus [1]. At hatching, energy is contained in the hatchling tissue and also in the residual yolk, which is the remainder of the original yolk that is absorbed into the abdominal cavity just before or immediately after hatching [1]. At hatching, turtles contain an average of 67% of the energy that was in the freshly laid egg [2], the remaining 33% being used to fuel metabolism in the developing embryo.

As energy in animals is obtained from the food they eat, and food in many environments is a limiting resource, it is frequently assumed that selection favors the efficient use of energy. In embryonic development, it is assumed that selection maximizes the amount of energy being transferred from the freshly laid egg into the hatchling [2]. Energy expenditure during embryonic development can be conceptually divided into two components: (1) Energy used to convert raw yolk and albumen material into cells and tissues, and (2) energy used to maintain the tissue once it has been synthesized which includes the energetic cost of further differentiation and maturation of cells [3,4]. If it is assumed that the energetic cost of manufacturing a gram of tissue from raw egg material is similar across a taxon (because in theory, the energy required to produce a quantity of embryonic tissue depends only on the composition of the tissue and the substrates used for anabolism [5,6]) the chemical reactions used to convert this raw material to tissue are similar across the animal kingdom and thus require the same amount of energy to drive them, despite differences in growth rate), then for hatchlings of the same size, the variable that can cause differences in total energy expenditure is the amount of energy used to maintain embryonic tissue during the incubation period. According to this logic, longer incubation periods that result in slower growth rates also require a greater amount of energy being spent on tissue maintenance because tissue, once it has been formed, is maintained for a relatively longer period before hatching occurs and this should lead to greater overall energy expenditure during embryonic development [3,4]. Hence the hatchling energetic production cost (total energy expended during embryonic development divided by the yolk-free hatchling dry mass, units J/g) should be greater in species that have longer incubation periods. Here I test this hypothesis by measuring the energy expenditure during embryonic development of the Eastern snake-necked turtle *Chelodina longicollis* and comparing it to the energy expenditure previously reported for the Brisbane river turtle *Emydura macquarii* [7]. Both species lay similar sized eggs (5-10g), but *C. longicollis* has an incubation period of 82d when incubated at 26ºC [8] compared to just 62 d for *E. macquarii* [7]. Both species can be found nesting in the same area at the same time of year (November – January) and construct nests of a similar depth (10-15cm) and thus experience similar nest hydric and thermal conditions.

2. MATERIALS AND METHODS

Twelve freshly laid eggs *C. longicollis* eggs were obtained on 13 Nov 2009 from the University of Queensland's St Lucia campus (27º 32'S, 153º 00'E). Eggs were weighed and then incubated buried in moist river sand (24g of water added to 2000g dry sand corresponding to a water potential ~ -50 kPa) in a plastic container with a loose fitting lid and placed in a constant temperature cabinet set at 26ºC. After two weeks of incubation, oxygen consumption of eggs was measured weekly. During oxygen consumption measurements, the water lost from sand was replaced and the sand thoroughly mixed to insure a relatively constant water potential throughout incubation.

Oxygen consumption of eggs was measured using closed respirometry. A 60ml syringe had a 0.1ml water drop added to it (to insure the atmosphere inside the syringe would be saturated with water vapor) and an egg placed inside it. Syringes containing eggs were placed in a 26ºC incubator for periods of between 50-130 min (as embryos became older they were left in the syringe for shorter periods). At the end of this measurement period a 40mL gas sample was injected through soda lime (to absorb carbon dioxide) and then drierite (to remove water vapour) into a previously calibrated (zero with high purity nitrogen; span with carbon dioxide free, water vapor free room air) paramagnetic oxygen analyzer (PAROX 1000, MBE Electronic AG, Switzerland). Oxygen consumption was calculated according to equation 9 of Vleck [9] assuming the syringe atmosphere was saturated with water vapor and that egg density was 1g/mL. Oxygen consumption of *E. macquarii* eggs was also measured by closed system respirometry using the methods described for *C. longicollis* eggs.

