Wildl. Res., 1991, **18**, 225–31

Hatchling Sex Ratios are Independent of Temperature in Field Nests of the Long-necked Turtle, *Chelodina longicollis* (Testudinata : Chelidae)

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Abstract

Eastern long-necked turtles, *Chelodina longicollis*, are known to lack heteromorphic sex chromosomes and to lack temperature-dependent sex determination when incubated under constant conditions. This study determined whether sex ratios of hatchlings emerging from natural nests of *C. longicollis* were different from that expected from constant temperature experiments. Temperatures in the eight nests monitored varied considerably each day (by 1·7–12·6°C), with eggs at the top of the nest experiencing the greatest variation (mean range 9·0°C) and eggs at the bottom experiencing least variation (mean range 5·3°C). Temperatures experienced by the top and bottom eggs differed by as much as 5·7°C at any one time. No monotonic seasonal trend was evident, but rainfall caused a sharp drop in nest temperatures. Sex ratios in hatchlings from 14 field nests of *C. longicollis* did not differ significantly from 1 : 1, a result in agreement with previous studies conducted at constant incubation temperatures in the laboratory.

Introduction

The influence of temperature on the outcome of sexual differentiation in turtles is now well established, having been demonstrated for at least one species from each of eight families (see Bull 1980, 1983; Ewert and Nelson 1990). For most species, females are produced at high temperatures and males at low temperatures. A very narrow range of temperatures, referred to as the threshold or pivotal temperature, produces both males and females and divides the male-producing temperatures from the female-producing temperatures (Bull 1983). A few species have upper and lower thresholds with females produced at both extremes (Yntema 1976; Gutzke and Paukstis 1984). The critical period for sex determination, during which embryonic sex can be irreversibly influenced by temperature, is generally considered to lie in the middle third of development (Yntema 1979; Bull and Vogt 1981).

Although widespread, not all species of turtle have temperature-dependent sex determination (TSD). Three species with heteromorphic sex chromosomes (Bull *et al.* 1974; Sites *et al.* 1979; Carr and Bickham 1981) are assumed to have genotypic sex determination (GSD) (confirmed for *Staurotypus*, Ewert and Nelson 1990). Four species that lack heteromorphic sex chromosomes, and also lack TSD, include the Australian *Emydura signata* (Bull *et al.* 1985), *E. macquarii* (Thompson 1988*a*) and *Chelodina longicollis* (Georges 1988) of the family Chelidae.

1035-3712/91/020225$05.00

All experimental studies on sex determination in chelid turtles have been conducted in the laboratory at constant temperatures. There is very little information on sex ratios of chelid hatchlings emerging from natural nests. In natural nests, temperatures may vary as the season progresses and fluctuate widely each day in response to ambient variation in air temperatures and solar irradiation (Packard *et al.* 1985; Thompson 1988*b*). Results of field experiments on species with TSD have been as expected from constant temperature experiments (Bull 1985; Georges 1989), but similar comparisons have not yet been made for species that lack heteromorphic chromosomes and lack TSD, such as the Australian chelid turtles. Smith and Wood (1985) reported a 1:1 hatchling sex ratio from a nest of *Emydura victoriae* from the Northern Territory, which is in agreement with the predictions of genotypic sex determination, but these data from one nest are insufficient to eliminate the possibility that hatchling sex ratios of chelid turtles are affected by the complex thermal regime of natural nests in a way quite different from constant temperature experiments.

The eastern long-necked turtle, *C. longicollis*, inhabits permanent freshwater lakes and rivers and ephemeral ponds, rainpools and swamps in eastern and south-eastern Australia where its ecology has been much studied (Chessman 1984*a*, 1984*b*, 1988; Parmenter 1985; Georges *et al.* 1986; Kennett and Georges 1990). It is abundant in artificial ponds of the CSIRO Division of Wildlife and Ecology at Gungahlin, Canberra, where it leaves the water each November to deposit between 13 and 24 eggs in a shallow chamber (Vestjens 1969). The present study was designed to determine sex ratios of hatchlings emerging from natural nests of *C. longicollis* at Gungahlin and to relate these ratios to the thermal conditions that prevail during incubation. The aim of the study was to see whether sex ratios from natural nests of *C. longicollis*, a species lacking heteromorphic sex chromosomes (Bull and Legler 1980), differed markedly from those predicted on the basis of constant temperature experiments (Georges 1988).

