Zoological Studies

Effects of Incubation and Rearing Temperatures on *Caiman latirostris* Growth

María Virginia Parachú Marcó[1,2,*], Carlos Ignacio Piña[1,2,3], Melina Simoncini[1,2], and Larriera Alejandro[1,4]

[1]*Proyecto Yacaré Laboratorio de Zoología Aplicada: Anexo Vertebrados (FHUC - UNL/MASPyMA), A. del Valle 8700, (PC3000) Santa Fe, Argentina. E-mail:yacare@arnet.com.ar*
[2]*CIC y TTP - CONICET, Dr. Matteri y España, (PC3105) Diamante, Entre Ríos, Argentina*
[3]*Facultad de Ciencias y Tecnología, Universidad Autónoma de Entre Ríos (UAdER), and Facultad de Ciencias de la Alimentación, Universidad Nacional de Entre Ríos (UNER), Entre Ríos, Argentina*
[4]*Facultad de Humanidades y Ciencias, Universidad Nacional del Litoral. Secretaría de Estado de Medio Ambiente y Desarrollo Sustentable de la Provincia de Santa Fe, Aristóbulo del Valle 8700 - (PC3000), Santa Fe, Argentina*

María Virginia Parachú Marcó, Carlos Ignacio Piña, Melina Simoncini, and Larriera Alejandro (2010) Effects of incubation and rearing temperatures on *Caiman latirostris* growth. *Zoological Studies* **49**(3): 367-373. Different studies found that incubation temperature has an influence on the size of *Caiman latirostris* hatchlings and determines their gonadal sex. Experimental manipulations revealed that the effects of temperature on growth are independent of sex. Ambient temperature after hatching is also an influential factor: juvenile caiman had lower growth at a cool (29°C) vs. a warm temperature (33°C) independent of incubation temperatures. Two-month-old caiman were raised at 2 different temperatures (29 and 33°C) for 100 d. These animals were subjected to different incubation treatments: 29, 31 (females), 33 (males), and 33°C with 17β-estradiol (females). No differences were found in growth based on incubation temperature or sex. Our data indicate that the evolutionary advantage of a temperature-dependent sex determination, if it exists, is not bound to differential growth between the sexes nor to incubation temperature, at least in the 1st stages of life before hatchlings reach their 1st winter. Temperatures during the development and grow-out phases have direct effects on the size of the broad-snouted caiman. http://zoolstud.sinica.edu.tw/Journals/49.3/367.pdf

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Ambient temperatures influence the biology of organisms, but such effects are especially pronounced in ectotherms (Rhen and Lang 1999). Reptiles have a wide range of sex-determination systems, including genotypic sex determination (GSD) and environmental sex determination (ESD; Wibbles et al. 1991). Temperature-dependent sex determination (TSD), in which egg incubation temperatures determine the sex of the developing embryos, exists in many reptiles including all crocodilians (Piña et al. 2003, Deeming 2004),

most turtles (Ewert et al. 2004), some lizards (Harlow 2004), and Tuatara (*Sphenodon* spp.; Nelson et al. 2004).

Although a very small temperature difference is sufficient to induce one sex or the other, this difference in temperature must be maintained for several days during a period referred to as the temperature-sensitive period (TSP; Lance 2008). In alligators, the TSP was shown to occur during the 3rd quarter of development (Lang and Andrews 1994, Milnes et al. 2002) and in the middle 3rd in

*To whom correspondence and reprint requests should be addressed. E-mail:virginiapara2@yahoo.com.ar

Caiman latirostris (Piña et al. 2007b). Incubation temperatures outside this window have no effect on the sex of the embryo, but may affect other phenotypic characteristics (Lance 2008). It is during the TSP that a gonad commits to either ovarian or testicular development (Lang and Andrews 1994). However, in many studies, exogenous estrogens administered prior to the TSP can override the effects of male incubation temperature, thus inducing females. This was examined in a freshwater turtle (*Trachemis scripta*; Sheehan et al. 1999), lizard (*Eublepharis macularis*; Tousignant and Crews 1995), crocodiles (*Crocodilus porosus*, *Cro. palustris*, and *Cro. johnstoni*; Lang and Andrews 1994), and alligators (*Alligator mississippiensis*; Lance and Bogart 1994 and a caiman *C. latirostris*; Imhof and Piña 2005). This indicates that the undifferentiated gonad responds either directly to estrogens or indirectly through some kind of estrogen-sensitive or extragonadal tissue (Milnes et al. 2002). While progress has been made with respect to the developmental mechanism of TSD (Lance 1997), little is known about how temperatures affect the growth with respect to other traits (Du and Ji 2003, Piña et al. 2003).

