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## Nest temperature and respiratory gases during natural incubation in the broad-shelled river turtle, *Chelodina expansa* (Testudinata : Chelidae)

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### Abstract

Temperature was monitored in three natural nests, and oxygen and carbon dioxide partial pressure monitored in one natural nest of the broad-shelled river turtle, *Chelodina expansa*, throughout incubation. Nest temperature decreased after nest construction in autumn, remained low during winter and gradually increased in spring to a maximum in summer. In a nest where temperature was recorded every hour, temperature typically fluctuated through a 2°C cycle on a daily basis throughout the entire incubation period, and the nest always heated faster than it cooled. Oxygen and carbon dioxide partial pressures in this nest were similar to soil oxygen and carbon dioxide partial pressures for the first 5 months of incubation, but nest respiratory gas tensions deviated from the surrounding soil over the last three months of incubation. Nest respiratory gas tensions were not greatly different from those in the atmosphere above the ground except after periods of rain. After heavy rain during the last 3 months of incubation the nest became moderately hypoxic ( $P_{O_2} \sim 100$  Torr) and hypercapnic ( $P_{CO_2} \sim 50$  Torr) for several successive days. These short periods of hypoxia and hypercapnia were not lethal.

### Introduction

All turtles bury their eggs in soil or sand and leave them to incubate with no further assistance. The thermal and hydric nature of the nest environment is very important to the incubating eggs, influencing embryonic mortality, and has been shown in laboratory experiments to affect hatchling quality in many species, as reflected by hatchling sex (e.g. Bull 1980), size (e.g. Packard *et al.* 1987), and locomotor performance (e.g. Miller *et al.* 1987; Janzen 1995). Hence selection of a nest site with an appropriate microenvironment is likely to be an important factor in a species' reproductive strategy because this can influence the quality of hatchlings emerging from the nest. Soil environments are often sufficiently moist to prevent desiccation during embryonic development, but underground environments can become hypoxic and hypercapnic due to the metabolism of soil micro-organisms and the developing embryos (Seymour and Ackerman 1980). However, because most turtles construct shallow nests (often less than 20 cm deep), the proximity of the eggs to the soil's surface probably prevents the gas tensions of oxygen and carbon dioxide in the nest environment from deviating too far from atmospheric levels (Ackerman 1977), although currently there is a paucity of data to confirm this prediction. Another consequence of incubation in shallow nests is that there are bound to be diurnal fluctuations in nest temperature, particularly in nests that are exposed to direct sunshine for at least part of the day.

Here I report data on temperature in three natural nests and on oxygen and carbon dioxide tensions in one of these nests throughout incubation in the broad-shelled river turtle, *Chelodina expansa* Gray. *C. expansa* is distributed in rivers and streams of south-east Queensland and the Murray–Darling river system (Cogger 1996) and is peculiar amongst Australian turtles in having an extremely prolonged natural incubation period (longer than 300 days: Goode and Russell 1968; Georges 1984). This species also breeds in late autumn or early winter compared with the more usual spring and early summer of other Australian freshwater turtle species with a non-tropical distribution.

### Material and Methods

A small population of *Chelodina expansa* inhabits the waste-water treatment ponds at the University of Queensland's St. Lucia campus located near Brisbane (27°32'S, 153°00'E). During 1995 I excavated two freshly constructed *C. expansa* nests and placed a miniature temperature-sensitive transmitter amongst the eggs 16 cm below the ground's surface. These nests were reconstructed and left until being excavated at the end of incubation. Nest 2 was constructed on 22 April and exposed to sunshine in the morning but was shaded by a tree in the afternoon while Nest 1 was constructed on 20 August and exposed to sunshine all day. Nest 3 was constructed on 2 June 1996 in the same location as Nest 2. This nest was excavated and a miniature data-logger programmed to log temperature every hour was placed amongst the eggs 16 cm below the surface along with a length of fine-bore tygon tubing that led to the surface. A 22-gauge needle stopped with a stop-cock was fitted to the tube end at the surface. At the same time, I placed another length of tubing 16 cm below the ground's surface 50 cm from the nest so that gas tensions in the soil adjacent to the nest could be measured. On days when gas samples were taken, the stop-cock was removed, the dead space in the tubing removed by withdrawing 5 mL of gas from the nest, and then a 60-mL sample of nest gas was taken into a syringe. The same process was applied to the soil-sampling tube. Gas in the syringes was immediately returned to the laboratory where 10 mL was injected into a previously calibrated thermostated oxygen electrode cell for measurement of oxygen partial pressure, and 50 mL was injected through water-absorbent (drierite) into a previously calibrated infra-red carbon dioxide analyser to measure carbon dioxide concentration. This measurement was converted into a carbon dioxide partial pressure using ambient barometric pressure and water vapour saturation pressure at the nest temperature (assuming that the nest and soil atmosphere was always saturated with water vapour).