Study Site

The Gungahlin ponds are a series of farm dams constructed to service the Gungahlin property before it became the CSIRO research station. The ponds are shallow (*c.* 1·5 m) with turbid waters and with emergent vegetation on the periphery. Vegetation adjacent to the pond is dominated by *Rumex* sp. and *Polygnum* sp. The emergent vegetation comprises *Juncus articularis*, *J. usitatus*, *J. bufonius*, *Cyperus* sp. and various *Eleocharis* spp. Vegetation in the areas used for nesting by the turtles is dominated by *Phalaris* sp. and other introduced grasses. The ponds are fenced, which prevents the entry of foxes and other predators in most years. Foxes prey heavily on both nesting female turtles and their nests in years when they gain access to Gungahlin (personal observation) and elsewhere (Thompson 1983). Soils surrounding the ponds are loamy clays (principally yellow podzols).

The climate of the Australian Capital Territory is temperate, with mean monthly maximum air temperatures ranging from 11°C in July to a peak of 27°C in January and February. Mean monthly minimum air temperatures ranged from 0°C to 13°C in the same months. The mean annual rainfall is 600 mm with a mean of 37 mm for July and a mean of 62 mm in February. It falls typically as showers and storms in summer and as gentle but prolonged rainfall in winter. Winds blow predominantly from the north-west in January with easterly sea breezes in the afternoons, and from the north-west in July (Canberra Airport, 1940–1974).

Materials and Methods

The study site was searched thoroughly for signs of nesting following rain, and at least twice per week in the absence of rain, between 20 November and 21 December 1987. Each of the 15 nests found was opened, taking care not to damage the nest plug which was placed to one side. Depth to the top egg was noted and the eggs were removed and numbered with a soft pencil. Egg length and width was measured (to ±0·1 mm) and the eggs were weighed (to ±0·1 g). Chamber depth was recorded. Eight fresh nests were chosen as typical of the range of nesting sites selected by the turtles and the eggs were replaced in order of their removal together with a temperature probe positioned at the centre of the egg mass. Nest 4 had probes placed immediately above the uppermost egg, in the centre of the nest and immediately below the deepest egg. The nest plug of each nest was replaced and sealed with a small quantity of moist soil.

Nest probes were NTC Bead Thermistors (1·259 Kohms at 20°C) calibrated against a mercury thermometer before the experiments began. Signals from the probes were recorded continuously from 16 to 24 December and from 6 to 23 February on a Chino Multichannel Electronic Temperature Recorder (Model ET 3200S, thermistor type), and traces were transferred by digitising tablet to computer for analysis.

The study site was checked regularly during late February and March for signs of hatching. When hatching occurred in an unmonitored nest on 6 March 1988, all monitored nests were opened and the eggs were returned to the laboratory to complete incubation at 28°C (±0·5°C) on moist vermiculite (4 g water per 3 g vermiculite). These eggs would all have developed beyond the middle third of incubation during which sex, if influenced at all by temperature, is considered to be determined (Yntema 1979; Bull and Vogt 1981).

Hatchlings were weighed (±0·1 g) after yolk internalisation and killed by intracranial injection of sodium pentabarbitone (Nembutal). The hatchlings were dissected and the urionogenital system examined. The condition of the paramesonephric duct (Mullerian duct) was scored as absent (0), very fine and visible at the anterior of the kidney only (1), fine but visible for the entire length of the kidney (2), or prominent (3). [These scores have been shown to correlate well with histological sex (Georges 1988) and were used to confirm assessment of sex based on histology.] The right gonad, kidney and associated ducts were removed, embedded in wax, sectioned, and dyed with haemotoxylin and eosin. The sex of each gonad was determined using a light microscope, by comparison with published photomicrographs of gonads (Leblond and Clermont 1952; Georges 1983; Miller and Limpus 1981).

Nest 14 contained a high proportion of hatchlings with abnormal bladders. The bladders were large and had adhered to the surface of the kidney obliterating the paramesonephric duct and, in some cases, the gonad. The sex of these hatchlings could not be determined. The sex of three hatchlings from the remaining nests also could not be determined histologically or from the condition of the paramesonephric duct, and was classed as indeterminate.

Chi-square analyses follow those recommended by Sokal and Rohlf (1981). Means are given with standard errors unless otherwise specified.

Results

Egg and Nest Characteristics

C. longicollis lays white hard-shelled ellipsoid eggs in a shallow chamber constructed with its hind limbs. Nest chambers were, on average, 10·4±0·7 cm deep ($n = 15$) with a depth to the uppermost egg of 3·7±0·3 cm ($n = 8$). Clutch size ranged from 9 to 16 eggs (mean 11·3±0·6, $n = 15$). The eggs were, on average, 31·2±0·1 mm long, 20·2±0·1 mm wide and weighed 7·3±0·1 g ($n = 169$).

