Thermic Variation in Incubation and Development of Pantanal Caiman (*Caiman crocodilus yacare*) (Daudin, 1802) Kept in Metabolic Box

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ABSTRACT

*Thirty-two eggs of Pantanal caiman (*Caiman crocodilus yacare*) (Daudin, 1802) were incubated at 30°C. After 20 days of incubation at 30°C, the remaining eggs were distributed in the following way: seven at 34°C, seven at 32°C, six at 30°C and six at 28°C. At 32°C and 34°C a 100% hatchability was obtained while at 30°C 83.3% and at 28°C 66.6%. After hatching, the animals were kept for 120 days at the same temperature of their final incubation. Weight (g) control and morphometric (cm) analysis were carried out. The statistic design was totally randomized and the data were treated by regression analysis showing a quadratic behavior (p<0.05). It was found that weight, total length (TTL), tail length (TL), snout- vent length (SVL), head length (HL), eye length (EL), snout length (SL), mid-point snout width (MSW), head width (HW), width of snout at nostrils (NW), distance between limbs (DBL) showed a higher development at the temperature range from 31.2°C to 31.8°C and circumference of belly at 30.0°C.*

Key words: Caiman, development, incubation, temperature, thermic variation

INTRODUCTION

The *Caiman crocodilus yacare* (Daudin, 1802) can be found in Bolivia, Paraguay, Uruguay, Argentina and Southwestern of Brazil, more specifically from Mato Grosso Sul (Paraguay river) to the state of Paraná – Paraná river (King and Burke, 1989). It is considered a medium size alligator, reaching 2.5 to 3.0 meters at adult age. In Brazil, nesting takes place in the rainy season, with an average of 21 to 38 eggs being laid (Groombridge, 1987). In the reproductive handling, the artificial incubation is a stage to increase caiman culture. This method is widely used other crocodilian cultures (Joanen

and McNease, 1975; Joanen and McNease, 1987; Joanen et al., 1987; Breyer, 1987; Verdade et al., 1992; Larriera, 1993). Generally, artificial incubation is processed with constant temperatures from the beginning to the hatching of the eggs. This aspect deserves discussion once that under natural nest conditions constant temperature does not occur during the whole incubation period.

In each nest occurs a temperature variation in relation to the position of the eggs and to room or environment temperature and this variation determines differences in development and sex (Joanen, 1969; Ferguson and Joanen, 1983). During the natural incubation, these occurs a

gradual temperature increase from the beginning to the hatching (Webb and Smith, 1984). According to these authors, the gradual temperature may facilitate development of eggyolk integralization and hatching.

Investigations on thermic variation during incubation have been carried out in order to verify the effects of temperature on the characteristics of the young alligators which were confirmed to increase in the last third of the embryo development, the phase of its fastest growth (Congdon et al., 1995). The embryo formation was divided into three general stages: the first two comprise the initial formation until the sexual definition period, and the last stage corresponds to its growth (Ferguson, 1985). Ferguson, 1987, evaluated the stages of embryonic development of crocodilians by working with eggs of three different species (*Alligator mississipiensis, Crocodilus johnstoni, Crocodilus porosus*) at different temperatures. In All*igator mississipiensis*, the incubation increase in temperature, (taking into account the viability limits for these species), accelerated the development of organogenesis initial phases. On the other hand, at 34°C the gonadal development was decelerated in relation to the development at 30°C, but the embryonic development was faster at 34°C when compared with that of 30°C and 28°C.

Data of the thermic influence on the initial growth have also been obtained. Joanen et al., 1987 observed significant effects of incubation temperature (from 29.4°C to 32.8°C) on final embryonic development and weight gain in animals kept at 30°C, which showed to be even more intense at higher incubation temperatures. Pinheiro et al (1992) analyzed the effects of 22.8, 26, 29.5 and 32°C temperatures on the development of Pantanal caiman and found a low food uptake and growth at 28.8°C but high food uptake and growth between 29.5 and 32°C up to 71 days of life. On the other hand, Vianna et al., 1995, studying the effect of 30, 32 and 34°C temperatures on the growth of young of *Caiman latirostris,* observed a higher total growth between 32 and 34°C but they did not observed difference between 32 and 34°C temperatures.