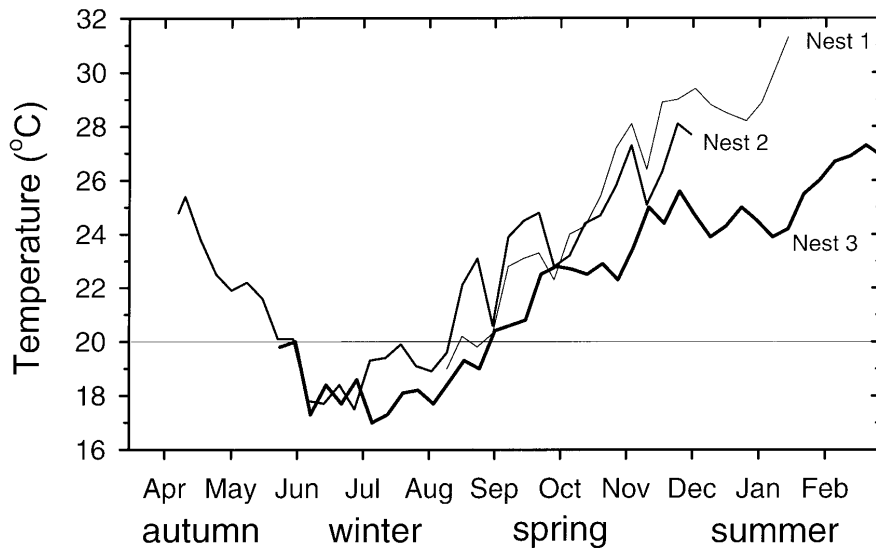
Water vapour conductance of nine *C. expansa* eggs collected from two non-monitored nests was measured in the laboratory after 180 days of incubation at 26°C, which was three-quarters of the way through embryonic development. Eggs were placed on 100 g of Drierite in 150-mL sealed vials (one egg per vial) for 30 min, after which they were weighed on an electronic balance. Barometric pressure was recorded at this time. They were then placed back in the sealed vials and incubated for a further 60 min at 26°C before being weighed again. Water vapour conductance was calculated from the mass loss of eggs during this second period according to standard methodology (Booth 1992). The metabolism of embryos within the eggs would have tended to increase egg temperature slightly above ambient. In contrast, the evaporation of water from the eggs would have tended to decrease egg temperature. The net effect would have been a slight cooling of the egg. Even if the egg temperature was 0.5°C cooler than ambient air only a slight error (<2%) would have been made in calculating water vapour conductance.

### Results

Nests 1, 2 and 3 contained 14, 16 and 12 eggs, respectively. At the end of the monitoring period (Nest 1, 155 days; Nest 2, 277 days; Nest 3, 285 days), nests were excavated. The contents of eggs in Nests 1 and 2 had dried, but embryonic development had been well advanced before desiccation killed the embryos. In Nest 3, 10 of the 12 eggs hatched successfully and the hatchlings were alive and vigorous when the nest was excavated but had not dug their way out of the nest.

