INCUBATION PERIOD AND SEX RATIO OF

Testudo hermanni boettgeri

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ABSTRACT

In many tortoise and turtle species the incubation temperature of the egg determines the sex of the hatchling. For Hermann's tortoises (*T. hermanni*) however, such data are fragmentary or unpublished. In this paper, duration of incubation of hatchlings from 312 eggs laid by a group of 10 female *T. hermanni boettgeri* are described. This incubation period did not vary linearly with incubation temperature but rather was independent of temperature in the range 29℃ to 34℃, being ~57 days throughout. Incubation period changed abruptly to ~83 days for temperatures of 26℃ with the transition range of temperatures lying between 26℃ and 28℃. No significant correlation between incubation period and other environmental parameters could be detected. Sex ratios were also measured as a function of incubation temperature, with a sex ratio of 50% at a threshold temperature of 31.5℃ and an abrupt change from virtually all males to all females over a narrow temperature range.

INTRODUCTION

The influence of temperature on the incubation period and sex of chelonians, especially aquatic species, has been studied by different authors. Extensive studies on *Emys orbicularis* have been published by Pieau (1971) and on various Emydinae species by Bull *et al.* (1982a). A recent review of temperature-dependent sex determination (TSD) in squamate reptiles is given by Spotila *et al.* (1994) and of TSD in turtles by Ewert *et al.* (1994). However little detailed work has been published on measurements of the effect of the incubation temperature on the incubation period and sex ratio of tortoises. In this paper the author reports on a study of the incubation of a total of 741 eggs of *T. hermanni boettgeri* from which 312 tortoises hatched successfully. These eggs were laid during the period 1982-1992 by a group of 10 female *T. hermanni boettgeri* kept in an outdoor terrarium. The study was planned to investigate the effects of environmental parameters on the incubation period and sex ratio of *T. hermanni boettgeri*. Apart from the temperature, many other parameters were studied, e.g. characteristics of the parental female, nest location, time of nesting, order of laying within a clutch, egg weight and humidity during the incubation period.

MATERIALS AND METHODS

Nesting by the colony of captive *T. hermanni boettgeri* took place in the months of May, June and July, usually between 10 am and 12 noon. All eggs were removed from the nests, marked with a pencil, weighed, inspected, and placed in incubators within one to three hours after laying. Three different types of incubators were used. One incubator used bi-metal control, whereas the other two used NTC-sensed temperature elements and a temperature sensing bridge or TRIAC triggering circuit as control units. All these incubators usually regulated the intended temperature to within about 0.2℃. To achieve this result the bi-metal controller had to be adjusted almost daily.
Within an incubator the eggs were placed on a foam rubber subsoil and positioned in a Latin square design. Horizontal and vertical thermal gradients within the incubator were minimised by careful distribution of the heating cables. Variation from the intended temperature was limited to ~0.2℃, as monitored by movable thermocouple probes. In view of the control of temperature gradients the approach of the Latin square design as intended by Bull *et al.* (1982b) is not necessary. To reduce the influence of unknown genetic or environmental parameters the eggs of one clutch were equally distributed over the three incubators.

The "incubation period" as used in this study is defined as the time interval between ovi-position and emergence of the full-grown hatchling. The length of time from pipping of the eggshell to the emergence of the hatchling is about one day. Therefore the influence on the determination of the incubation period in small. Immediately after laying, the eggs were inspected for the possible development of blood-vessels which are normally visible after 6-8 days of incubation. No sign of blood-vessels were detected, so a hypothetical developmental process within the female is unlikely and will practically not influence the results. In exceptional cases (~2%) the hatchling did not hatch after the expected time. In these cases the egg was opened by hand two weeks after the end of the predetermined incubation period. These exceptional cases were not included in the used data.

The hatchlings were marked individually by making notches in the marginal scutes according to the system used by the Charles Darwin Station on Santa Cruz Island (Thonton, 1991). Most of these hatchlings were distributed among members of the Dutch Tortoise Association. Only a relatively small number (~25%, random selected) were kept until sex determination could take place. These juveniles were kept in the same outdoor terrarium as the colony of full-grown *T. hermanni boettgeri*.

The sex of the juveniles was determined by external characteristics such as the shape of the tail, carapace, plastron or anal scutes (Stubbs *et al.*, 1981). In most cases this determination can take place with a carapace length of ~10 cm at an age of 3-4 years. Juveniles were defined as male or female not before at least two different sex characteristics, in general the shape of the tail and anal scutes, were deciding.

