

Effects of Incubation Temperature on the Size of *Caiman latirostris* (Crocodylia: Alligatoridae) at Hatching and after One Year

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ABSTRACT.—We investigated the effects of incubation temperature (29°C, 31°C, and 33°C) on total length (TL) and body mass (BM) of *Caiman latirostris*, a crocodylian with temperature-dependent sex determination (TSD), at hatching ($N = 180$) and in a sample of hatchlings ($N = 40$) after one year of raising. Size at hatching was strongly clutch-specific. Animals incubated at 31°C (100% females) were larger than at 29°C (100% female) and 33°C (100% males). Absolute growth to one year was higher for females (eggs incubated at 29°C and 31°C) than for males (eggs incubated at 33°C). The possibility that constant 33°C incubation temperature had compromised embryological development cannot be rejected. If so, it confirms that high incubation temperatures can have long-lasting effects on posthatching growth. If not, possible advantages of females growing more rapidly than males are discussed.

Incubation temperature can affect the phenotype of hatchling reptiles (e.g., Allsteadt and Lang, 1995; Rhen and Lang, 1995; Booth, 2006; Nelson et al., 2006), including sex in species with temperature-dependent sex determination (TSD; most turtles [Rhen and Lang, 1998; Wibbels et al., 1998; Freedberg et al., 2006], some lizards [Harlow and Shine, 1999; Rhen and Crews, 1999; Ji et al., 2006], and all crocodylians studied to date [Lang and Andrews, 1994; Piña et al., 2003]). However, independent of sex, high- and low-incubation temperatures cause abnormalities and are known to compromise survival to hatching (Webb and Cooper-Preston, 1989; Zhu et al., 2006). Incubation temperature and sex can independently influence posthatching growth rates (Ferguson and Joanen, 1983; Webb and Cooper-Preston, 1989) and hypotheses about the possible evolutionary advantages of TSD in reptiles (Ferguson and Joanen, 1983; Webb and Cooper-Preston, 1989; Girondot and Pieau, 1999; Shine, 1999) often link sex to posthatching growth potential in various ways. In the context of crocodylians, adult males are much larger than adult females (Webb et al., 1978; Ferguson and Joanen, 1983; Verdade and Sarkis, 1998; Verdade et al., 2003), and small adult females may not be as reproductively disadvantaged as

small adult males (Webb and Cooper-Preston, 1989).

Temperature-dependent sex determination has been demonstrated in *Caiman latirostris* (29–31°C gives 100% females, 33°C 100% males, 34.5°C males and females but with low survivorship to hatching [Piña et al., 2003]). This study provides new information on the effects of incubation temperature on total length (TL) and body mass (BM) at hatching and on posthatching growth rates (ΔTL and ΔBM) to one year of age.

MATERIALS AND METHODS

We collected 10 young clutches of *C. latirostris* eggs ($N = 260$ eggs) from the wild, in northern Santa Fe Province, Argentina, during four nesting seasons (1996/97, $N = 4$; 1997/98, $N = 3$; 1998/99, $N = 1$; 1999/2000, $N = 2$). No eggs were opened to confirm the age of embryos, but back calculation from time to hatching (80.9 days at 29°C; 73.4 d at 31°C, and 69.9 d at 33°C [Piña et al., 2003]), indicated that five were <1 week, three were 1–2 weeks old, and two were <3 weeks old when collected. Thus, eggs were collected before sex was determined (Piña et al., 2007), which was confirmed by the distribution of sexes matching those expected from TSD (Piña et al., 2003).