#### *Nest temperature*

On three occasions (16 June, 8 September and 2 December) temperature was measured every hour from 0500 to 2200 hours in Nests 1 and 2. On each occasion temperature minima were recorded at 0800 hours and temperature maxima recorded at 1600 hours in both nests. For the rest of the monitoring period nests were visited daily at 0800 and 1600 hours so that temperatures close to daily minima and maxima were recorded. In these nests the mean of the



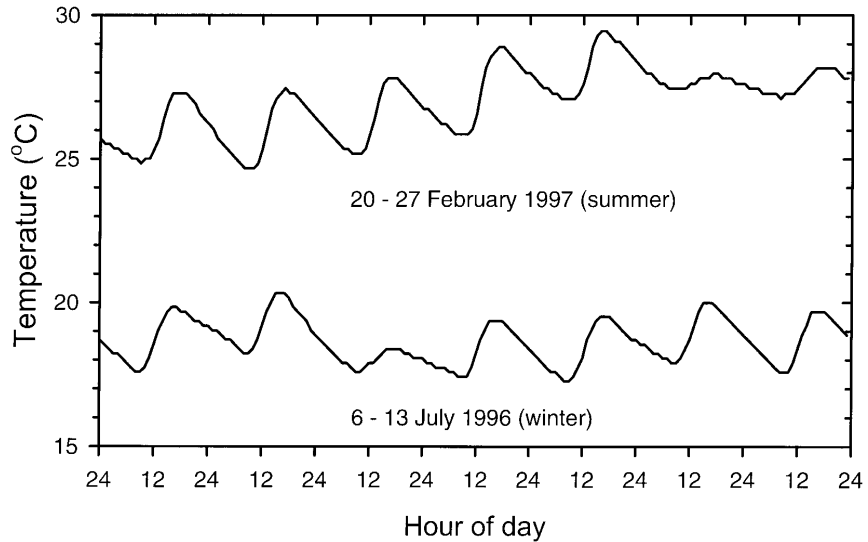
**Fig. 1.** Average weekly temperature of three *Chelodina expansa* nests. Nests 1 and 2 were constructed in 1995 and nest temperature was measured twice daily by temperature sensitive transmitters so each weekly average is based on 14 data points. Nest three was constructed in 1996 and nest temperature recorded every hour, so each weekly average is based on 168 data points. Thin horizontal line at 20°C indicates the temperature below which embryonic development is extremely slow.

minima and maxima temperatures was calculated to estimate the average daily nest temperature. A weekly mean of average daily nest temperature was then calculated and plotted for the duration of the monitored period (Fig. 1). Hourly temperature from Nest 3 was averaged each week and plotted for the duration of the monitored period (Fig. 1). In Nest 3 the mean daily nest temperature estimated from daily minima and maxima was within 0.5°C of mean daily nest temperature estimated from all 24 hourly readings. Thus the method used for estimating mean daily nest temperature for Nests 1 and 2 was appropriate. The general pattern of seasonal nest temperature was the same for all three nests: temperature decreased in autumn from April to June, remained low throughout winter from June through August, and then increased through spring and summer from August to February (Fig. 1). The greater temporal resolution of the temperature record from Nest 3 enabled some more generalisations. Nest temperature fluctuated cyclically by 2°C on most days, including both winter and summer (Fig. 2), except on overcast or rainy days, when the daily temperature cycle was less than 2°C (Fig. 2). In both summer and winter minimum temperatures were recorded at 0800 or 0900 hours and maximum temperatures at 1600, 1700 or 1800 hours (Fig. 2). The nest always heated faster than it cooled, taking 8–9 hours to heat from the minimum to maximum temperature, and 15–16 hours to cool from the maximum to minimum temperature (Fig. 2).

