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Effect of Incubation Temperature on Incubation Period, Sex Ratio, Hatching Success, and Survivorship in *Caiman latirostris* (Crocodylia, Alligatoridae)

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ABSTRACT.—Temperature-dependent sex-determination has been reported for all extant crocodylians. We present information about incubation temperature effects on incubation period, sex ratio, hatching success, and hatchling survivorship during the first year of life for *Caiman latirostris*. Incubation period was negatively related to temperature. Sex of hatchlings were related to incubation temperature. Only females were produced at 29°C and 31°C, only males were produced at 33°C, and both males and females hatched at 34.5°C. Hatching success and survivorship were unaffected by incubation temperature.

Reptiles have a wide range of sex-determination systems, including genotypic sex determination (GSD) and environmental sex determination (ESD; Wibbles et al., 1994). Temperature-dependent sex determination (TSD), a form of ESD, is present in some turtles

(Ewert et al., 1994) and lizards (Rhen and Crews, 1999), but all crocodiles studied to date (11 of 22 extant species, Lang and Andrews, 1994) show only TSD. It is relevant to know whether temperature is involved in sex determination of all crocodile taxa because, if all the extant species have TSD, it contrasts with the diversity found in other reptile groups. Moreover, the species studied showed different responses to incubation temperature, for example; *Crocodylus johnstoni* never produced more than 40% males under constant temperature incubation, whereas *Alligator mississippiensis* produced 100% males at certain temperatures (Lang and Andrews, 1994).

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Currently there are ranching programs under way in Argentina in which eggs of *Caiman latirostris* are collected and subjected to artificial incubation, and a percentage of hatchlings are reintroduced into the wild. Consequently, inappropriate management could be detrimental to wild populations. For example, the 30% rate of infertile eggs of the turtle *Dermochelys coriacea* (a TSD species) in Malaysia is attributed to lack of males in the population because of reintroduction of an inadequate number of males (Mrosovsky, 1994).

There are no published data on sex determination in *C. latirostris* under laboratory conditions, and the pattern of TSD is unknown. The purposes of this study were to determine for *C. latirostris* if constant incubation temperature: influences incubation period, sex ratio, hatching success, or hatchling survivorship during the first year of life.

MATERIALS AND METHODS

Eggs of *C. latirostris* came from two different sources. Nine nests from Proyecto Yacaré breeding stock (Santa Fe province, Argentina) were collected within 12 h of egg-laying during 1996 to 1998, and the other four nests used were harvested within seven days after laying from natural areas during 1998 and 1999. For the experiment, we used 401 eggs from 13 clutches. Incubators consisted of a plastic container with water and one aquarium heater. Inside the container, above water, there was a grid containing nest material where eggs and a Hobo Data Logger were placed. Each incubator was covered with a styrofoam lid. Incubators were set at selected temperature $\pm 0.5^\circ\text{C}$. Humidity at all treatments was high but was not measured.

We incubated eggs at four constant temperature treatments (29°C , 31°C , 33°C , and 34.5°C). Every clutch was randomly divided across treatments, to control for clutch effects. Animals were marked on both hind feet using Monel tags (#001; Natl. Band and Tag Co., Newport, KY) after hatching, and the day of hatching was noted. Hatching success was measured as number of hatchlings/number of eggs for each treatment. Incubation period was the number of days from the beginning of incubation to hatching, plus the estimated "age" of the nest derived from the opaque band (if the oviposition date was unknown). First-year survivorship was recorded.

Sex was assessed by observing secondary sexual characters (Webb et al., 1984; Allsteadt and Lang, 1995). We dissected at least one animal from each of the 13 nests, at the three lower incubation temperatures. We determined gonadal sex macroscopically by shape, texture, and color of the gonads, and by the presence/absence of Müllerian ducts of randomly selected newborn caimans from different treatments, and sexing (whenever possible) embryos that failed to hatch ($N = 30$).

Hatchlings were maintained as described in Larriera (1993). Animals hatched in 1996 and 1999 were not included in survivorship to one year results. In 1996, we used only one tag per animal, and some tags were lost. Survivorship could not be calculated for the 1999 cohort because the study was completed before the caimans attained one year of age. Hatching success, survivorship, and sex ratio were analyzed using a Chi-square test and incubation period by a two-way