RESULTS

Incubation period

In this study incubation periods of *T. hermanni boettgeri* were measured at constant incubation temperatures. From the 741 eggs incubated at a constant temperature, 515 eggs were visibly fertilized, from which 312 tortoises hatched successfully. Table 1 shows the results of incubation temperatures of 25°C up to 34°C. Other environmental parameters such as parental female, nest location, time of nesting, order of laying within a clutch, egg weight and humidity during the incubation period were studied, but no significant correlation of these parameters with the incubation period could be found. The numbers however were too small to justify definite conclusions. The eggs were weighed with an accuracy of ± 0.5 g. Loss of weight of eggs during incubation is on the average 30-40%. In 4 out of 120 clutches exceptional loss of weight (more than 60%) resulted in either dead embryos or rather short incubation periods. These exceptions are not included in the numbers given in Table 1. The results, also presented in Figure 1, show a relatively abrupt change from a low-temperature incubation period of ~83 days to a high-temperature period of ~57 days. The transition period is defined as the average of those two periods, and the corresponding temperature is called the transition temperature. A possible curve that correlates the measure points was found to be the following mathematical approximation;

$$y = 70 - 26\arctan(3(x-27.5))p^{-1} \quad [1]$$

where:
- y = incubation period [days]
- x = incubation temperature [°C]
- 27.5 = transition temperature [°C]
- 70 = transition period [days]

Mortality rates for incubation temperatures of 26°C up to 32°C were in the range 20% to 30%. For incubation temperatures of 25°C, 33°C and 34°C the mortality rate was about 50%, whereas at 24°C and 35°C (~20 eggs) the mortality rate was almost 100%. Hatchlings incubated at a constant temperature of 24°C and 25°C are generally small, weak and not able tot hatch successfully, so the definition of the incubation period at these temperatures is questionable. For that reason the exact shape of the curve for incubation temperatures below 27°C in Figure 1 cannot be determined.

Sex ratio

During the study it was found that in some cases *T. hermanni boettgeri* incubated at temperatures of 30℃ - 33℃ showed their secondary sex characteristics only after a long time. In exceptional cases it took 8-10 years, which is long compared with the normal situation. This effect can lead to a male-oriented shift in the measured sex ratio if not all the hatchlings of a specific year (or even better a specific clutch) are sexed definitively. For that reason only the number of males and females of clutches from which the sex of all hatchlings was determined were taken into account. A random selected group of ~25% of the hatchlings (72 out of 312) were kept until sex determination could take place. Table 2 and Figure 2 give some results of the measured sex ratios (defined as the percentage male hatchlings) of 72 *T. hermanni boettgeri*. Figure 2 shows a sharp transition between 30℃ and 33℃, with a sex ratio of 50% at a temperature of 31.5℃. The temperature producing a sex ratio of 50% is defined as the threshold temperature.

DISCUSSION

Detailed information on the influence of incubation temperatures on the incubation period and sex ratio has been reported for *Emys orbicularis* by Pieau (1971), Pieau and Dorizzi (1981) and for various Emydinae species by Bull *et al.* (1982a). Since then temperature dependent sex determination (TSD) is described in about eight families of turtles. A recent review of TSD is given by Ewert *et al.* (1994). For *T. hermanni* only sparse measurements are reported. Kirsche (1967) found an average incubation period of 63 days for an incubation temperature of 28℃ - 30℃, which is in accordance with the results of this study. Much longer periods of more than 100 days are reported for wild populations by Swingland & Stubbs (1985). In the authors outdoor terrarium, some *T. hermanni boettgeri* hatched after 110 to 120 days, thanks to a relatively warm and dry Dutch summer.

In this study incubation temperatures of 24℃ to 34℃ were used. Constant temperatures below 26℃ and above 32℃ lead to increasing mortality rates.
It appears that damaging incubation temperatures are similar to those that are avoided in wild populations by careful nesting site selection (Meek and Avery (1988), Willemsen (1991)).

The incubation period was almost constant in the range 29℃ to 34℃, being ~57 days throughout. This result indicates that embryonic developmental rates are almost constant in that range. A mortality rate of almost 100% at an incubation temperature of 24℃ indicates that the minimum temperature for embryonic development for *T. hermanni boettgeri* is about 23℃ to 24℃. Using these results to test the model suggested by Georges (1989) to predict hatchling sex ratios when nest temperatures fluctuate will be difficult. In nature nest temperatures will regularly drop below this minimum temperature of embryonic development.

Sex determination of hatchlings that were incubated at temperatures between 30℃ and 33℃ was in some exceptional cases possible only after a long time. This phenomenon can be explained by the study of Pieau (1975), who has reported on the formation of intersexes of *Emys orbicularis*, incubated at a threshold temperature of 28℃ to 29℃. Observations of the author however indicate that "external" sex characters will appear with time in almost all cases. Zaborski *et al.* (1982) suggested the possibility that some individuals which were intersexes as hatchling become phenotypic females afterwards. Whether sex ratio persist until appearance of sexual dimorphism cannot be determined because sex determination was based only upon external sex characteristics.

Comparison with the results for the emydines shows a similar change from virtually all males to all females over a narrow temperature range. The threshold temperature for *T.hermanni* however was found in this study to be 31.5℃ instead of 28℃ to 30℃ for Emydinae studied by Bull *et al.* (1982a).