Each of the 10 clutches was subdivided evenly among 29°C (87 eggs), 31°C (86 eggs), and 33°C (87 eggs). Incubation was carried out in 150-liter plastic containers (Piña, 2002) in a cooled room (<29°C). Eggs were placed

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in vegetative nest material in the upper half, separated by a grid, with water in the lower half heated with thermostatically controlled aquarium heaters. Because metabolic heat among the eggs can increase nest temperatures by a few degrees (Webb and Cooper-Preston, 1989; Ewert and Nelson, 2003; Zbinden et al., 2006), temperature among the eggs was monitored daily with calibrated Hobo Data Loggers (Onset Computer Corporation, Pocasset, Massachusetts), and water temperature adjusted as necessary to maintain the desired mean temperature ($\pm 0.5^\circ\text{C}$) among the eggs. Humidity among the eggs, above the water, was high (no desiccation occurred) but was otherwise not measured.

At hatching, body mass (BM ± 0.1 g) and total length (TL ± 1 mm) were recorded, and each individual was numbered with one or two no. 1 Monel Tags (National Band and Tag Co., Newport, Kentucky) in the webbing of the hind feet. Sex was determined by inspection of the cliteropenis when animals were older than six months or by autopsy if they died (Piña et al., 2003).

Each year, hatchlings from all temperatures were raised together in the same concrete raising pen (5 \times 7 m; half water half land) inside a greenhouse. They were fed ad libitum three times per week. Body mass and TL were measured after one year and absolute growth (ΔTL or ΔBM) since hatching calculated. Because not all the hatchlings were the same age, there was a variance of ± 20 days in age on the day of measurement.

Standard descriptive statistics were used to quantify TL and BM at hatching and after one year, from which mean growth rates (ΔTL and ΔBM) were quantified. To test for significant differences in size, the MANOVA procedure (Wilk's criterion) was used (InfoStat, 2004), with incubation temperature, clutch, and their interaction as grouping factors and TL and BM as correlated response variables. We also performed ANOVAs for TL and BM with the same model as MANOVA.

Clutch effects on survival and growth were so profound that, in the analyses after one year, we only analyzed data from clutches ($N = 3$) that had surviving hatchlings from all three temperatures (40 hatchlings: 29°C , 15; 31°C , 10; 33°C , 15). In this case, the MANCOVA procedure (Wilk's criterion) was followed with incubation temperature, clutch, and their interaction as grouping factors, BM as covariate, and ΔTL and ΔBM as response variables. Differences among incubation temperature treatments were determined with Hotelling's Test (InfoStat, 2004).

Given that 29°C and 31°C both produced females, and 33°C males, within the MANOVA, we did a contrast (33°C vs. $29 + 31^\circ\text{C}$) to

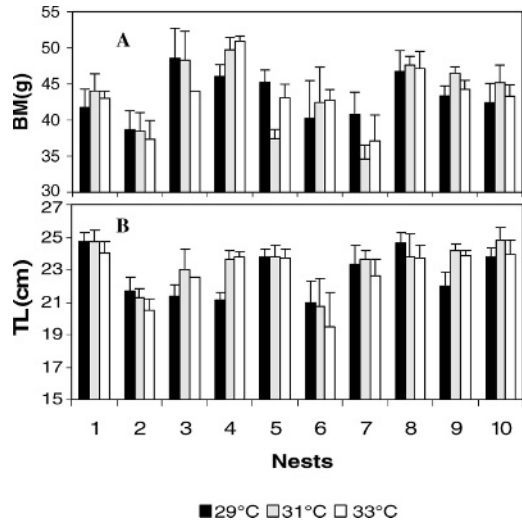


FIG. 1. Mean hatching size for 10 *Caiman latirostris* nests ($N = 180$ hatchlings) incubated at different incubation temperatures. (A) Body mass (BM), (B) total length (TL). Nests 5, 6, and 7 had animals remaining after one year from each incubation treatment.

compare males and females at hatching ($N = 180$) and after one year ($N = 40$). This provides a measure of whether males and females from the 10 nests (180 hatchlings; 44 males, and 136 females) had the same sizes at hatching and from the three nests that have survivors at all the incubation treatment ($N = 40$) have the same absolute growth rates during the first year. Means are given ± 1 SE.