The bibliographical survey revealed very few works on thermic variation in incubation and no work on the particular species *Caiman crocodilus yacare*. Therefore, the present experiment to evaluate the effect of thermic variation during the incubation period and the effects of different temperatures on after hatching development was understaken.

MATERIALS AND METHODS

The experiment, was carried out for 175 days during April 1st through September 22rd, 1998.

The stage from incubation to hatching was carried out at Criadouro de Animais Silvestres Jacará in the district of Guaragi, municipality of Ponta Grossa, state of Paraná, Brazil, and the growing stage was carried out at the Laboratory of Animal Reproduction of Universidade Estadual de Maringá.

On April 4th, 1998, thirty-two eggs were collected from the same nest, with an average weight of 61.28 g each, showing opaque bands. Two of the eggs were opened to verify the embryonic stage of the nest and later used to analyze the embryonic development. The remaining thirty eggs were weighted, measured, marked with pencil and placed in an incubator, according to the model proposed by Verdade et al., 1992, at 30°C. An 82-L 643x403x290mm polystyrene foam box was used as incubator containing a 7cm watersheet, two perforated silverware supporters on which was placed a plastic tray containing moistened vermiculite as support and cover for 25-Watt lights were fastened and controlled by 0.2°C- precision thermostat, made and checked in the laboratory of physics, at Universidade Estadual de Maringá. An orifice to place the thermometer was made at the middle height of one of the walls.

During the incubation, other four eggs were opened, to evaluate the embryonic development, two on April 8th and the two on April 18th. The six embryos (the two opened earlier and two just mentioned) were examined, weighted and described according to the model proposed by Ferguson, 1987.

After 20-day incubation at 30°C, the remaining eggs were redistributed at four different temperatures: seven each at 34, and 32 and six each at 30 and 28°C. Each egg was placed individually in a plastic ice-cream box containing vermiculite, which was a modified incubation model different from that created by Verdade, 1992, thus facilitating the identification control of each egg at the hatching moment.

After hatching, the newborn animals were kept in a growing box containing water warmed to their original hatching temperature: seven animals in a box at 34°C, seven at 32°C, five at 30°C and three at 28°C. Each animal was identified with colored beads fastened by smooth thin wire at the final third of the tail, fortnightly weighted and morphometrically analyzed. The measurements taken were the following: total length (TTL)–from the extreme snout front to the extreme tail tip; tail length (TL)-from the front end of vent to the extreme tail tip; snout-vent length (SVL)–from the extreme snout front to the front end of vent; head length (HL) from the extreme snout front to the back surface of occipital condyle; eye length (EL)–total eye socket length; snout length (S)– from the front end of the snout to the front eye socket edge; width of snout at mid –point (MSW); head width (HW)–distance between the lateral surfaces of jawbone condyles; width of snout at nostrils (NW)–distance between the lateral surfaces of jawbone front end; distance between members (DBM); circumference of belly (CB)– belly circumference at the middle region of the animal. For weighting, a 0.01g–precision Sartorius 1202 MP balance was used and for morphometric analyses, an Italian 0.05 mm–precision M-Metrica caliper, a millimetric ruler, a transparent 1mm-precision ruler and a 1mm-precision tape measure. Each growing box was kept tilted, providing 2/3 of the bottom area moistened and 1/3 dry. 150-Watt aquarium electric heaters coupled to a 0.2°C–precision thermostat were used to keep and control temperature. Water temperature was checked every day through an Incoterm 0.1°C–precision thermometer for the possibly necessary adjustment of the thermostat, which was always made within the precision limits, once the experiment was carried out in a nonairconditioned room which suffered the influence of room temperatures higher than the temperatures tested in the experiment. The open part of the boxes was covered with a plastic screen fastened with elastic cord to prevent the animals from escaping.

The diet was constituted of ground cattle meat including 0.01% vitaminic premix Jacaré-Phós. The animals were fed *at libitum* five days a week taking into account the minimal food requirement proportionate to 25% live weight, the animal's appetite and losses during feeding. It must be emphasized that the vitaminic premix contained zinc bacitracine, an antifungic active principle.