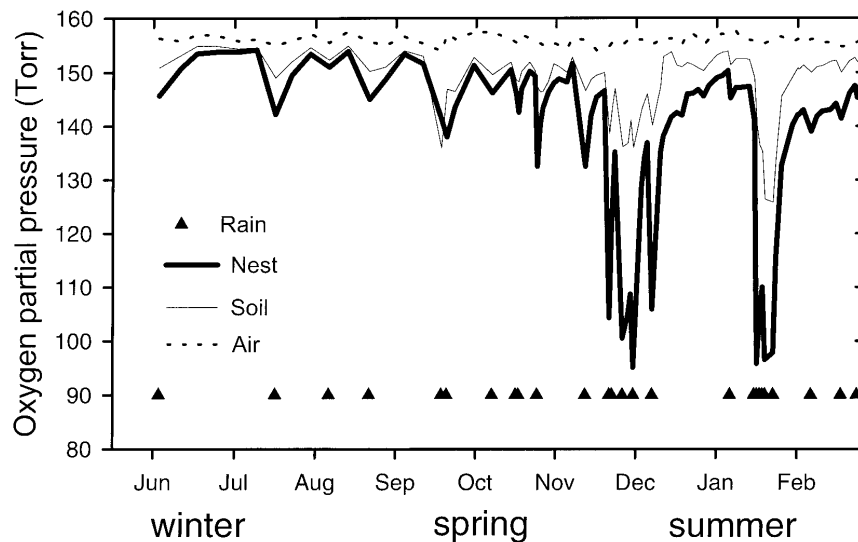
#### *Nest gases*

Except for one occasion after rain in September 1996, oxygen partial pressure ( $P_{O_2}$ ) in Nest 3 was always lower than in the soil, and this difference became greater towards the end of the incubation period (Fig. 3). Carbon dioxide partial pressure ( $P_{CO_2}$ ) in the nest and soil mirrored the changes in  $P_{O_2}$ , and the difference between the soil and nest  $P_{CO_2}$  also became greater at the end of incubation (Fig. 4). Rain always caused oxygen and carbon dioxide tensions in the nest and soil to become more extreme, with heavier rains causing greater perturbations. These perturbations were most extreme during the last three months of incubation (Figs 3, 4). Gaseous

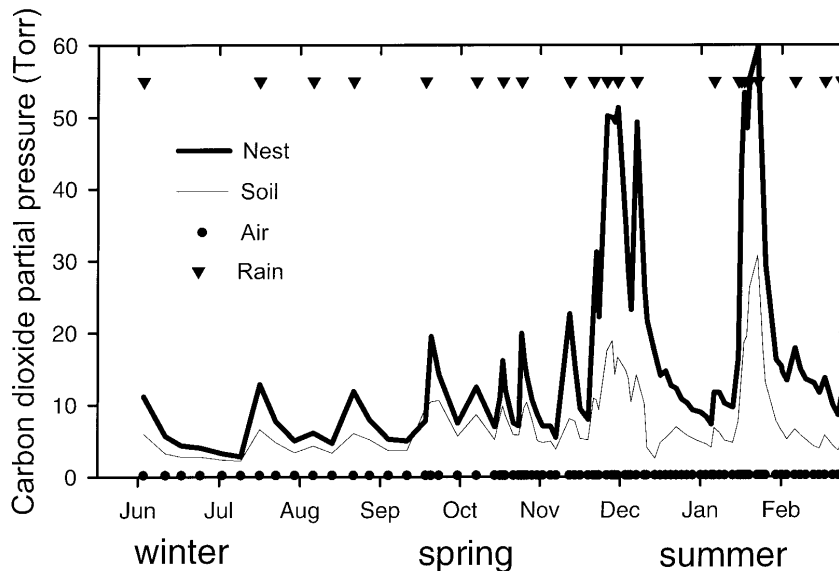
conditions within the nest became moderately hypoxic ( $P_{O_2} < 100$  Torr) and moderately hypercapnic ( $P_{CO_2} > 50$  Torr) for several days after heavy rain during the last three months of incubation (Figs 3, 4).



**Fig. 2.** A sample of hourly nest temperature over a week period in winter and summer in *Chelodina expansa* nest three. Note daily cyclic temperature fluctuations of approximately  $2^{\circ}\text{C}$  in both winter and summer, and that heating occurs faster than cooling. Also note that minimum and maximum temperatures occur at the same time of day in summer and winter.