The day of pipping (when the eggshell is first broken) and the day of hatching (when hatchling has completely left the shell) were recorded. Hatchlings were then weighed and euthanized by first chilling to 5ºC and then being placed in a freezer. Hatchlings were dissected while still frozen, and the residual yolk in the abdominal cavity was removed and weighed along with the carcass. The residual yolk and carcass were dried to constant mass in a drying oven at 50ºC for 48h.

Total energy consumed throughout incubation for each embryo was calculated by integrating the area under the oxygen consumption verses days of incubation plot to obtain the total volume of oxygen consumed by each embryo. This volume of oxygen was converted to joules assuming the principal substrate metabolized was lipid and using a oxy-joule equivalent of 19.79J/mLO2 [3]. Energetic production cost was calculated by dividing the total energy consumed by a hatchling during embryonic development by its dry yolk-free mass.

Results are presented as means ± SE. Statistical comparisons between *C. longicollis* and *E. macquarii* eggs and hatchling were made using student's t-tests, with statistical significance being assumed at $\alpha<0.05$.

3. RESULTS AND DISCUSSION

C. longicollis eggs (n=12) had a mean mass of 6.88±0.18g, and all hatched successfully. Time to pipping was 81.5±0.5 d and time to hatching 83.1±0.5 d (Table 1). Oxygen consumption of *C. longicollis* embryos increased steadily between day 14 and day 40, increased rapidly between day 40 and day 63, reached a peak on day 70, and then decreased steadily until hatching (Fig. 1). Initial egg mass, hatching mass, dry yolk-free body

mass and dry residual yolk mass were similar in *C. longicollis* and *E. macquarii* (Table 1), but incubation period, peak oxygen consumption, total energy consumed during incubation and hatchling production cost were significantly different (Table 1).

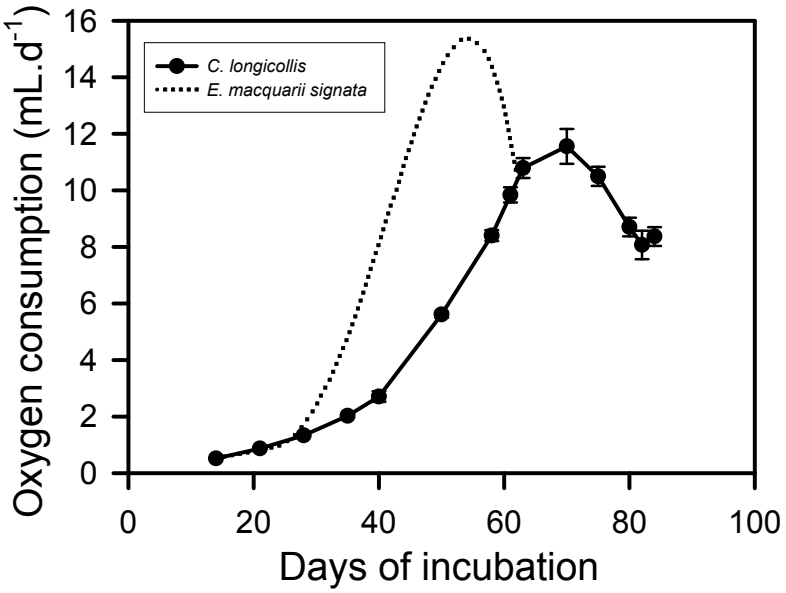

Fig. 1. Oxygen consumption of *C. longicollis* embryos incubated at 26°C. Data points represent the means and standard errors of the means for 12 eggs. Dotted line represents the oxygen consumption of a 6.9g *E. macquarii* egg incubated at 26ºC [7] for comparison

Table 1. Incubation and hatchling parameters of *C. longicollis* and *E. macquarii* eggs incubated at 26ºC. Values for *E. macquarii* are from data recorded from 11 eggs originally reported in Booth [7]. All data for both species passed the Kolmogorov-Smirnov test for normality. Means were compared for significant differences using Student's t-tests