For feeding a polystyrene recipient was placed on the dry area of the box. The animals were fed at 5 p.m. and the next morning they were taken out of the boxes which were cleaned and new clean water was added at the appropriate temperature to avoid thermic shocks.

The experimental design was totally randomized, in which the different temperatures constituted the treatments and the hatched animals were the repetitions. The collected data were submitted to regression analysis taking into account the treatment and animal ages, using Secretary of State for Agriculture's software, after Euclides (1993), in the following statistical model:

$Y_{ijk} = b_o + b_1 T_i + b_2 T_i^2 + b_3 I + b_4 I^2 + e_{ijk}$, in which:

Y_{ijk} = observation made to animal j at temperature i at age k

b_0 = constant

b_1 = linear regression coefficient of the observation Y in function of temperature i

b_2 = quadratic regression coefficient of observation Y in function of temperature i

b_3 = linear regression coefficient of observation Y in function of age j

b_4 = quadratic regression coefficient of observation Y in function of age j

T_i = water temperature i = 1; 2; 3; 4

I_j = animal age j; j=1; 120 days

e_{ijk} = aleatory error associated to each observation y_{ijk}

RESULTS

General data on artificial incubation are presented in Table 1. In Table 2, a brief description of the six embryos collected in the period of the first 18 days of incubation at 30°C is presented. The embryos collected increased five times their weight from the first to the 18th day of artificial incubation. In Table 3, the most important correlations of the parameters measured such as weight x TTL, weight x TTL, weight x TL, weight x SVL, weight x CB, TTL x TL, TTL x HL, SVL x HL and SVL x SL are shown. The correlations showed to be significant (p<0.01).

Miranda, M. P. et al.

Table 1 - Data of the effect of thermic variation on Pantanal caiman(Caiman crocodilus yacare) eggs

IT (°C)	FT (°C)	N	AT (days)	H (%)	MT(%)	NSA
30	28	6	69.2	66.63	50	3
30	30	6	61.4	83.3	16.6	5
30	32	7	57.2	100	0	7
30	34	7	56.7	100	0	7

IT= Initial incubation temperature (°C); FT= Final incubation temperature (°C); N= Number of eggs; AT= Average incubation time (days); H= Hatchability (%); MT= Pre and post-hatching mortality(%); NSA= Number of post-hatching surviving animals

Table 2 - Morphological description of six embryos of Pantanal caiman (*Caiman crocodiles yacare*) collected during the initial 18 days of incubation at 30 °C

Egg opening day	Egg n°	Embryo weight (g)	Morphological stage (Ferguson,1987)	Description of morphological stage *
April 1st 1998	6	1.19	17	Paddle form hands and feet presenting mesodermic condensations with defined digits; transparent head.
	13	0.83	17	
April 8th 1998	24	2.80	20	Small portion of interdigital membranes still remaining between toes and between digits; whitened body.
	29	2,77	19	Presence of interdigital membranes that characterize the paws as paddles in which digits are already internally defined.
April 18th 1998	01	5,03	22	Dorsal skin pigmentation; absence of interdigital membrane; definite round claws; there are four protuberances on the head between the eye sockets.
	07	5,36	22	

* descriptions made during the experiment by the author

Table 3 - Correlations of the parameters weight, total length, tail length, snout-vent length, circumference of belly, head length, snout length of *Caiman crocodilus yacare* sample specimens

Variables	Correlations
Weight x TTL	0.86
Weight x TL	0.82
Weight x SVL	0.84
Weight x CB	0.87
TTL x TL	0.96
TTL x SVL	0.95
TTL x HL	0.89
SVL x HL	0.87
SVL x SL	0.92

The eggs whose incubation temperature was reduced from 30°C to 28°C suffered the severest damages. Two of the six eggs were found dead after 51 days of incubation: the embryos were already formed but dead. The hatching of the four remaining eggs resulted in premature animals that still had a small portion of the vitelinic sac exposed outside the abdominal cavity. One of the eggs hatched on the 68th day of incubation, but the animal showed a spent aspect and died the following day. The other three animals hatched, on the 68th, 69th and 71st day of incubation, respectively. From the eggs kept at 30°C, four hatched with 61 to 62 days of incubation, while the remaining egg was opened on June 2rd and showed the presence of a living embryo, though extremely retarded in relation to viteline sac absortion, needing approximately other 20 to 25 days to hatch. The eggs incubated at 32°C and 34°C presented a 100% hatchability rate and the incubation time for each egg was, 54, 55, 56, 57, 58, 60 and 60 days in one case and 55, 56, 57, 57, 58 and 58 in the other case, respectively. Data on the initial development were collected for 120 days.