RESULTS

Eggs from all nests at each temperature produced hatchlings in all years (1996–97, 74; 1997–98, 46; 1998–99, 20; 1999–2000, 40; total $N = 180$). Embryonic survival to hatching was similar for all treatments: at 29°C , it was 79.3% (of 87); at 31°C 77.9% (of 86); and at 33°C 50.6% (of 87; $\chi^2 = 4.14$; $\text{df} = 2$; $P = 0.126$). When 29°C and 31°C (all females: 78.6% survival of 173 eggs) were combined, there was higher survival than at 33°C ($\chi^2 = 21.23$, $\text{df} = 1$, $P < 0.001$).

Independent of incubation temperature, the size of hatchlings (TL and BM) was highly and obviously clutch specific (MANOVA "Incubation temperature \times Nest" Interaction $P < 0.001$; Fig. 1). Variation in TL between clutches (Fig. 1B), which is an index of somatic growth, did not mirror variation in BM (Fig. 1A). For example Clutch 1 produced the longest hatchlings but not the heaviest ones (Fig. 1).

Incubation temperature (MANOVA: $F_{4,292} = 4.365$; $P = 0.002$) and clutch ($F_{18,292} = 30.7$; $P <$

TABLE 1. Mean size of *Caiman latirostris* ($N = 40$) at hatching and mean absolute growth after one year (F). Δ = absolute growth increments. Means \pm SE.

Treatment	TL (cm)	BM (g)	Δ TL (cm)	Δ BM (g)
29°C (15)	23.9 \pm 0.2	44.2 \pm 0.9	21.1 \pm 2.1	290.7 \pm 44.6
31°C (10)	23.3 \pm 0.3	40.2 \pm 1.9	23.1 \pm 3.1	328.2 \pm 75.0
29°C + 31°C (25)	23.7 \pm 0.2	42.6 \pm 1.0	21.9 \pm 1.7	305.7 \pm 39.4
33°C (15)	23.4 \pm 0.2	42.0 \pm 1.3	14.5 \pm 1.5	140.8 \pm 26.4

0.001) affected hatchling size. The interaction between incubation temperature and clutch was significant for size (TL and BM; $F_{36,292} = 3.481$; $P < 0.001$), confirming clutches did not respond to temperature in the same way (Fig. 1). No significant difference in the size (TL and BM) of male (33°C) and female (29 + 31°C) hatchlings existed (MANOVA: contrast $F_{2,144} = 0.94$; $P = 0.39$), but ANOVAs with TL and BM ($N = 180$) as response variables and nest, incubation temperature, and their interaction as grouping variables, confirmed that 31°C individuals had longer TLs than those produced at 29°C or 33°C, which were similar to each other ($F_{2,150} = 7.27$; $P = 0.001$), but we found no difference in BM among incubation temperatures ($F_{2,150} = 0.03$; $P = 0.97$).

Three of the 10 clutches (56 individuals at hatching) had survivors ($N = 40$) from all temperature treatments, to one year of age. Hatching size of animals that died (TL 23.8 \pm 0.2 cm, BM 42.1 \pm 1.5 g; $N = 16$) were not significantly different from those that survived (TL = 23.6 \pm 0.1 cm, $N = 40$; BM = 42.4 \pm 0.8 g, MANOVA $F_{2,49} = 1.58$; $P = 0.2171$).

Within this restricted subsample of survivors (Table 1, $N = 40$) TL and BM at hatching of males and females were similar (MANOVA: contrast $F_{2,30} = 1.12$; $P = 0.34$). But within the first year (Table 1), animals incubated at 33°C (all males) grew much slower than those from 31°C and 29°C (MANOVA: Contrast $F_{2,29} = 5.73$; $P = 0.008$). Initial BM (covariate), clutch, and incubation temperature by clutch interaction had no effect on posthatching growth ($P > 0.52$). Mean growth of 33°C incubated animals (Table 1) was 33.7% (TL) and 53.9% (BM) less than the mean growth of animals from 29°C and 31°C combined.

