THE PHYSIOLOGY AND ECOLOGY OF THE EGGS
OF THE PLEURODIRAN TORTOISE
EMYDURA MACQUARII (GRAY), 1831

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SUMMARY

The eggs of the Pleurodiran short-necked tortoise, *Bmydura maquarii*, are intermediate between the hard-shelled and hard, expansible-shelled eggs studied to date. Therefore aspects of the physiology and ecology of the eggs of *E. maquarii* were studied and compared to Cryptodiran species from other parts of the world. Measurements of physical characteristics of the eggs, including their mass, length, breadth, surface area, shell and membrane thicknesses, ultrastructure of the shell and membranes, and of the pores in the shell, were made to help describe and enable interpretations of experiments on the gas and water relations of the embryos.

Eggs of *E. maquarii* are 36.11 mm long x 22.02 mm wide (mean) and the mean mass is 10.423 g. There are positive semilogarithmic correlations between egg length and breadth, length and mass, and breadth and mass. Elongation is constant over the range of egg sizes. Egg mass is positively correlated with female size supporting the suggestion that egg breadth is limited by the dimensions of the mother's pelvis. Hatchlings are 48.06% of the fresh egg mass regardless of egg size or the conditions experienced during incubation. The egg is made up of yolk (37%), albumen (47%) and shell (14%). The yolk is 68% water, albumen 95% water and shell 22% water. The water content of fresh eggs and hatchlings is similar. The water content of embryos falls from 95% to 77% between the middle of the incubation and hatching. In unhatched eggs the shell is thickest around the equatorial plane (0.191 mm) and thinnest at the poles (0.155 mm). Shell from the equatorial plane is thinner (0.177 mm) and at the poles thicker (0.166 mm) in hatched eggs than unhatched ones. Membranes from the equator are as thick (0.06 mm) as those from poles of the egg.
As the structure of the shell is similar to that of other species of chelonians with well organised shells, the process of shell formation is the same. Pores are Types 1a and 3a(ii) of Board *et al* (1977) and are concentrated in the equatorial regions of the shell. There is no external cuticle. Counts of the number of pores per egg ranged from 103 to 17,720 ($\bar{x} = 3,673$) and the mean minimum diameter is $18.5 \mu$. Lines of intense dissolution on the inner surface of the shell of hatched eggs are assumed to be adjacent to blood vessels in the chorioallantois. The shell membranes are composed of five layers of fibres of different diameter.

The pattern of $O_2$ consumption ($\dot{V}_{O_2}$) of eggs of *E. macquarti* increases exponentially during the first 80% of the incubation, peaking at 87% of the way through the incubation and then falling to 61–82% of the peak value before hatching. There is a slight rise in the rate of respiration at hatching due to the efforts of the hatchling to free itself from the egg. Equal amounts of $O_2$ are consumed during incubation at $25^\circ C$ and $30^\circ C$ (580 ml), and mass-specific $\dot{V}_{O_2}$ is equal at both temperatures (110 ml.g$^{-1}$ of hatchling mass). Embryonic growth, indicated by mass, reflects the change in $\dot{V}_{O_2}$, increasing exponentially for the first 70–80% of the incubation and then slowing prior to hatching. $\dot{V}_{O_2}$ is related to embryonic mass (corrected to the water content of hatchlings) raised to the power 0.862, similar to the exponent for avian embryos (0.92), which supports the idea that the cost of biosynthesis in embryos raises this exponent above that for adult animals in general (0.75). Respiratory exchange ratio (RE) of 0.61 is not significantly different at $25^\circ$ and $30^\circ C$. Most lipid (94.2%) found in fresh eggs is contained in the yolk; lipid accounts for 76.7% of the energy used during incubation. Mean $Q_{10}$ is 2.87.

The different patterns of embryonic respiration shown by chelonians may allow synchronous hatching, as in some birds. Thus deeply buried
eggs, which experience essentially the same temperatures in any one clutch during incubation, grow at the same rate and hatch simultaneously, have an exponential pattern of embryonic respiration. In contrast, eggs at intermediate depths, e.g. *E. macquarii*, experience a range of temperatures in different parts of the nest and therefore develop at different rates. The period of time between the peak of respiration and hatching may be varied in different individuals to allow the synchronous hatching of eggs at slightly different stages of development.