The parameters measured after the statistical analysis resulted in regression equations correlating treatment and age of the animals which are in the growing curve obtained through regression analysis presented in Figure I to Figure XI. The regression equations were quadratic and statistically significant ($p<0.05$), except for the parameter width of nostrils, in which no effect occurred.

Through equation derivation the following temperatures were obtained: weight= 31.2°C; TTL= 31.6°C; TL=31.7°C; SVL=28°C; HL=31.8°C; EL=31.6°C; SL=31.3°C; HW=31.8°C; DBL=31.8°C; SW=31.3°C; CB=30.6°C. The variable NW did not vary ($p>0.05$) in the treatments at no age.

DISCUSSION

The problems met in the final incubation stage at 28°C probably occurred because of the temperature reduction from 30 to 28°C. The egg incubation at constant temperature of 28°C (Breyer, 1987) resulted in 94% hatchability rate, which indicated that 28°C not the problem *per se* but the reduction during incubation from 30°C to 28°C. On the other hand , Webb and Cooper-Preston,1989, verified that nests with temperatures between 28.1°C and 29°C, and between 33.1°C and 35°C showed a high mortality rate. According to these authors, from 11 eggs incubated in laboratory at 28°C eight embryos hatched prematurely but were normal, one embryo died before hatching and two during the hatching process. As for the egg that did not hatch at the sam e time with the others nested at 30°C and had its development retarded, it may be concluded that the eggs in the same nest are differently affected by the thermic treatment depending on each developing potential. As for the temperature increase from 30 to 32 and 34°C a 100% hatchability rate was obtained, but two embryos (one from the 32°C nest and one from the 34°C nest) hatched prematurely presenting incomplete absorption of the viteline sac and incomplete closure of abdominal cavity. It could be possible that external noises and the voicing of the other animals within the eggs may have affected the premature hatching of embryos as described by Joanen, 1969. Incubations at constant temperatures from 28 to 34°C were carried out with alligator eggs. At 31°C, the hatchability was 18% higher that at 29.4°C and 60% higher than at 33.9°C (Joanen and McNease, 1979), which showed the highest hatchability within the temperature limits from 31°C to 31.7°C. Other temperatures as 32.8 with 83% hatchability rate, 31.7 with 82.1%, 30.6 with 76.2% and 29.4 with 68% were also tested (Joanen and Mcnease, 1991). The results of 100% hatchability found at 32 and 34°C in the present experiment were in disagreement with those of the author cited, but this success come be due to thermic variation once that a higher temperature used in the final growing phase of the embryo facilitated the hatching of animals with minimum of characteristic variation. It might have result in a more homogeneous hatching (Congdon et al., 1995).

In the twenty-second morphological stage of the embryos here collected and compared to those of Ferguson, 1987, is experiment, they showed to be completely formed and growing in size and weight. The embryonic developments of *Alligator mississipiensis, Crocodilus johnstoni* and *Crocodilus porosus* were compared through morphometric analysis by Deeming and Ferguson, 1990.
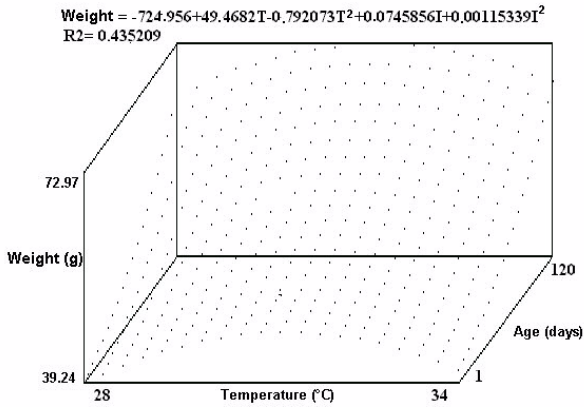
Weight = -724.956+49.4682T-0.792073T^2+0.0745856I+0.00115339I^2
R2= 0.435209

