



Temperature-dependent sex determination in the salt-water crocodile, *Crocodylus porosus* Schneider

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One clutch of 45 eggs of the estuarine crocodile, *Crocodylus porosus*, was divided into two groups and incubated at $33 \pm 0.1^\circ\text{C}$ and $34 \pm 0.1^\circ\text{C}$. The per cent hatching was 69 and 79 at 33 and 34°C respectively. All the hatchlings incubated at 33° developed into females and all the hatchlings incubated at 34°C developed into males, indicating thereby that temperature-dependent sex determination takes place in this species, and that the critical temperature lies between these two temperatures.

It is well known that sex determination in most reptiles, specifically in the primitive ones, is temperature-dependent¹. Since in our laboratory² we have demon-

strated temperature-dependent sex determination (TSD) in the olive ridley sea turtle, *Lepidochelys olivacea*, it was decided to attempt the same technique and incubate the crocodilian eggs artificially at different temperatures with the hope to produce sufficient number of males for rear and release in the sanctuary.

A clutch of 45 eggs was collected on 13 June 1987, around 11.00 a.m. from a nest in Forest Block VIII, near Junajera creek of Bhitarkanika river flowing in the Bhitarkanika Wildlife Sanctuary. The eggs were collected after 15 days of egg laying. The average temperature of the nest, atmosphere and surrounding water was 33, 34 and 30°C , respectively. The nest size was 152 cm \times 137 cm \times 82 cm (length \times width \times depth) and its materials consisted of aquatic fern, *Aerostichum aureum* mixed with soil. The size (length) of the nesting female was about 25 cm. The mean size and weight of the eggs during collection were 70 \times 55 cm (length \times width) and 110 g, respectively. During collection, the eggs were irregularly arranged. After collection, the eggs were placed in a plastic container along with ferns from the nest and transported to Utkal University on 15 June 1987. The eggs were washed, divided into two batches and kept in enamel trays with moist cotton (Figure 1). While one batch of eggs (16), incubated at $33.0 \pm 0.1^\circ\text{C}$ – which was the nest temperature – yielded females, the other batch of eggs (29), incubated at

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Figure 1. Oval eggs of *C. porosus*.Figure 2. Hatchlings of *C. porosus*.Table 1. Incubation, hatching and sex of *Crocodylus porosus*

| Temperature (°C) | Eggs incubated (no.) | Incubation period (days) | Hatching (%) | Sex |
|------------------|----------------------|--------------------------|--------------|--------------|
| 33 | 16 | 52 | 69 | Females only |
| 34 | 29 | 51 | 79 | Males only |

$34 \pm 0.1^\circ\text{C}$ in B.O.D. incubator yielded males. Since higher temperature yielded males, the second batch with 29 eggs was incubated at a higher temperature to obtain males. The eggs were examined every 24 h and moist cotton was replaced as and when necessary.

The incubation data are presented in Table 1. There was no change in egg morphology until 12 days of incubation, after which, longitudinal cracks appeared on the shell and the cracks widened on the following days. A total of 34 hatchlings emerged (Figure 2) and hatching at 33 and 34°C was 69% and 79%, respectively. Examination of unhatched eggs revealed prenatal death in two embryos at higher temperature incubation and the rest of the 9 eggs were infertile. Hatching time was remarkably synchronous and occurred within 48 h in each group. The average time for hatching was 51 and 52 days at higher and lower temperatures, respectively. Considering the fact that the eggs were collected from the nest 15 days after laying and were in transit for 3 days, the total hatching period can be taken as 70 and 69 days at low and high temperatures incubation, respectively.

Since the middle third of development appears to be the time when the temperature exerts its effect on sex determination, the incubation in the laboratory happened to be in this sensitive period between 23 and 46 days after laying. Within 24 h of hatching, the hatchlings

produced the vocal sound, characteristic of the species. Four days after hatching, the hatchlings were transported to Research Station at Bhitarkanika and were released into two separate hatchling pools. Attempts were made to sex the hatchlings by the anal probing method⁴, but this method was not quite effective until the hatchlings were 32.5 cm long. Initially, the examination of genitalia with blunt forceps indicated that all the 23 hatchlings from high temperature incubation were males, and all the 11 hatchlings from low temperature incubation were females. After completion of one year, the hatchlings (yearlings) reached 75 to 90 cm in length and 2.5 to 3.0 kg in weight. To confirm the sex of the hatchlings, anal probing method was repeated on completion of one year and two years of rearing and the observation agreed with the previous findings; low temperature yielding all females and high temperature yielding all males.

The present study is in confirmation with the reports by Ferguson and Joanen³ who reported the hatching of males at warm temperatures and females at cool temperatures in alligators. Since 100% of females and males were obtained at 33 and 34°C incubation, it is reasonable to conclude that the critical temperature lies in between these two.

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