Caiman latirostris eggs incubated at 29 and 31°C produced 100% females; incubation at 33°C produced 100% males; and a higher temperature (34.5°C) produced both sexes (Piña et al. 2003). Thus the broad-snouted caiman has pattern II of the TSD (TSD II; female-male-female, as defined by Ewert et al. 1994) as do other crocodiles (Lang and Andrews 1994, Deeming 2004). The TSD II mode, results in females being selected for at low and high temperatures extremes, and males being selected for at intermediary temperatures. This mode occurs in species where adult females are smaller in size than males, or where there is no sexual dimorphism (Ewert and Nelson 1991).

The thermal environment can influence stages in the life history other than the adult stage, such as the embryo and hatchling stages (Iungman et al. 2008, Kuo et al. 2009). Many factors affect the genetic potential of a crocodile's growth during the 1st yr of life. Incubation temperature and sex can independently influence post-hatching growth rates; and there are hypotheses about the possible evolutionary advantages of TSD in reptiles (Piña et al. 2007a, Crews and Bull 2008). Recently, Warner and Shine (2008) found that reproductive success of each sex was optimized by the incubation temperature that produces that sex under natural conditions. Given that the incubation temperature determines gonadal sex and influences many other traits, it is critical to acknowledge the potentially interactive effects of incubation temperature and gonadal sex on phenotypes in species that exhibit TSD (Rhen and Lang 2004), and try to determine if it does so differently for males and females. In oviparous species, this difficulty can be overcome by hormonally manipulating embryos to reverse the gonad's phenotype and break the link between offspring sex and incubation temperature (Rhen and Crews 1999).

Incubation temperatures also influence post-hatching patterns of crocodilian thermoregulation (Lang 1987), growth, and survivorship (Joanen et al. 1987, Piña et al. 2003). In these animals, TSD represents an evolutionary advantage because it allows males and females to grow at different rates, which could be related to the greater size of adult males (Lang and Andrews 1994). But Piña et al. (2007a) found that absolute growth for up to 1 yr was higher for *C. latirostris* females (from eggs incubated at 29 and 31°C) than for males (from eggs incubated at 33°C).

TSD is often so extreme that only 1 sex is produced across a wide range of incubation temperatures. Thus, a test of the theory requires producing females at unnatural temperature extremes. This experiment allowed us to separate the potentially confounding effects of embryonic incubation temperature and sex on the neonatal growth of the resultant hatchlings, and to make same-sex comparisons across a range of different incubation temperatures. The experiment was conducted for 100 d beginning when the animals were 2 mo old. In temperate climates, there are hot summers and cold winters. The winter is the most critical period for hatchlings, and if they are able to survive, they can probably survive their 1st yr of life (Larriera and Imhof 2006). For this reason, rapid growth during the 1st mo of life might provide advantages for tolerating winter temperatures. Our objective was to determine if there are any effects of sex and incubation temperatures on the post-hatching growth rate. On the other hand, we tried to determine if there are influences of incubation treatments and grow-out conditions on the snout-vent length and body mass. Finally, we discuss if there are implications of these results for the evolution of TSD.

MATERIALS AND METHODS

Caiman latirostris eggs were collected shortly after oviposition (48 h) from 2 different wild nests (17 viable eggs for nest A and 14 viable eggs from nest B) during the 2000 reproductive season (Dec. 1999-Jan. 2000) in Santa Fe Province, Argentina. Prior to the removal of the nest, the upper surface of each egg was marked with a graphite pencil so we could maintain the original nest orientation during the experimental incubation. Eggs were collected, and those not viable were discarded. Both nests were transported to the laboratory and divided in groups that were assigned to 4 incubation treatments 29, 31 (female-producing temperature), 33 (male-producing temperature), and 33°C with topical application of 17β-estradiol during the TSP (Piña et al. 2007b) resulting in 100% females (Stocker et al. 2003, Imhof and Piña 2005; Table 1). To control for the clutch effect (Piña et al. 2007a), eggs from different clutches were randomly distributed among treatments.

Incubators consisted of plastic containers (65 × 40 × 50 cm; 2 for each incubation treatment) filled to a depth of approximately 10 cm with water. An aquarium heater with a thermostat control was placed in the water, with a thin sheet of plastic above the water. Onto the plastic sheet was placed damp nesting material, into which the eggs and a temperature data logger (Onset Computer Corp., Pocasset, MA, USA) were put. The data loggers were programmed to record temperature every 10 min and were checked daily, because the metabolic heat among the eggs can increase incubation temperature by a few degrees (Zbinden et al. 2006). Each incubator was covered with a Styrofoam lid and sealed with plastic wrap to ensure high humidity. Using these incubators, the temperature was maintained within ± 0.5°C, and the relative humidity remained close to saturation.