Figure 1 - Weight (g) of caiman due to temperature (ºC) up to 120-day age. T = Temperature, A = Age, R2 = Regression coefficient.
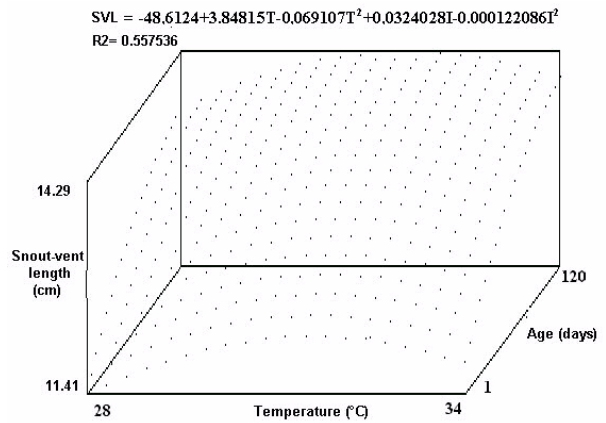
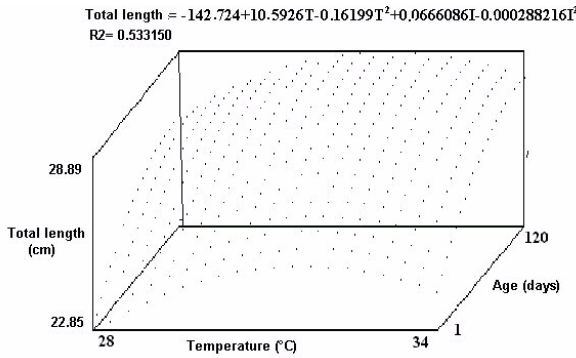Total length = -142.724+10.5926T-0.16199T^2+0.0666086I-0.000288216I^2
R2= 0.533150

Figure 2 – Total length (cm) of caiman due to temperature (ºC) up to 120-day age. T = Temperature, A = Age, R2 = Regression coefficient.
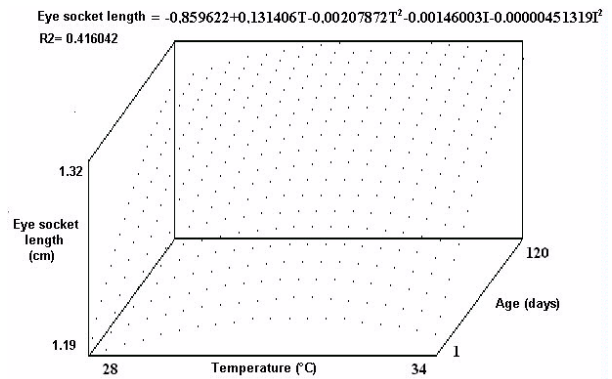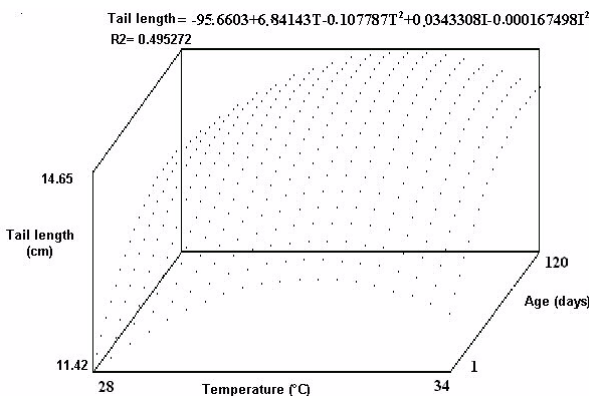
Tail length = -95.6603+6.84143T-0.107787T^2+0.0343308I-0.000167498I^2
R2= 0.495272

Figure 3 - Tail length (cm) of caiman due to temperature (ºC) up to 120-day age. T = Temperature, A = Age, R2 = Regression coefficient.