**Fig. 3.** Oxygen partial pressure in the nest, in soil adjacent to the nest and in the atmosphere above the ground throughout the incubation period of *Chelodina expansa* nest three. Filled triangles indicate rainfall events. Sharp decreases in oxygen partial pressure in the soil and nest correspond with rain, with deeper troughs being caused by heavier rain.



**Fig. 4.** Carbon dioxide partial pressure in the nest, in soil adjacent to the nest and in the atmosphere above the ground throughout the incubation period of *Chelodina expansa* nest three. Filled inverted triangles indicate rainfall events. Sharp increases in carbon dioxide partial pressure in the soil and nest correspond with rain, with higher peaks being caused by heavier rain.

#### Water vapour conductance

Water vapour conductance of nine *C. expansa* eggs three-quarters of the way through development at 26°C and corrected to 1 atmosphere of pressure was  $134.4 \pm 4.0$  (s.e.)  $\text{mg day}^{-1} \text{Torr}^{-1}$ .

#### Discussion

##### Nest temperature

As expected, the seasonal pattern of nest temperature follows that of soil temperature, which is decreasing when most nests are constructed in autumn, remains at a minimum over winter and increases during spring and summer. Trends of increasing nest temperature with time are common in turtle species that nest in spring and early summer (Congdon *et al.* 1987; Thompson 1988). Despite the general similarity of the pattern of nest temperature in all three nests there are some differences. Nest 1 and 2 existed at the same time, but Nest 1 received sunshine for the entire day while Nest 2 was shaded by a tree in the afternoon. As a consequence, the average temperature experienced in Nest 1 from October onwards was greater than in Nest 2 (Fig. 1). This effect of shade on nest temperature has previously been noted (Congdon *et al.* 1987; Thompson 1988; Bodie *et al.* 1996). Differences between years were also apparent. Nests 2 and 3 were constructed within one metre of each other but in different years and both received shade in the afternoon. Temperatures in these nests were similar from June to mid-July, but Nest 3 was notably cooler from mid-July onwards (Fig. 1), presumably because 1996 experienced more cloudy and rainy weather than 1995.

In the laboratory, early-stage *C. expansa* embryos develop at a very slow rate at temperatures between 18°C and 20°C (author's unpublished data). Also, most embryos of this species diapause at the beginning of development (author's unpublished data). Hence, during autumn when nest temperature is dropping and during winter when the temperature remains below 20°C

there is little embryonic development and this contributes in part to the unusually long incubation period of *C. expansa*. However, from September onwards mean nest temperature exceed 20°C and reach 27–31°C during summer (Fig. 1), but embryos in Nest 3 still took five months to reach the hatching stage. Growth and development of *C. expansa* embryos is inherently slower than that of other species of Australian turtles (Goode 1966; Goode and Russell 1968), and this is another reason for the prolonged natural incubation period in *C. expansa*.

Nest 1 was constructed at the very beginning of spring, much later than is usual for this species and may represent a female's second clutch for the year (Booth 1998). Although this clutch of eggs was laid some 4–5 months later than usual, the hatching date of such a clutch may not be greatly different from clutches laid in autumn if the nest is exposed to sun all day. For example, embryos in Nest 2, which was constructed in April, were probably not very advanced in development when eggs in Nest 1 were laid in August because of the cold nest temperatures over the autumn–winter period. However, from October onwards embryos in Nest 1 experienced warmer temperatures and would have developed faster than embryos in Nest 2. Thus it is probable that the two clutches would have reached the hatching stage of development at a similar date if field conditions had been wetter and the embryos had not perished by desiccation. Indeed, clutches of *C. expansa* eggs laid in September on the Murray River at Patho, Victoria (35°57'S, 144°14'E), were observed to hatch at 'about the same time' as clutches laid five months earlier (Goode and Russell 1968).