Between 19 and 25 d of development, prior to the TSP (Piña et al. 2007b), 5 μg of 17β-estradiol dissolved in 5 ml ethanol was applied topically to the exterior of the eggs of 1 group of eggs (at 33°C) to manipulate the hormone levels in order to override the effect of temperature (Crews et al. 1991). This process was reported to cause a 100% occurrence of females (Stocker et al. 2003, Imhof and Piña 2005).

At hatching, all individuals were weighed, measured, and identified by 2 serially numbered tags (National Band and Tag, Newport, KY, USA) on their hind feet. When hatchlings reached 2 mo of age, 31 animals were randomly distributed into 2 temperature environments (29 and 33°C). Individuals from every incubation treatment (29, 31, 33, and 33°C with hormone) were included in each temperature group. The temperature was continuously monitored with a Hobo Temperature Data Logger (Onset Computer Corporation, MA, USA) on each tank. Animals were fed chicken supplemented with vitamins and minerals ad libitum (Vionate S®, Novartis, São Paulo, Brazil), 3 times a week. After 12 h, the remaining food was removed and chambers were cleaned. At the end of the experiment, weight (to a precision of 0.5 g) and snout-vent length (SVL; to a precision of 0.5 cm) were recorded. Animals were sacrificed at the completion of the study to examine the gonads and determine hatchling sex. Results are expressed as the mean ± standard error (SE).

Hatchling size was analyzed with analysis of variance (ANOVA) where body mass (BM) and SVL were response variables, and incubation temperature (29, 31, 33, and 33°C with hormone) and nest of origin were the grouping variables; we also considered the influence of incubation temperature on the growth rate (incubation temperature by nest interaction; It × N). Contrasts were made between: 1) females (29, 31, and 33°C with hormone) and males (33°C); 2) females incubated at 29 and 31°C and those produced at 33°C with hormone; and 3) eggs incubated at 33°C with and without hormone treatment. Our alpha value for significance was 0.05.

Hatchling growth was determined by subtracting the initial measurement from the final one and was examined by analysis of co-variance (ANCOVA), where BM and SVL growth were the response variables. Incubation treatment and growing temperature (29 and 33°C) were grouping variables, and nest of origin was used as a blocking factor. We considered the interactive effect of incubation temperature and rearing temperature on the growth rate (rearing temperature by the incubation temperature interaction; Rt × It) in order to evaluate potential effects.

RESULTS

Hatchling sex

Incubation at 29, 31, and 33°C with 17β-estradiol resulted in all hatchlings being females. Hatchlings produced at 33°C were all males.

Hatchling size

Incubation temperature effects were evident on the SVL (F = 5.17; p = 0.0073; Fig. 1A) and BM (F = 5.76; p = 0.0047; Fig. 1B). Hatchlings incubated at 31°C had higher BM and SVL than hatchlings incubated at 29°C (Tukey's test: p < 0.05). The interaction between incubation temperature and nest was not significant for SVL (interaction It × N: p = 0.1086), but nests responded to temperature in different ways for the BM increase (Fig. 2; interaction It × N: p = 0.0287), showing that nest B produced heavier hatchlings at the intermediate incubation temperature (31°C). No difference in body size was found between males and females from several incubation treatments (contrast 1, p > 0.5329). Females incubated at 29 and 31°C were of similar size compared to those produced at 33°C with the hormone (contrast 2, p > 0.2931). The BM and SVL of male hatchlings produced at 33°C were similar to those of females produced at 33°C with 17β-estradiol (contrast 3, p > 0.3180).