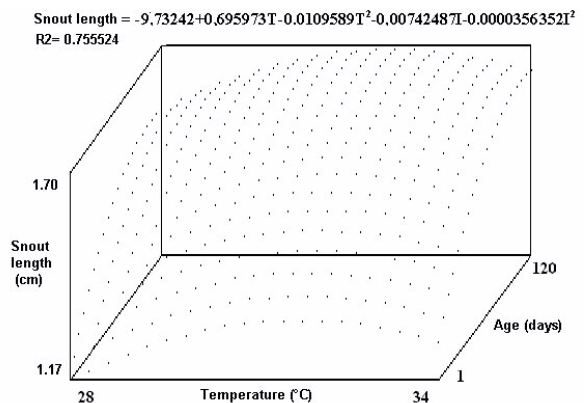SVL = -48.6124+3.84815T-0.069107T^2+0.0324028I-0.0001220861^2
R2= 0.557536

Figure 4 - Snout-vent length (cm) of caiman due to temperature (ºC) up to 120-day age. T = Temperature, A = Age, R2 = Regression coefficient.

Eye socket length = -0.859622+0.131406T-0.00207872T^2-0.00146003I-0.00000451319I^2
R2= 0.416042

Figure 5 - Eye length (cm) of caiman due to temperature (ºC) up to 120-day age. T = Temperature, A = Age, R2 = Regression coefficient.

Snout length = -9.73242+0.695973T-0.0109589T^2-0.00742487I-0.0000356352I^2
R2= 0.755524

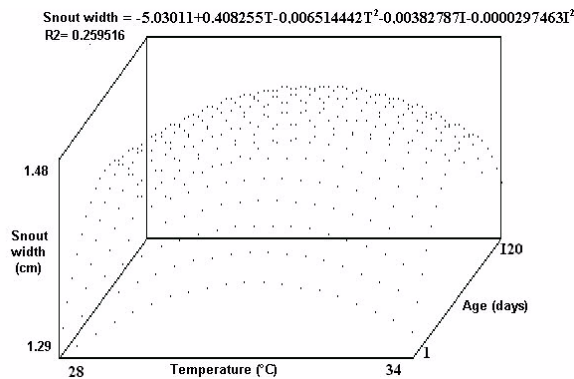**Figure 6** - Snout length (cm) of caiman due to temperature (ºC) up to 120-day age. T = Temperature, A = Age, R2 = Regression coefficient.

Figure 7 - Snout width (cm) of caiman due to temperature (ºC) up to 120-day age. T = Temperature, A = Age, R2 = Regression coefficient.
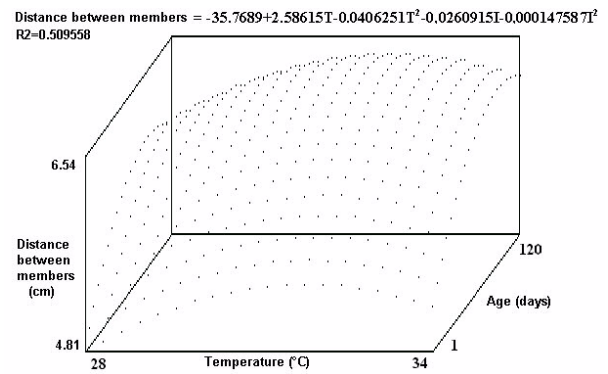

Figure 8 - Head width (cm) of caiman due to temperature (ºC) up to 120-day age. T = Temperature, A = Age, R2 = Regression coefficient.

Figure 9 – Circumference of belly (cm) of caiman due to temperature (ºC) up to 120-day age. T = Temperature, A = Age, R2 = Regression coefficient.