An unexpected finding was that the daily fluctuation in Nest 3's temperature was typically 2°C in both winter and summer (Fig. 2) and that the peak and trough of temperatures occurred at the same time of day in both winter and summer (Fig. 2). Because solar radiation is more intense and day length longer in summer than in winter, I expected to see a greater daily fluctuation in nest temperature and the peak temperature occurring later in the day in summer than in winter. A possible explanation for not finding these differences is that during summer this nest was shaded by a tree from about 1400 hours onwards, but due to a different sun angle the nest did not become shaded until about 1600 hours during winter. There were many days when peak temperature did not rise more than 0.5°C above the overnight low temperature. On these occasions heavy cloud cover or rain prevented the sun from warming the ground to its usual degree. Cloud and rain have also been reported to decrease diurnal temperature fluctuations in nests of the long-necked turtle, *Chelodina longicollis* (Palmer-Allen *et al.* 1991). Typically, the reported daily fluctuation in nest temperature of freshwater turtles is greater than 2°C, with values of 6–10°C being reported (Thompson 1988; Palmer-Allen *et al.* 1991; Georges 1992; Bodie *et al.* 1996). The magnitude of the daily fluctuation is chiefly affected by nest depth, shallower nests having greater fluctuations (Shul'gun 1957). *C. expansa* constructs deeper nests than most of the species referred to in the studies listed above, and this partly explains the difference, but the fact that the monitored nest was shaded for part of the day probably also caused a smaller fluctuation in nest temperature.

The finding that daily nest heating was always faster than daily nest cooling (Fig. 2) is of interest because theoretical models of eggs incubating in shallow nests usually assume that heating and cooling rates are identical (e.g. Georges *et al.* 1994). Previous studies that have examined temperatures in turtle nests have also found that nests heat faster than they cool (Palmer-Allen *et al.* 1991; Georges 1992; Bodie *et al.* 1996) but the authors of these studies failed to comment on the phenomenon. Obviously, heat entered the soil surrounding the nest faster during heating than it left the soil during cooling because of differences in the complicated interchange of heat within the soil and between the soil and the above-ground environment. The biological significance of the heating phase being faster than the cooling phase is unknown, but it should lead to shorter incubation periods compared to a situation where the heating and cooling rates are the same because the embryo will spend a greater portion of the day at a higher temperature, and development is more rapid at higher temperature.

*Respiratory gases in the nest*

During the first six months of incubation (i.e. until December) the  $P_{O_2}$  and  $P_{CO_2}$  in Nest 3 remained above 150 Torr and below 10 Torr, respectively, in between rain episodes (Figs 3, 4). Nest gas tensions were similar to those in the surrounding soil and no more than 10 Torr different from the atmosphere above the ground. This result was expected because the nest was relatively shallow (middle of nest 16 cm below ground surface) and the clutch mass (156 g) small enough that the total oxygen demand of, and the carbon dioxide production by, the entire clutch of early- and mid-stage embryos was relatively small. However, during dry periods over the last three months when the oxygen consumption of, and the carbon dioxide production by, the embryos reached its peak, oxygen tensions within the nest became lower and carbon dioxide tensions higher than in the surrounding soil, and averaged 140 Torr and 14 Torr respectively. These deviations from atmospheric levels were relatively minor and probably had no effect on embryonic metabolism. However, even in this shallow nest moderate hypoxia and hypercapnia were experienced by embryos for several days at a time during and immediately after heavy rain from December onwards (Figs 3, 4). Whitehead (1987) also observed a marked increase in hypoxia and hypercapnia after heavy rain in a nest of the crocodile *Crocodylus johnstoni*. The oxygen and carbon dioxide tensions experienced by developing *C. expansa* embryos inside the shell during these periods can be estimated using Fick's law of diffusion (for oxygen):

$$\text{Oxygen consumption} = G_{O_2} \times (P_{O_2} \text{ inside shell} - P_{O_2} \text{ in nest}) \text{ (Paganelli } et al. \text{ 1978).}$$