Thermal Characteristics of Nests

Nest temperature varied considerably each day, with the pattern shown for nest 4 on 13 February (Fig. 1) typical of the daily cycle for days where temperatures were unaffected by recent rain. Minimum daily core temperature (taken at centre of egg mass) occurred consistently after sunrise in mid-morning whereas the maximum was generally achieved in the late afternoon or early evening. Core temperatures fluctuated between 1·7 and 12·6°C each day (Table 1). The hottest eggs at the top of nest 4 (monitored by three probes), experienced the greatest daily range in temperature (mean 9·0±0·6°C, range 2·7–15·2°C, $n = 27$) whereas the coolest eggs at the bottom experience least variation (mean 5·3± 0·3°C, range 2·1–8·5°C, $n = 27$). Temperatures experienced by the top and bottom eggs differed by as much as 5·7°C at any one time (mean daily maximum differential 3·25±0·3°C, $n = 27$).

No pronounced monotonic seasonal trend in nest temperatures was evident, although the thermal characteristics of nests were profoundly altered by rain. Rainfalls of 8 mm on 21 December and 18·4 mm on 22 December caused a sharp drop in nest temperatures (Fig. 2).

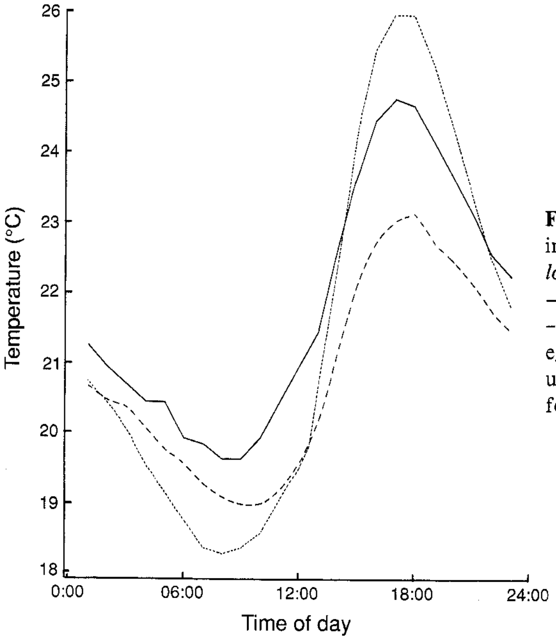

Fig. 1. Typical pattern of daily variation in temperatures within a nest of *Chelodina longicollis* at Gungahlin (December 1987). ——, variation in core temperature; - - - -, temperature adjacent to the deepest egg; ·········, temperature adjacent to the uppermost egg. The temperatures shown are for a day uninfluenced by recent rainfall.

Table 1. Temperatures (°C) and sex of hatchlings from nests of *Chelodina longicollis* at Gungahlin

M, males; *F*, females; *I*, indeterminate; *U*, unsexed mortalities. Mean core temperatures are based on 667 hourly nest temperatures taken over 29 days

Nest	Mean core temp.	Mean daily range (Range)	Mean daily min. (Range)	Mean daily max. (Range)	*M*	*F*	*I*	*U*
1	18·8	4·3 (1·7–7·7)	17·2 (13·9–24·1)	21·5 (18·2–26·7)	5	4	0	1
2	19·9	4·4 (2·3–9·8)	18·2 (15·0–22·8)	22·6 (18·6–27·6)	7	4	0	3
3	22·4	8·1 (2·9–11·0)	19·1 (13·6–25·0)	27·3 (19·8–32·2)	3	4	2	0
4	22·5	6·1 (1·8–11·2)	19·9 (16·4–26·7)	26·1 (20·6–32·1)	4	3	0	4
5	20·7	7·2 (2·5–10·8)	17·9 (14·3–22·0)	25·0 (21·1–30·0)	4	5	0	2
13	19·1	5·2 (2·3–8·5)	17·0 (13·9–22·0)	22·2 (18·1–28·1)	4	2	1	4
14	21·4	4·5 (1·7–9·2)	19·5 (16·3–25·4)	24·0 (20·0–31·9)	–	–	14	–
15	20·9	6·3 (2·9–12·6)	18·2 (14·5–21·5)	24·5 (20·2–29·0)	4	6	0	0

Sex Ratios

Sex ratios and thermal characteristics of nests are shown in Table 1. There was no significant heterogeneity in the sex ratios among individual nests ($\chi^2 = 2 \cdot 22$, d.f. = 6, $P = 0 \cdot 90$) so the data were pooled across nests for an overall test of deviation of the sex ratio from 1:1. Of the animals that could be sexed, 31 were male and 28 were female