Figure 10 – Distance between members (cm) of caiman due to temperature (ºC) up to 120-day age. T = Temperature, A = Age, R2 = Regression coefficient.
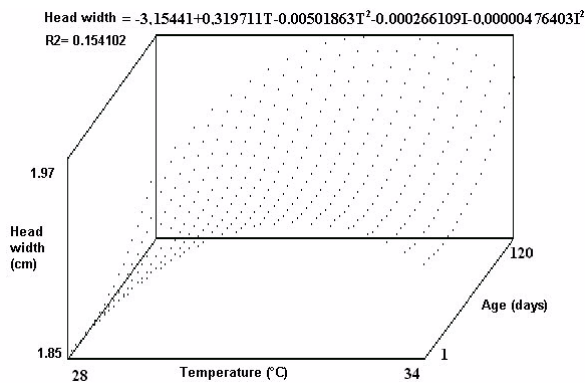

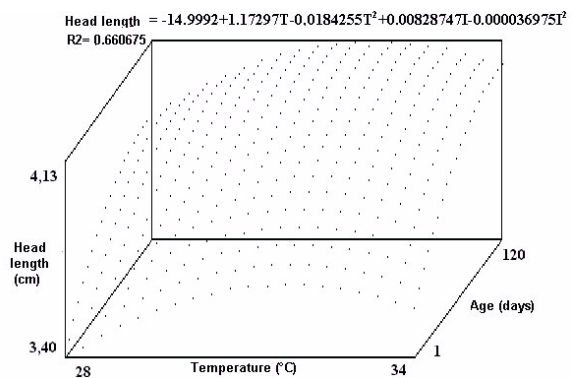**Figure 11** - Head length (cm) of caiman due to temperature (ºC) up to 120-day age. T = Temperature, A = Age, R2 = Regression coefficient.
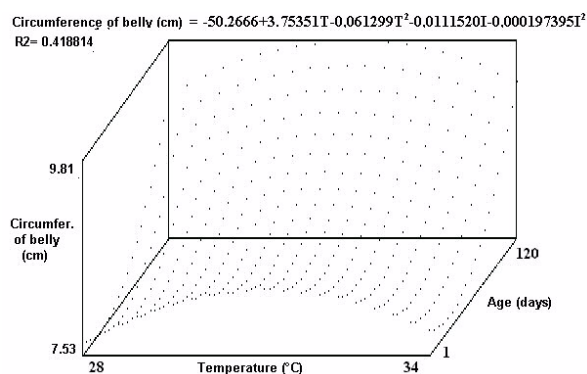
These authors reported that the fast increase in embryonic mass initiated in morphological stage 20, with species-specific differences in morphological stages 23, 24 and 25. The differences in relation to total length initiated in stage 10 reaching their maximum increase in morphological stages 24 and 25. Taking into account that at collecting the embryos had already reached stage 17, it may be possible that the sex of the animal was already defined, though no embryonic tissue analysis was then made. As for post-hatching development, the animals kept at 34°C showed the strongest appetite while the animals kept at 28°C showed the minimum appetite, but both had their development retarded when compared to the other treatments which evidences that extreme thermic treatments are detrimental to the animal's growth. The best

temperature treatment for weight gain was 31.22°C, close to the best temperature 29.5°C, followed by the second best, 32°C, found by Pinheiro et al., 1992. The weight gain verified in this experiment, however, is lower than 91.21g found by Pinheiro et al., 1992, probably due to the high population density. The best temperature, 31.6°C, for total length (28.89 cm) was similar to that found in experiments with alligators(30.6 °C and 31.7 °C) by Webb and Cooper-Preston, 1989, and 32 oC by Vianna et al., 1995, for *Caiman latirostris.* The tail length depends on the size of the egg (Deeming and Ferguson, 1990). A high correlation (0.96) between total growth and tail length was observed, which may explain the best temperature, 31.7°C, found in the present experiment, close to 31.6°C, observed by those authors for total length. The greatest tail length was 14.65 cm which indicates an increase of 3.23 cm from the beginning to the end of the experiment. Snout-vent length represents approximately half of the total length of the animal and this explains the same 31.6°C for snout-vent length and total length found in this experiment. The increase of 2.88 cm in SVL, in this experiment, however, showed to be lower than 3.8 cm observed by Santos et al., 1993, when the animals were fed with fish and kept at 32°C water temperature. The animals presented the greatest head length (4.13 cm) and greater head width (1.97 cm) at 31.8°C (Figures 8 and 11). The best performance in snout length (1.70 cm) and snout width (1.48 cm) was observed at 31.3°C (Figures 6 and 7). The values found for head length are similar to those found by Santos et al., 1993, who observed heads with 4.1 to 4.4 cm length at 32°C temperature. As for head development, particularly regarding snout, it is a species-specific feature (Deeming and Ferguson, 1990) but differences from animal to animal may not be excluded, in the same nest, because different sizes of heads are features normally attributed to sexual dimorphism (Verdade et al., 1997). The longest distance between members (6.54 cm) was observed at 31.8°C, which is similar to the best SVL growth temperature at 32°C (Pinheiro et al., 1992) and the best total length temperature also 32°C (Vianna et al., 1995). The development of the eyes only influences the size of the embryos after morphological stage 24, which corresponds to the period of 45 to 50 days of life (Ferguson, 1987; Webb and Manolis, 1987; Deeming and Ferguson,