Eggshell oxygen conductance ( $G_{O_2}$ ) can be estimated from water vapour conductance measurements through the use of the ratio of the diffusion constants of water vapour and oxygen (Paganelli *et al.* 1978). Water vapour conductance of *C. expansa* eggs at this stage of development was  $134.4 \text{ mg day}^{-1} \text{ Torr}^{-1}$ , which corresponds to an oxygen conductance of  $142.1 \text{ mL day}^{-1} \text{ Torr}^{-1}$ . Peak oxygen consumption of a *C. expansa* egg at  $26^\circ\text{C}$  is  $15.84 \text{ mL day}^{-1}$  (author's unpublished data) and  $P_{O_2}$  nest is 100 Torr. These values give a difference in  $P_{O_2}$  across the shell of just 0.1 Torr, resulting in a  $P_{O_2}$  inside the shell of 99.9 Torr. Similar calculations for carbon dioxide (assuming an RQ of 0.75 for embryonic metabolism) gives a  $P_{CO_2}$  inside the shell of 50.1 Torr, just 0.1 Torr greater than for the nest gas. Clearly, the highly porous nature of the eggshell late in incubation, as indicated by the large water vapour conductance, results in negligible differences in respiratory gases between the inside and outside of the eggshell.

The extremes in respiratory gas tensions found in the *C. expansa* nest after heavy rain during the last three months of incubation ( $P_{O_2} \sim 90\text{--}100$  Torr;  $P_{CO_2} \sim 50\text{--}60$  Torr) are similar to those found in nests of the sea turtles *Chelonia mydas* and *Caretta caretta* during late incubation (Ackerman 1977). However, the sea turtle nests were constructed in sand, were much deeper (middle of nest 50 cm from the surface) and contained a much larger mass of eggs so that the peak oxygen demand of these nests ( $300\text{--}390 \text{ mL h}^{-1}$ ; Ackerman 1977) was about 50 times greater than the peak oxygen demand of the *C. expansa* nest examined ( $6.6 \text{ mL h}^{-1}$ ). In sea turtle nests the large oxygen demand and carbon dioxide production of the embryos late in incubation are responsible for the large difference in respiratory gas tensions between the nest and the above-ground atmosphere. In contrast, it is a change in the gas conductance of the soil associated with rain that is responsible for the extreme respiratory gas tensions in the *C. expansa* nest. Gas conductance of the soil decreases with increased water content because the tiny gas-filled interstitial spaces surrounding soil particles become filled with liquid water. This greatly impedes the movement of oxygen and carbon dioxide through the soil because gas movement occurs by diffusing through the interstitial spaces between soil particles (Ackerman 1977; Seymour *et al.* 1986) and diffusion through water is several orders of magnitude slower than through air. In the case of Nest 3, how extreme the respiratory gas tensions in the nest became was related to how much rain fell: the heavier the rain, the greater the increase in the water content of the soil, which caused a greater decrease in the gas conductance of the soil and



resulted in more extreme respiratory gas tensions. Once rain ceased, liquid water drained downwards away from the top soil or was lost by evaporation to the atmosphere above the soil and resulted in a decreased water content of the soil, which caused the gas conductance of the soil to increase again. Hence, after rain ceased, the respiratory gas tensions in the nest gradually became less extreme. The temporary exposure of embryos to moderate hypoxia and hypercapnia was not lethal, but probably caused a temporary slowing of embryonic metabolism, as occurs in other turtle embryos (Kam 1993; Kam and Lillywhite 1994), and thus may have retarded embryonic growth during this time.

Respiratory gas tensions at the end of incubation during non-rain periods in the nest of *C. expansa* examined here ( $P_{O_2} \sim 140$  Torr,  $P_{CO_2} \sim 14$  Torr) are similar to those found in the nests of the freshwater turtle *Emydura macquarii* at the end of incubation (Thompson 1981) and in the nesting mounds of the crocodylians *Alligator mississippiensis* (Booth and Thompson 1991) and the nests of the hole-nesting crocodylians *Crocodylus acutus* (Lutz and Dunbar-Cooper 1984) and *C. johnstoni* (Whitehead 1987). These limited data suggest that, with the exception of turtles that lay large numbers of eggs in the same nest, the respiratory gas tensions within turtle and crocodile nests usually deviate by a maximum of 15 Torr from those in the atmosphere above the ground. Such small deviations are unlikely to affect the physiology of developing embryos, but during heavy rain respiratory gases may become more extreme and this may impede embryonic development.

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