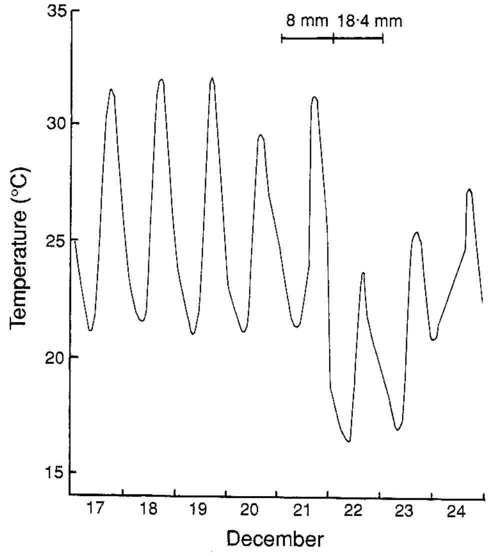

Fig. 2. Effect of rainfall on core temperatures in nests of *Chelodina longicollis* at Gungahlin. Five relatively warm days precede two days of rain (denoted by horizontal bar with falls in mm), at which time nest temperatures dropped sharply.

yielding a ratio that was not significantly different from 1 : 1 ($\chi^2 = 0 \cdot 15$, d.f. = 1, $P = 0 \cdot 70$) or significantly different from results of constant temperature experiments (Georges 1988) ($\chi^2 = 1 \cdot 53$, d.f. = 1, $P = 0 \cdot 22$). Nor did hatchling sex ratios depend on whether eggs were in the top, middle or bottom stratum of eggs within nests ($\chi^2 = 1 \cdot 93$, d.f. = 2, $P = 0 \cdot 38$).

Discussion

Mean core nest temperatures ranged from 18·8°C to 22·5°C (Table 1), well below those chosen for study of sex determination in the laboratory (Georges 1988). The absence of an effect of temperature on hatchling sex ratios in the present study serves to extend the results of the laboratory study to lower temperatures. Furthermore, eggs of *C. longicollis* fail to complete development when incubated at constant temperatures below 24°C (Georges, unpublished data), so the field embryos must be relying on the relatively short period each day (*c.* 5 hours) when temperatures are conducive to metabolism necessary for development. Thus, eggs in the field may experience temperatures, albeit only for part of each day, that are outside the range for which development is possible at constant temperatures. This may place limitations on the degree to which results of constant temperature experiments in the laboratory can be extended to field situations for species with TSD, especially in species which may have a second low threshold for sex determination (as in *Chelydra serpentina*; Yntema 1976).

The thermal characteristics of nests of *C. longicollis* are in close agreement with those previously described for other shallow-nesting species, such as *Chelydra serpentina* (Packard *et al.* 1985) and *Emydura macquarii* (Thompson 1988*b*). Eggs in shallow nests are subject to wide daily fluctuations in temperature and to thermal differentials between eggs within a single clutch. These features have consequences for both developmental rates and hatching synchrony (Thompson 1988*b*) and sex determination in species with TSD (Wilhoft *et al.* 1983).

The only field study of sex determination in a pleurodire is that of Alho *et al.* (1985) who found that temperature influenced hatchling sex ratios in modified field nests of *Podocnemis expansa*. At the time of the experiments described in their paper, TSD had not been demonstrated in the laboratory for any pleurodire, so the field studies of Alho *et al.* (1985) raised the distinct possibility that the complex regime of temperatures experienced by embryos in field nests may influence sex in a way not anticipated from constant-temperature laboratory experiments. No evidence of such an effect was found in the present study as all nests produced both male and female hatchlings, and the hatchling sex ratio could not be shown to differ from 1 : 1. While subtle effects of temperature on sex ratios may have been missed because the nests studied, and the number of eggs per nest, were few, a marked effect similar to that recorded for *P. expansa* (Alho *et al.* 1985) and other species with TSD (Bull 1985), where eggs in most nests are all of one sex or the other, would not have passed unnoticed by this study. It appears that sex determination in *C. longicollis* is independent of incubation temperatures both under constant temperature regimes in the laboratory (Georges 1988) and in the field.

Acknowledgments

We thank Peter Fullagar and Chris Davey of the CSIRO Division of Wildlife and Ecology for allowing us access to the Gungahlin ponds and for loan of temperature recording equipment. Colin McQueen provided advice on histological procedures. Frank Krikowa drew the figures and Roger Welsh repaired the temperature recorder as required.

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Manuscript received 15 May 1990; revised 12 October 1990; accepted 21 December 1990