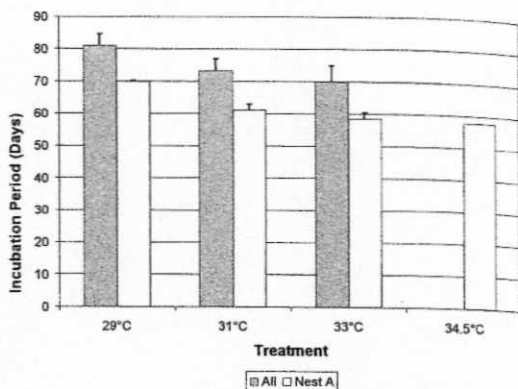


FIG. 1. Days of incubation period in laboratory at four constant incubation temperatures. The All series represents the mean of all nests used in the experiment (12), exception of nest A (other series) because this was the only one that produced hatchlings at 34.5°C . Analysis of nest A alone showed no significant differences in incubation period between 33°C and 34.5°C (N are $29^\circ\text{C} = 5$; $31^\circ\text{C} = 6$; $33^\circ\text{C} = 9$; $34.5^\circ\text{C} = 6$).

ANOVA using clutch and temperature treatment as factors.

RESULTS

Incubation Period.—Time required to complete development was 80.9 ± 3.7 (mean \pm SD) days at 29°C , 73.4 ± 3.5 days at 31°C , 69.9 ± 5.1 days at 33°C , and 69 days at 34.5°C . Incubation period differed among temperature treatments (Fig. 1). Increasing temperature from 29°C to 33°C reduced the incubation period ($F = 414.3$, $P < 0.001$), but no differences were observed between 33°C and 34.5°C . Only one clutch incubated at 34.5°C produced hatchlings (shown in Fig. 1). Clutch was a significant source of variation ($F = 373.5$, $P < 0.001$).

Sex Ratios.—Temperature during incubation had a significant effect on sex determination of *Caiman latirostris* ($\chi^2 = 163.68$, $df = 3$, $P < 0.001$). Eggs incubated at 29°C ($N = 52$) and 31°C ($N = 54$) produced 100% females. Incubation at 33°C produced 100% males ($N = 58$). Highest temperature treatment (34.5°C) produced both sexes, in a ratio of 6 males : 4 females ($N = 10$). Similar results were obtained in other experiments, incubating eggs at 29°C and 33°C , carried out in the same laboratory (results not reported here). There were twins in one egg incubated at 34.5°C , and both were males. No variation was found in sex ratios between nests or among years at the incubation temperatures studied in these experiments. The sex of animals that failed to hatch was the same as hatchlings produced at same temperatures, indicating that temperature does determine sex and does not act via differential mortality of males or females at different temperatures.

Hatching Success.—No differences in hatching success were found among treatments at 29°C , 31°C , and 33°C ($\chi^2 = 3.90$, $df = 2$, $P = 0.143$), but there were differences among years (Table 1). During 1996, there

TABLE 1. Hatching success of four treatments in each year of experiment. Total HS refers to hatching success of each treatment in the period 1996/1999. N.D. = no data. Sample size in parentheses.

| Year | Treatments | | | |
|----------|------------|------------|------------|-----------|
| | 29°C | 31°C | 33°C | 34.5°C |
| 1996 | 52.9 (70) | 57.1 (70) | 12.9 (70) | N.D. |
| 1997 | 76.2 (21) | 76.2 (21) | 66.7 (21) | N.D. |
| 1998 | 33.3 (15) | 60 (15) | 87.5 (16) | 33.3 (18) |
| 1999 | 80 (15) | 93.3 (15) | 80 (15) | 0 (19) |
| Total HS | 57.9 (121) | 65.3 (121) | 40.2 (122) | 16.2 (37) |

TABLE 2. Percentage of survivorship of *Caiman latirostris* hatchlings during their first year of life. N.D. = no data, Total = survivorship of both years. Sample size in parentheses.

| Year | Incubation temperature | | | |
|-------|------------------------|-----------|-----------|--------|
| | 29°C | 31°C | 33°C | 34.5°C |
| 1997 | 81.3 (16) | 56.3 (16) | 71.4 (14) | N.D. |
| 1998 | 80 (5) | 44.4 (9) | 50 (14) | 0 (6) |
| Total | 81 (21) | 52 (25) | 60.7 (28) | 0 (6) |

was low hatching success at 33°C, but during 1998 the same treatment had the highest hatching rate. The best mean hatching success occurred in 1999. We assume low hatching success was because of excess humidity condensed to drops of water in the environment. Incubation at 34.5°C produced a lower percentage of hatchlings than any other treatment: 16.2%, just six animals from 37 eggs ($\chi^2 = 9.16$, $df = 3$, $P = 0.028$).

Survivorship.—Survivorship to one year was unaffected by incubation temperature (Table 2; $\chi^2 = 4.64$, $df = 3$, $P = 0.201$). We must note that incubation at 34.5°C had zero survivorship (76% of χ^2 -value, 3.53/4.64); this indicates temperature effects were not detected because of small sample size.

