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Effects of depth in semi-controlled artificial incubation on egg hatching success of *Crocodylus acutus* (Cuvier, 1807) and hatchlings biometry

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Semi-controlled artificial incubation methods of crocodile eggs use low technology devices where one or more parameters cannot be controlled, but with the advantage that they are less expensive and logistically more feasible. This study evaluated the effect of different artificial incubation depths on the hatching success of eggs of *Crocodylus acutus*, under semi-controlled conditions, and analyzed biometric data of the hatchlings. The crocodile nests were collected from sandbanks of the two rivers (Río Negro and Río Santa Rosa), both located at Machiques de Perijá, Zulia state, Venezuela. The results showed a significant and positive effect on hatching success when the eggs were buried at 2 cm depth, compared to those at 10 cm and 20 cm. The hatching success at 2 cm depth was 82.43%. Also, we found that the size and weight of the hatchlings, as well as the relationships between these biometric variables, depend on the place of origin of the nests. The highest hatching percentage obtained at 2 cm was probably due to the effect of optimal incubation temperatures at this depth. The isolation and controlled heating system of the incubation room would prevent extreme fluctuations in temperature, favoring greater hatching at 2 cm depth. Differences in the biometric aspects of the hatchlings could be associated with differences in the size and the physiological status of the females from both sites.

Key words: crocodile eggs; incubation room; Machiques de Perijá; nests; Venezuela.

The American crocodile or Caimán de la Costa, *Crocodylus acutus* (Cuvier, 1807), has a wide distribution in the Neotropics, both on the Atlantic coast from South Florida in the United States, through Mexico, Central America, some Caribbean islands and northern South America (Colombia and Venezuela); as in the Pacific coast from Mexico to Ecuador and Peru (Thorbjarnarson, 1989; Ernst *et al.*, 1999). At present *C. acutus* is included in Appendix I of the Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES, 2021) and is classified as a vulnerable species on the Red List of the International Union for the Conservation of Nature (IUCN) (Ponce-Campos *et al.*, 2012). In Venezuela, it is considered an endangered species by decree 1496 (República de Venezuela, 1996) and en-

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dangered according to the Red Book of Venezuelan Fauna (SEIJAS *et al*., 2015).

The rescue and artificial incubation of crocodile eggs, for subsequent release of juveniles in their habitat, is one of the most effective actions for the conservation of these species (HUCHZERMEYER, 2003; BARROS *et al*., 2010; ESPINOSA-BLANCO *et al*., 2013). The purpose of these practices is to increase hatching success under controlled conditions with respect to natural conditions (HERNÁNDEZ-HURTADO *et al*., 2013), where nests are frequently threatened by natural events (e. g., floods, desiccation, extreme temperature variations), natural predators and looting of nests by rural inhabitants for the purpose of subsistence or to incorporate this item into the illegal traffic of wildlife (OGDEN, 1978; MAZZOTTI, 1989; BARROS *et al*., 2010).

The techniques of artificial incubation of crocodile eggs can be divided into those whose conditions are controlled, mainly in terms of substrate, temperature and humidity, and the semi-controlled, rustic, simple or homemade. The latter use methods with low technology where one or more parameters cannot be controlled, but with the advantage that they are less expensive and logistically more feasible (BARROS *et al*., 2010; ESPINOSA-BLANCO *et al*., 2013). This last type of artificial incubation has been used in several studies with the main purpose of determining hatching success (SEIJAS & GONZALEZ, 1994; BARROS *et al*., 2010; MANDUJANO, 2012; ESPINOSA-BLANCO *et al*., 2013; HERNÁNDEZ-HURTADO *et al*., 2013).

Under artificial incubation conditions (controlled or semi-controlled), hatching success will depend on intrinsic and extrinsic factors of the nest, the former being those corresponding to fertility, which in turn depends on the sexual maturity and health status of the reproducers; while the latter are related to handling during harvesting and transport, and the incubation process itself, where temperature and humidity are the key factors in this process (HUCHZERMEYER, 2003; PÉREZ & ESCOBEDO-GALVÁN, 2005; MANDUJANO, 2012).