Hatchling growth

Two animals from different incubation temperatures (29 and 31°C) died during the experiment (Table 1). The rearing temperature had a profound effect on hatchling growth. As expected, after 100 d, animals kept at 33°C had grown more than those kept at 29°C (p < 0.0040). For both variables (BM and SVL), animals incubated under different treatments responded in the same way to growth temperature (interaction Rt × It: p > 0.6919; Table 2). The clutch (p < 0.0215) but not incubation treatment (p > 0.3227) had an influence on caiman growth.
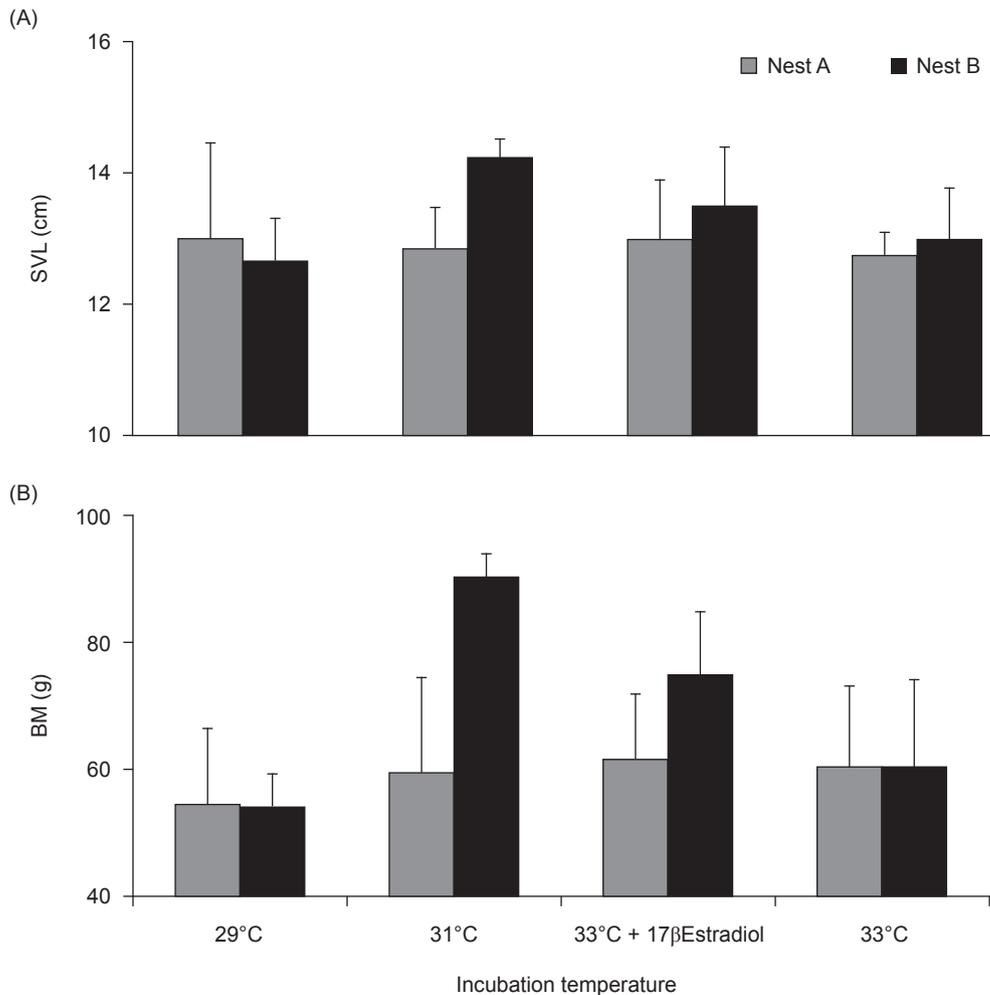

Fig. 1. Snout-vent length (SVL; A) and body mass (BM; B) of *Caiman latirostris* hatchlings at 60 d of age, incubated at different temperatures (29, 31, 33, and 33°C with 17β-estradiol).

DISCUSSION

In this experiment, we found that embryonic temperature affected hatchling size of *C. latirostris*. Hatchlings from eggs incubated at 31°C were heavier and longer than those incubated at 29°C. In agreement with this idea, many authors concluded that incubation temperature affects the rate of embryonic development in crocodilians in different ways (Lang and Andrews 1994, Piña

Table 1. Animals from 2 wild nests distributed at random at 4 incubation temperatures (29, 31, 33, and 33°C with 17β-estradiol) and 2 rearing temperatures (29 and 33°C)

Incubation temperature	Rearing temperature	
	29°C	33°C
29°C	4	4 (-1)*
31°C	4 (-1)*	4
33°C	5	2
33°C +17β-estradiol	4	4
Total no. of hatchlings	17 (16)	14 (13)

*Indicates a dead animal.

et al. 2007b, Iungman et al. 2008). Hatchling sizes show different relations to incubation temperatures, and there are reports of longer hatchlings at low (*Cro. johnstoni* and *Cro. porosus*, Webb et al. 1987), intermediate (*Cro. niloticus*, Hutton 1987; *C. latirostris*, Piña et al. 2007a; and *A. mississippiensis*, Allsteadt and Lang 1995), and high temperatures (*C. yacare*; Campos 1993). Some authors reported better growth at extreme temperatures (29.4 and 32.8°C) than intermediate ones (30.6 and 31.7°C; Joanen et al. 1987) in *A. mississippiensis*. The same results were reported in hatchling lizards, where thermal effects vary among species and do not exhibit a constant pattern (Chen et al. 2009). These differences could be the result of the nest of origin due to incubation temperature interactions (Allsteadt and Lang 1995, Piña et al. 2007b), and if the experiment were repeated with other nests, the results could completely change.