1990). Alligator eggs were incubated at 29°C, 31°C, 32°C and 33°C, but none of these temperatures affected the size of the eyes (Alsteadt and Lang, 1995), which is discordant from the optimal temperature, 31.6°C, found in this experiment and close to 32°C found by Pinheiro et al., 1992, for the total growth. According to Verdade, 1987, eye length may be related to sexual dimorphism features and, as in the present experiment only one nest was used as a sample, there may have been a predominance of one sex. The parameters of eye length and circumference of belly, however, have not been registered yet in literature for this species. The option to measure the circumference of belly was made because of the possibility to industrialize the animals hide from the trunk region for whose development the best temperature found was 30.6°C. This temperature is different from the values found by Pinheiro et al., 1992 and by Vianna et al., 1995, for other species. The significant quadratic effect (0.87) of the circumference of belly and weight (Table 3), at 31.2°C, close to that observed for circumference of belly (30.6 °C), may also serve as an indicative for weight gain. Although an optimal temperature for the best development of each parameter was found, it may be noticed that in comparison to the data observed in literature the growth values found here are lower. The differences between growth rates may be attributed to stocking density, nutritional variation and sexual differences. The stocking density used in this experiment for 30°C, 32°C and 34°C was approximately the double of that usually recommended (1 animal per 9 to 12 cm^2). Only one type of diet was used with the addition of mineral salt. It is highly probable that the mineral salt dose used was not sufficient to meet the total requirement of the animals. Different authors have also suggested that a diet variation may induce a higher growth rate in the animal. No parasitological analysis was made, therefore, health problems of the animals may not be discarded, though no occurrence of problematic animals was observed during the experiment, except for the death of one animal in the last week of the experiment, which was lean and refused to eat.

Initial artificial incubation temperature changes from 30 to 32 and 34°C, after the 18th day for *Caiman crocodilus yacare* resulted in a higher hatchability rate that at 28°C. The best post-

hatching growth rate was obtained at 31°C and 32°C temperatures.

RESUMO

Trinta e dois ovos de *Caiman crocodilus yacare* foram incubados, inicialmente, à temperatura de 30°C. Durante a incubação foram retirados seis ovos para se conhecer o estágio embriológico e peso, além de breve descrição dos embriões. Após 20 dias de incubação a 30°C, os ovos foram assim distribuídos: sete em 34°C, sete em 32°C, seis em 30°C e seis em 28°C. Em 32 e 34°C obteve-se 100% de eclodibilidade, em 30°C - 83,3%, e a 28 °C - 66,6 %. Após a eclosão os animais permaneceram durante 120 dias na mesma temperatura de incubação final. Foi realizado controle de peso (g) e análise morfométrica (cm). O delineamento estatístico foi inteiramente casualizado, e os dados foram analisados por regressão, que mostraram um comportamento quadrático (p<0,05). Constatou-se que o peso, comprimento total, comprimento da cauda, comprimento focinho-cloaca, comprimento da cabeça, comprimento do olho, comprimento do focinho, largura do focinho, largura da cabeça, largura das narinas, distância entre membros tiveram maior desenvolvimento na faixa de 31,2 a 31,8°C e, a circunferência da barriga a 30,6°C. A redução de 30 para 28°C, na incubação artificial, desencadeou eclosão de animais prematuros. Portanto, a redução da temperatura de 30 para 28°C, durante a incubação artificial não é recomendada, e maior crescimento pode ser obtido entre 31 a 32°C.

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