DISCUSSION

Incubation period in *C. latirostris* was negatively related to temperature. Results indicate that temperature could act by producing an increase in metabolism as temperature rises (Zug, 1993), thus reducing the time required for development within the 29–33°C range. Temperature does not modify incubation period linearly; effects were higher from 29°C to 31°C than from 31°C to 33°C (Lang and Andrews, 1994, and this experiment). Hatchlings from one nest at 34.5°C suggest that differences from 33°C to 34.5°C are insignificant. Our results are similar to previous studies reported for crocodylians: temperature affects incubation period up to 33°C. *C. latirostris* has the shortest incubation period reported at 29°C, and one of the longest at 33°C, exceeded only by *Caiman crocodilus* and *Crocodylus moreletii* (Lang and Andrews, 1994) and *Crocodylus porosus* (Webb et al., 1987).

Incubation temperature determinates sex in *C. latirostris*. Low incubation temperatures (29°C and 31°C) produce 100% females, 33°C produces only males (100%), but higher temperature (34.5°C) produces both males and females. It appears *C. latirostris* has pattern II of TSD (female-male-female, as defined by Ewert et al., 1994) as do other crocodylians (Lang and Andrews, 1994). We obtained animals incubated at 34.5°C from only one nest, so inferences concerning sex ratios are not limited this incubation temperature. Clutch is a significant source of variation for sex of hatchlings at temperatures that produce both sexes (Conley et al., 1997; Lang and Andrews, 1994; Rhen and Lang 1998). *Caiman latirostris* produces 100% males at constant incubation temperature of 33°C, contrasting with other species of crocodylians studied to

date, except *Alligator mississippiensis* (Lang and Andrews, 1994).

Incubation temperature influences hatching success in *C. latirostris*. We found the lowest hatching success at 34.5°C. Lang and Andrews (1994) reported that eggs of *Alligator* incubated at 34.5°C had a rate of hatching of 29%, but incubation at 35°C reduced hatching success to 11%. Results for other species reported by Lang and Andrews (1994), and Webb et al. (1987) show that incubation at 34°C is a lethal temperature for most species (*Caiman crocodilus*, *Crocodylus palustris*, *C. moreletii*, *Crocodylus siamensis*, *C. porosus*, and *Gavialis gangeticus*). These species produced no hatchlings at 34°C, or higher, incubation temperatures. It is interesting note that the two species of Alligatoridae having the highest latitudinal distributions (*Alligator mississippiensis* and *C. latirostris*) produce hatchlings at temperatures higher than 34°C. *Crocodylus johnstoni* develop at 34°C but do not produce more than 39% males at any constant incubation temperature. Some wild nests of *C. johnstoni* produce 100% male hatchlings. Webb et al. (1987) attributed this to daily fluctuation of temperature in nests of *C. johnstoni* and the steadily increasing temperature during natural incubation that allows eggs to develop at temperature as high as 34°C.

In this study, survivorship during the first year was unaffected by incubation temperature, but the lack of differences could be a result of low number of hatchlings produced at 34.5°C. Survivorship was highest at 29°C (89%) and 33°C (61%), female and male producing temperatures, respectively. *Caiman* eggs incubated at 31°C had a survivorship of 52%, and it was null at 34.5°C (0%). These results are similar to those reported by Janzen (1995), in which snapping turtles incubated at temperatures that produced mixed sex ratios had lower survivorship than hatchlings incubated at temperature that produced only males or females.

Our results suggest that hatchlings produced at 34.5°C have lower fitness than hatchlings incubated at 29°C, 31°C, or 33°C, because the former had the lowest hatching success (16%) and survivorship (0%). We did not use incubation temperatures between 31°C and 33°C (which probably produce both sexes) to determine whether lower fitness of animals incubated at 34.5°C was because of production of both sexes or because this incubation temperature is detrimental for *C. latirostris*. Further experiments are needed to answer this question.

Woodward and Murray (1993) suggested a possible

selective advantage because of TSD on the ability of crocodylians to produce skewed sex ratios. We found higher hatching success and survivorship from the eggs incubated at 29°C, which is consistent with this hypothesis, but recent data on alligators (Lance et al., 2000) challenge this idea. Results of our experiments do not provide evidence of a clear evolutionary advantage for TSD in *C. latirostris*, other than the lower fitness of those eggs incubated at 34.5°C. In this experiment, we demonstrated that another crocodylian species has TSD, rising the total to 12 of 22 extant species.

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