In natural nests in the field $P_{O_2}$ is depressed by about 10 torr and $P_{CO_2}$ elevated by about 4 torr over controls just prior to hatching. The maximum $\Delta P_{O_2}$ and $\Delta P_{CO_2}$ across the dry shell, calculated from weight loss data over silica gel at constant temperature are 2.1 and 1.6 torr and from conductance values across the normally partially hydrated shell and membranes measured directly are 19.0 and 7.5 torr. The shell membranes are a more significant barrier to the diffusion of $O_2$ and $CO_2$ than is the dry shell. However both shell and membranes dry out regionally during development resulting in the formation of a small white patch on the uppermost surface of the egg within 30 h of being laid. This patch grows to eventually cover the whole egg surface in some eggs, thus facilitating gas exchange through the eggshell in advance of the requirements of the embryo. The conductance of shell and membranes to $O_2$ and $CO_2$ increases with the area of the white patch reaching $2.6 \pm 1.1 \text{ cm}^3\text{ day}^{-1}\text{ torr}^{-1}$ and $4.7 \pm 1.3 \text{ cm}^3\text{ day}^{-1}\text{ torr}^{-1}$ at $30^\circ C$ for eggs with the white patch covering the whole shell.

*E. macquarii* does not appear to select nesting sites in the field on the basis of the hydric properties of the soil. The tolerance of the eggs to widely different hydric conditions during incubation reflects this. Eggs were incubated completely buried in, half buried in, and sus-
pended above substrates ranging in water potential from -50 kPa to -3,550 kPa. Eggs buried at -50, -110 and -220 kPa and those half buried at -220 kPa had reduced hatching rates; all other treatments recorded high hatching success. All eggs lose weight for the first third of the incubation regardless of the water potential of the substrate. Eggs in the wettest substrates gain water at an increasing rate until hatching, those on drier substrates lose water at a constant rate. All eggs suspended above the substrate sustain a net loss of water. The shells of eggs that gain water crack to accommodate it. Eggs that successfully hatched ranged from those that lost 27.5% of their fresh egg mass to those that gained 32.0%. An experiment using fluorescein and tritiated water confirmed that eggs contacting the substrate absorb liquid water from that substrate but liquid water does not flow between eggs. Water potential measurements of egg contents indicate that water is drawn into the egg against a water potential gradient of up to 320 kPa but it is not known how this occurs. Desiccating conditions experienced during incubation do not result in abnormal hatchlings in *E. maoquarii* as they do in other species. The term cleidoic is discussed with regard to the degree of water uptake by non-cleidoic eggs. Eggs such as *E. maoquarii*, which imbibe water under certain conditions, but the effect of this does not influence the size of the hatchlings, are termed "facultative cleidoic" eggs.

Nests of *E. maoquarii* in the field experience temperatures less severe than the soil surface but still experience diurnal temperature fluctuations up to 10°C. The temperature of nests rises throughout the incubation period as the summer progresses. Metabolic heat does not measurably raise nest temperatures. Incubation time in the laboratory is inversely proportional to incubation temperature. Incubation time in the field is influenced by the degree of shading of the nest. Adult sex ratio is significantly biased towards females. Sex determination in
E. macquarii is independent of incubation temperature, with a 1:1.9 sex ratio at incubation temperatures of 20, 25, 26, 28, 30 and 32°C. Temperature-dependent sex determination may be an adaptation that ensures outbreeding and has its greatest advantage in small discrete populations with relatively high rates of predation on the eggs.

On the River Murray in South Australia the introduced fox, Vulpes vulpes, takes 93% of chelid tortoise nests and other predators (water rats, goannas and ravens) take a further 2.7%. Death of eggs due to other causes is rare. The size structure of the population of E. macquarii in the Murray was compared to that of a closely related species from the Cooper Creek, which lives in the virtual absence of foxes, but which has essentially the same endemic nest predators that occur on the Murray. There are a significantly higher number of juveniles and young adults in the Cooper Creek than the Murray population. There appears to be a significant reduction in juvenile recruitment into the Murray population of E. macquarii resulting in a shift in the age structure of the population towards old individuals resulting in a gradual decline in the size of the population.