Joanen et al. (1987) reported that the post-hatching growth rate of *A. mississippiensis* was influenced by incubation temperature. In lizards, it was reported that temperatures can affect the hatchling phenotype (Harlow 2004). Warner and Shine (2008) showed that incubation temperature

Table 2. Mean increases in snout-vent length (cm) and mass (g) Bay tray after 100 d of experiments on *Caiman latirostris* hatchlings produced at different incubation temperatures (29, 31, 33, and 33°C with 17β-estradiol) and maintained at 2 rearing temperatures (29 and 33°C). P_{Rt}, *p* value of the rearing temperature; P_{It}, *p* value of the incubation temperature. The interaction "incubation temperature by rearing temperature" was not significant for BM or SVL (*p* > 0.6919)

SVL increase (cm) after 100 d of the experiment					
Rearing temperature	Incubation temperature				
	29°C	31°C	33°C + 17β-estradiol	33°C	P_{It} value
29°C	19 ± 1.1	18.9 ± 2.6	16.7 ± 2.8	17.2 ± 2.5	*p* = 0.3332
33°C	20.9 ± 1.2	21.1 ± 1.9	19.9 ± 1.6	18.5 ± 1.4	
P_{Rt} value	*p* = 0.0040				

Mass increase (g) after 100 d of the experiment					
Rearing temperature	Incubation temperature				
	29°C	31°C	33°C + 17β-estradiol	33°C	P_{It} value
29°C	240.9 ± 26.7	218.7 ± 111.7	170.5 ± 126.9	134.8 ± 76.6	*p* = 0.3227
33°C	302.3 ± 87.4	337.2 ± 115.3	240.2 ± 89.1	195.4 ± 107.2	
P_{Rt} value	*p* = 0.0029				

affects lifetime fitness, and does so differently for jacky dragon (*Amphibolurus muricatus*) males and females. On the other hand, authors studying turtles and oviparous lizards suggested that the influences of incubation environment on hatchling phenotypes (including growth rates) may be irrelevant to fitness (Qualls and Shine 2000, Steyermark and Spotila 2001). Caiman mortality is highly size-specific, and a large number of hatchlings die during the 1st winter (Larriera and Imhof 2006), so faster growth after hatching should be biologically relevant. Wild nests of the broad-snouted caiman exhibit differences in temperature during the incubation process, varying from 29.9 ± 1.4 to 33.1 ± 2.1°C (Piña and Larriera 2002). We assumed that because of these differences in incubation temperature, animals produced at lower temperatures would have an advantage if kept at lower temperatures, and that those incubated at high temperatures would have an advantage if kept at a high rearing temperature. Our data showed that incubation temperatures did not affect hatchling growth in a short time period previous to winter, regardless of the rearing temperature.

Crews and Bull (2008), based on the Charnov-Bull model, believed that for species that exhibit environmental sex determination, something must happen to the egg or embryo that carries over into adult fitness, and the effect must be one that exerts different effects on males and females. The puzzle is that so much growth occurs between hatching and maturity in a crocodilian; it would seem that all effects of embryonic temperature would be erased by adulthood (Crews and Bull 2008). In this paper, we found no effect of incubation temperature on hatchling growth for up to 160 d post-hatching. We suggest that egg incubation temperatures do not directly affect postnatal growth, but that growth is affected by differences in thermal preferences induced by egg incubation temperatures. However more-deliberate experiments are needed to test this hypothesis, because in most TSD species that are long-lived and mature after many years, like crocodiles, the overall effects on characters are difficult to determine (Teller 2007). Understanding reproductive fitness will reveal how varying activities and temperatures affect these animals (Rhen and Lang 1999). It is unlikely, though, that sex could have confounded the incubation temperature effects, for the same reason that growth was not influenced by egg incubation temperatures, and there was no effect of sex on growth. Due to the ongoing controversy over the continued use of TSD as a mechanism of sex-determination, and the unknown evolutionary significance, different additional research must be performed, and future studies must try to integrate numerous goals and aims to study the incubation effects in adult organisms.

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