DISCUSSION

The length and mass of a crocodylian embryo at hatching is primarily influenced by egg size, and secondarily by the degree to which energy, originally in the yolk, has been used for somatic growth or retained as internalized yolk (Webb et al., 1987). Thus, hatchling TL is an index of somatic growth by the time of hatching, whereas hatchling BM is a measure of the

combined yolk plus yolk-free embryo mass and is, thus, a total mass of two entities. Both vary as a function of incubation temperature, which drives embryonic development and metabolic rates (Webb et al., 1987; Whitehead et al., 1990).

With *C. latirostris* in this study, significant clutch effects were demonstrated. First, the clutches were internally inconsistent in the relationship between hatchling size (TL and BM) and incubation temperature (Fig. 1). Despite the general trends with size (31°C produced the longest hatchlings) against incubation temperature, in some clutches 29°C gave the longest, heaviest hatchlings, whereas in others it gave the shortest lightest hatchlings (relative to 31°C and 33°C; Fig. 1). Differences in mean hatchling TL and BM (at all temperatures) between clutches, may well reflect clutch-specific variation in mean egg mass (not measured here), which varies much more between than within clutches in *C. latirostris* (Piña et al., 2002).

The general trend between hatchling TL and incubation temperature (31°C hatchling being longer than 29 and 33°C; Fig. 1B) is inconsistent with results from *Crocodylus johnstoni* and *Crocodylus porosus* (Webb et al., 1987) where lower incubation temperatures resulted in longer hatchlings, that is, embryos with more somatic growth prior to hatching. Shorter animals from 33°C could be explained because at that temperature more yolk could be used to meet enhanced metabolic needs. Animals incubated at 29°C \pm 0.5°C had periods of development at 28.5°C, which could be expected to be stressful to the developing embryos (Webb and Cooper-Preston, 1989). Embryos so stressed alter their pattern of development with a view to enhancing escape from the egg. Hence, the yolk is internalized at an earlier stage of development, giving embryos with the same BM but shorter TL (less somatic growth prior to hatching). Incubation period at 29°C for *C. latirostris* is one of the shortest for crocodylians at this temperature (Piña et al., 2003), which may reflect embryological development rate adjustments, aimed at enhancing pipping and escape from the confines of a suboptimal thermal environment in the egg. A similar lack

of variation in hatchling BM has been reported in some other reptiles (Allsteadt and Lang, 1995; Du and Ji, 2003; Ji et al., 2006). However, other studies report larger hatchlings at medium incubation temperature (Allsteadt and Lang, 1995 [in TL]; Ji and Du, 2001a,b) as found in this work.

Some previous studies have not included clutch as a grouping factor, which hides the possible interaction, making it difficult to determine whether some nests produced bigger hatchlings at lower incubation temperatures than medium or higher incubation temperatures, as we found. Because of the incubation temperature \times nest interaction, results would depend on the selected nests for the experiment (Allsteadt and Lang, 1995). In the American Alligator (*Alligator mississippiensis*), Allsteadt and Lang (1995) reported that larger hatchlings occurred at 32°C than at 29°C, but their BM was reasonably constant across temperatures. Hutton (1987) found *Crocodylus niloticus* hatchlings to be larger (in TL) at 31°C than at either 28°C or 34°C. Our results are similar to those with the alligator and Nile Crocodile, in that longer hatchlings (more somatic growth) were produced at an intermediate incubation temperature (31°C), but they were of similar BM to those incubated at 29°C or 33°C (possibly animals with more internalized yolk; Webb et al., 1987). However, Campos (1993) suggested that length but not BM of *Caiman yacare* could increase as incubation temperature rises.

Incubation temperature has a profound effect on the sex of *C. latirostris* (Piña et al., 2003), a relatively minor effect on the size of individuals at hatching, but a significant effect on posthatching growth. Because our experimental design with 2°C shifts in incubation temperature resulted in 100% males or 100% females, we were unable to determine whether incubation affected growth independently of sex. This appears to be the case with *Crocodylus porosus* (Webb and Cooper-Preston, 1989), and *A. mississippiensis* (Allsteadt and Lang, 1995).