Parameter	*C. longicollis*	*E. macquarii*	Probability of a significant difference
Number of eggs used	12	11	
Initial egg mass (g)	6.88±0.18	6.94±0.20	*P*=0.800
Incubation period (d)	83.1±0.5	61.8±0.3	*P*<0.001
Peak oxygen consumption (ml/d)	12.1±0.4	15.1±0.4	*P*<0.001
Total energy consumed during incubation (kJ)	7.77±0.19	6.99±0.14	*P*=0.010
Hatchling body mass (wet, including residual yolk) (g)	3.769±0.169	3.977±0.088	*P*=0.332
Dry yolk-free body mass (g)	0.896±0.046	0.954±0.027	*P* =0.299
Dry residual yolk mass (g)	0.053±0.008	0.068±0.004	*P*=0.077
Production cost (kJ/g dry yolk-free hatchling)	8.94±0.52	7.33±0.11	*P*=0.008

Despite having similar egg mass, the incubation period of *C. longicollis* eggs was 34% longer than *E. macquarii* eggs. In the Murray river region of Victoria (35º56.5' S, 144º14' E) where female *E. macquarii* and *C. longicollis*, nest at the same time, natural incubation periods are reported to be 75d and 138d respectively [10]. These incubation periods are considerably longer than those reported here, implying that mean nest temperature in the Murray river region of Victoria is lower than 26ºC. Embryonic oxygen consumption of both species increased slowly for the first half of incubation, was followed by a rapid increase that reached a peak ~80% through incubation, which was followed by a decrease until hatching occurred. The peak rate of oxygen consumption was 34% higher in *E. macquarii* embryos, but *C. longicollis* embryos consumed 11% more oxygen over the entire incubation period and thus expended more energy during incubation than *E. macquarii* embryos. This pattern of oxygen consumption is typical for embryonic turtles and is best explained by an embryonic energy expenditure model [3,4] where embryonic growth (in terms of grams of tissue synthesized per day) starts off slowly, increases exponentially until 80% of incubation, and then slows dramatically during the last 20% of incubation (Fig. 2). Accordingly, the amount of energy used to synthesize tissue from yolk and albumen is similar in both *C. longicollis* and *E. macquarii* (~3.1 kJ), but *C. longicollis* embryos use more energy for maintenance (~4.8 kJ) than E. macquarii embryos (~3.7 kJ) and this accounts for the difference in total energy consumed during development between the two species. The greater maintenance component of *C. longicollis* embryos results in a 22% higher production cost compared with *E. macquarii*. This finding supports the hypothesis first proposed by Vleck et al. [3] for bird embryos, in which embryos that have longer incubation periods incur a higher energetic cost because their embryonic tissue must be maintained for a longer period of time than embryos with a shorter incubation period. A similar conclusion was made with embryonic development of komodo dragon (*Varanus komodoensis*) eggs which have a prolonged incubation period and higher energetic cost of development compared to other lizard eggs [11].

The production cost of *C. longicollis* (8.9kJ/g) was significantly greater than that of *E. macquarii* (7.3kJ/g) and this could be attributed to the greater maintenance cost. Interestingly, in birds, embryonic production cost (mean 15.4kJ/g, [4]) is significantly greater (*P*=0.008, student's t-test) than that of turtles (9.9kJ/g, [2] and the two species reported here). The higher production cost of avian embryonic development can be attributed to the higher maintenance metabolism component caused by the higher metabolic rate of avian tissue [12,13].

Given that longer development times can result in greater embryonic energy expenditure, and that energy is usually at a premium, the question arises: why hasn't development time been minimized in all turtle species? The answer to this question is bound to be complex, and probably different for individual species. Goode and Russell [10] examined the embryonic development of *C. longicollis* and *E. macquarii* and suggested that the difference in embryonic development rates of these two species was due to differences in orientation of the embryos within the egg. *E. macquarii* embryos have their vertebral axis parallel to the longest axis of the egg, whereas *C. longicollis* embryos have their vertebral axis at right angles to the longest axis of the egg which results in a tighter curvature of the body and thus might prolonged the yolk absorbance process and results in a prolonged incubation period [10]. However this hypothesis is unlikely because the pattern of oxygen consumption indicates that growth of *C. longicollis* embryos is slow throughout incubation, not just at the end of incubation when the residual yolk is being absorbed into the abdominal cavity.