As suggested by Bull and Vogt (1979), different mortality rates of the embryos are not likely to occur because mortality rates were independent of the incubation temperatures within the region of 26°C to 33°C.

Based on the effect of temperature on sex determination one could expect that relatively cold regions or periods may lead to temporarily lower threshold temperatures or favored male differentiation. This theory is not supported by field investigations as performed for Emydinae by Bull *et al. (1982b).* This lack of evidence could be explained by compensation with other environmental effects. However in this study the incubation temperature was the only environmental parameter that had a detectable influence on the incubation period and sex ratio. Other parameters, such as genetically determined differences, nest location, time of nesting, order within a clutch, egg weight, and humidity during the incubation were investigated, but no correlations with the incubation period or sex ratio were found. Further investigations are needed to confirm these preliminary conclusions.

The most conspicuous difference with incubation in nature are the dayly and seasonal variations in the incubation temperatures versus the constant incubation temperatures used in this study. In nature during daylight the soil temperature at the egg location can be up to 18°C higher than at night (Pieau, 1982) and this effect may easily overrule the influence of a small change in a constant average temperature. The results of this study show that fluctuations of the environmental temperature above 28°C does not influence the development rate, but can have a large effect on sex ratios. To what extent the number of hours of incubation or the embryonic development above the threshold temperature are decisive for sex determination is still an open question.

For that reason this study is being continued by an investigation on the influence of variations of the incubation temperature on the sex ratio of *T. hermanni boettgeri*.

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TABLES AND FIGURES

Table 1 : Incubation period of Testudo hermanni boettgeri

Incubation Temperature [℃]	Number of eggs	Mean Incubation Period [days]	Standard deviation
25	2	82.0	0.0
26	7	83.0	3.6
27	3	72.0	2.7
28	20	65.7	3.2
29	10	56.1	0.9
30	31	57.8	1.3
31	48	57.7	2.9
32	64	55.6	3.7
33	101	56.1	3.3
34	26	56.4	3.2

Table 2 : Sex ratios of Testudo hermanni boettgeri

Incubation Temperature [℃]	Number of males	Number of females	Sex ratio %
25	2	0	100
26	4	0	100
28	5	0	100
30	11	0	100
31	11	3	79
32	6	17	26
33	0	11	0
34	0	4	0

Fig. 1 shows the incubation period of Testudo hermanni boettgeri as a function of the incubator temperature. Data are shown as mean and S.D

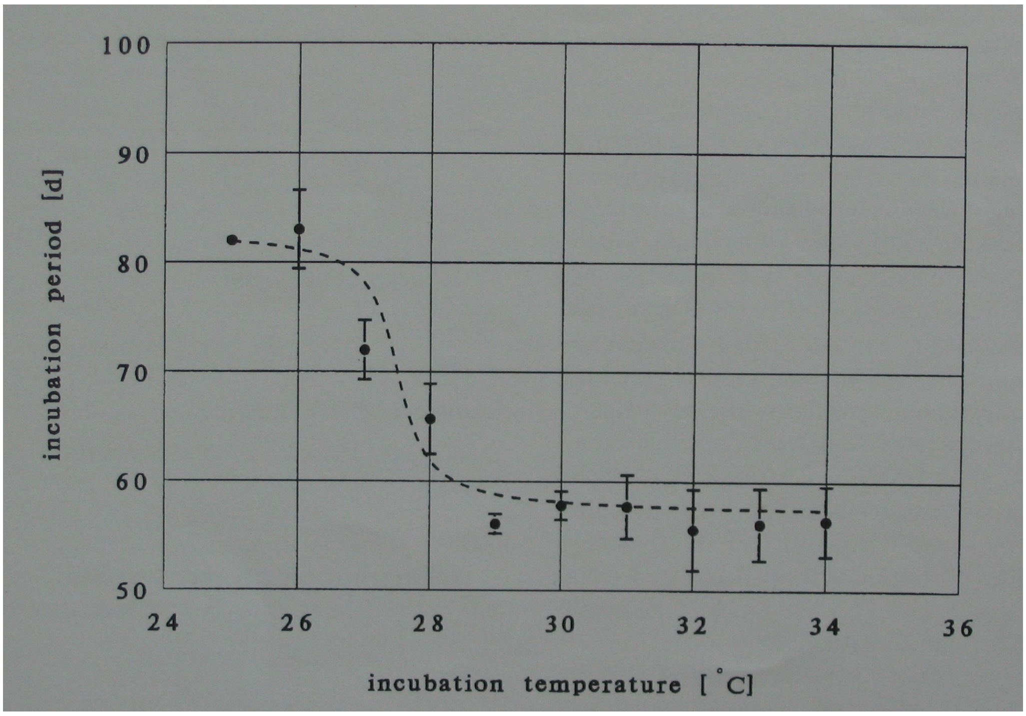

Fig. 2 shows the sex ratio of *Testudo hermanni boettgeri* as a function of the incubator temperature. Data are shown as mean sex ratios.