After one year of growth, we found relatively minor differences in the extent of growth that had taken place in animals incubated at 29°C and 31°C (females) but a significant reduction in growth of those incubated at 33°C (males; Table 1). In an experiment carried out by Ferguson and Joanen (1983), similar results were obtained. After one year of growth in *A. mississippiensis*, females from 30°C weighed more and were longer than males incubated at 34°C. However, Joanen et al. (1987) showed that the relationship between size at two years of age and incubation temperature in *A. mississippiensis* was curvilinear (see analysis in Webb and Cooper-Preston, 1989).

Peak male size (from 31.5°C incubation temperatures) was 6–7% higher than peak female size (30.5°C incubation temperature) and 12% higher than male size from 33°C incubation. That is, 33°C incubation produced males, but males whose posthatching growth could be constrained. In their study, as in ours, 33°C incubation produced males but may be too high to produce males with optimum posthatching growth.

Webb and Cooper-Preston (1989) argued that independent of sex, incubation temperature affects the potential of hatchlings to grow fast and to attain larger sizes after they hatch. In all crocodylians, adult males are larger than adult females, sometimes appreciably. They argued that TSD is a considerable advance on genetic sex determination in allowing maleness to be allocated only to embryos developing at a rate that appears optimal for posthatching growth. The same was suggested for the Snapping turtle (Rhen and Lang, 1995, 1999). This would only be consistent with the results reported here if the 33°C incubated animals (all males with reduced growth rates in the first year) were compromised. To test this hypothesis for the species, incubation at lower male-producing temperatures would be needed.

During the first year in captivity, we found that hatching size of animals that died or survived (from the three nests that have surviving hatchlings at the three incubation temperatures) was similar. It was reported that hatchlings incubated at 29°C, 31°C and 33°C had similar survival, but those incubated at 34.5°C had low hatching success and zero survival (Piña et al., 2003). Hatchling size has been demonstrated as having an effect on survival in turtles (*Trachemys scripta elegans*; Janzen et al., 2000) and lizards (Agamidae: *Calotes versicolor*; Ji et al., 2002), with small hatchlings having lower survivorship. However, in other lizards (Iguanidae: *Sceloporus undulatus*; Warner, 2001; Warner and Andrews, 2002) and snakes (Pythonidae: *Liasis fuscus*; Madsen and Shine, 1998), no such relationship could be demonstrated. Survivorship was affected by size in laboratory experiments with *Podocnemis expansa* (Testudines: Pelomedusidae; Valenzuela, 2001) and with *Phrynops hilarii* (Testudines: Chelidae) as mentioned by Piña and Argañaraz (2003), again with smaller hatchlings having lower survivorship.

Shine (1999) listed six different possible models for the adaptive significance of TSD in reptiles. The “matching sex to the time of hatching” seems unrealistic for crocodylians, because they produce only one clutch per year and differences in incubation period caused by incubation temperature are lower than differ-

ences in laying time within a local population, at least in *C. latirostris*. The "matching sex to phenotype" model could not apply since there is an interaction between nest and incubation temperature at hatching and some nests produced hatchlings with larger size at male incubation temperatures and others at temperatures that induce female development; but our results support this model in that, in general, females grew faster than males (but this appears to be an artifact of 33°C being a high incubation temperature as reported in the alligator). The existence of the clutch by incubation temperature interaction could support the "matching sex to egg size" model as well (Shine, 1999) since some clutches did better at some incubation temperatures. Unfortunately, we did not measure the eggs. Recently it was reported that sex is related to egg size in a lizard (Flatt et al., 2001). Our data are not sufficient to test the "nest philopatry" model (Shine, 1999) nor the models involving interactions: the "sex by temperature interaction for offspring survival" or the "sex by temperature offspring phenotypes."

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