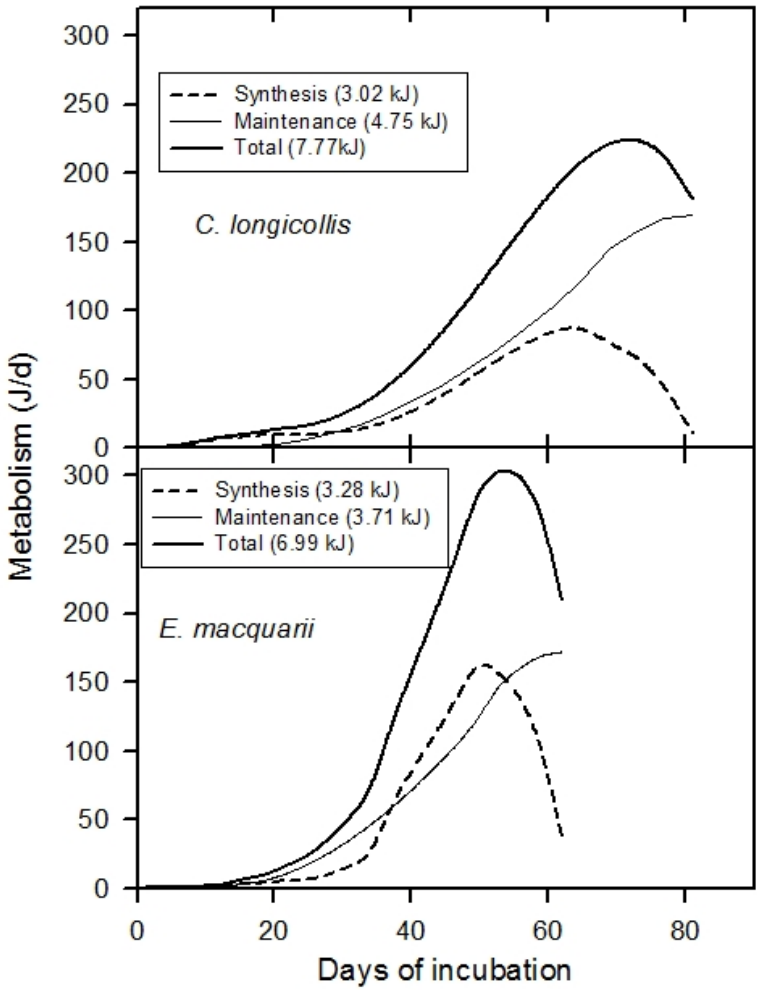

Fig. 2. The Vleck et al. [3] model of embryonic energy expenditure applied to *C. longicollis* and *E. macquarii* embryos incubated at 26ºC. Total metabolism curves calculated from oxygen consumption data assuming an oxy-joule equivalent of 19.79J/mLO_2 [3]. Maintenance metabolism curves derived assuming sigmoidal embryo growth and maintenance metabolism equivalent to the standard metabolic rate of turtles at 20ºC (allometric equation from Bennett and Dawson [14] adjusted to 26ºC assuming a Q_{10} of 2.5). Synthesis metabolism was calculated as the difference between total metabolism and maintenance metabolism. Total energy expenditure for each component was calculated by integrating the area under each curve and is reported as a number beside the line legend within the figure

4. CONCLUSION

Longer incubation periods result in a greater energetic cost of embryonic development in turtles. This added cost is not caused by greater tissue synthesis cost, but because embryonic tissue must be maintained for a longer period of time which results in higher maintenance cost.

ETHICAL APPROVAL

This work was approved by a University of Queensland animal ethics committee (approval number SBS/529/09) and under permit from the appropriate Queensland Government Department (Scientific Purposes permit WISP06725910) and complies with Australian Government animal experimentation regulations.

COMPETING INTERESTS

The author declares he has no competing interests in the publication of this paper.

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