In different studies both in natural environments and in artificial incubation of *C. acutus* and other species of crocodilians, the effect of temperature on embryo survival and hatching success has been highlighted (WEBB *et al*., 1983; LÓPEZ-LUNA *et al*., 2015; BARRAGÁN *et al*., 2021). Under natural conditions it has been found that the variation in the temperature of crocodile nests is influenced, among other factors, by the depth at which the females lay their eggs (HUTTON, 1987); however, little is known about the effect of depth under experimental or artificial incubation conditions. Therefore, the objective of this study was to evaluate the effect of different artificial incubation depths on the hatching success of eggs of *C. acutus*, under semi-controlled conditions. It was based on the hypothesis that depth affects hatching success under conditions when it is not possible to control variables such as temperature and humidity. Additionally, biometric data of neonates are reported and analyzed.

MATERIALS AND METHODS

Study area

The nests of *C. acutus* were collected from the sand banks of the rivers Santa Rosa and Río Negro, both of which are tributaries of the Santa Ana river, located in the Machiques de Perijá municipality, Zulia state, Venezuela (Fig. 1). Its riparian forests are severely intervened by agricultural activity, leaving some wooded patches on their margins. In this area there are also a series of rural, peasant and indigenous settlements (mainly of the Barí and Wayúu ethnic groups). The Río Santa Rosa presents a great sinuosity, favoring the formation of beaches in most of its extension, especially in times of drought. The Río Negro is equally sinuous but a bit narrower in its cross section and it is encased, so that most of its banks are gorges and ravines usually covered with secondary vegetation of both grasslands and thorny shrubs. The climate of the area corresponds to a sub-humid tropical forest with an average annual rainfall of 1544 mm and an average temperature of 27.8 ºC. It is characterized by a dry period from December to March. The rainy period begins in April and ends in November, reaching maximum rainfall in the month of October (MARNR, 1997). In this area, the presence of *C. acutus* and its nests on the banks of both rivers has been previously reported (BARROS *et al.*, 2010; VALERIS & BARROS, 2011).

Egg collection

The search and collection of the eggs of *C. acutus* was carried out between January and March 2008, with the help of local indigenous people, specifically from the Senkay community (Barí), who have notable experience in tracking and locating the nests. The eggs were collected during the early morning to avoid direct exposure to solar radiation. For the transfer of the eggs, a plastic container (paint kegs) was prepared with a first layer of wet sand (taken from the nest) in the bottom. The same sand was laid between each layer of eggs, and between the eggs of each layer, to protect them and prevent their movement during transportation (PÉREZ, 2001; JIMÉNEZ-ORAÁ *et al.*, 2007; JOANEN & MCNEASE, 2009). Two nests located on the bank of the Río Santa Rosa were left in the same place as a natural control.

Semi-controlled artificial incubation

The room where the semi-controlled artificial incubation was carried out was established a few kilometers from the collection sites. This enclosure is 5 m^2, whose walls are made of cement blocks and friezed inside and out, with a terracotta floor and basic electrical supply. Three incandescent lamps were placed, one of 150 and two of 220 watts to try to maintain the temperature in the appropriate incubation interval (29-33 ºC). To verify the adequate temperature fluctuation, it was measured once a week during the experiment on different hours of the day (e.g., 3 am, 9 am, 2 pm and 8 pm); using a thermometer inside the incubators. During the day (between 12 and 3 pm), two lamps were turned off so that the temperature did not rise above 33 ºC.

The eggs were placed in the incubators the same day they were collected. The incubators consisted of polystyrene boxes with the following measurements of length: 55 cm, width: 32 cm and height: 28 cm, filled with the same sand from the nest to carry out the incubation. The test at different depths of artificial incubation (DAI) consisted of placing the eggs within

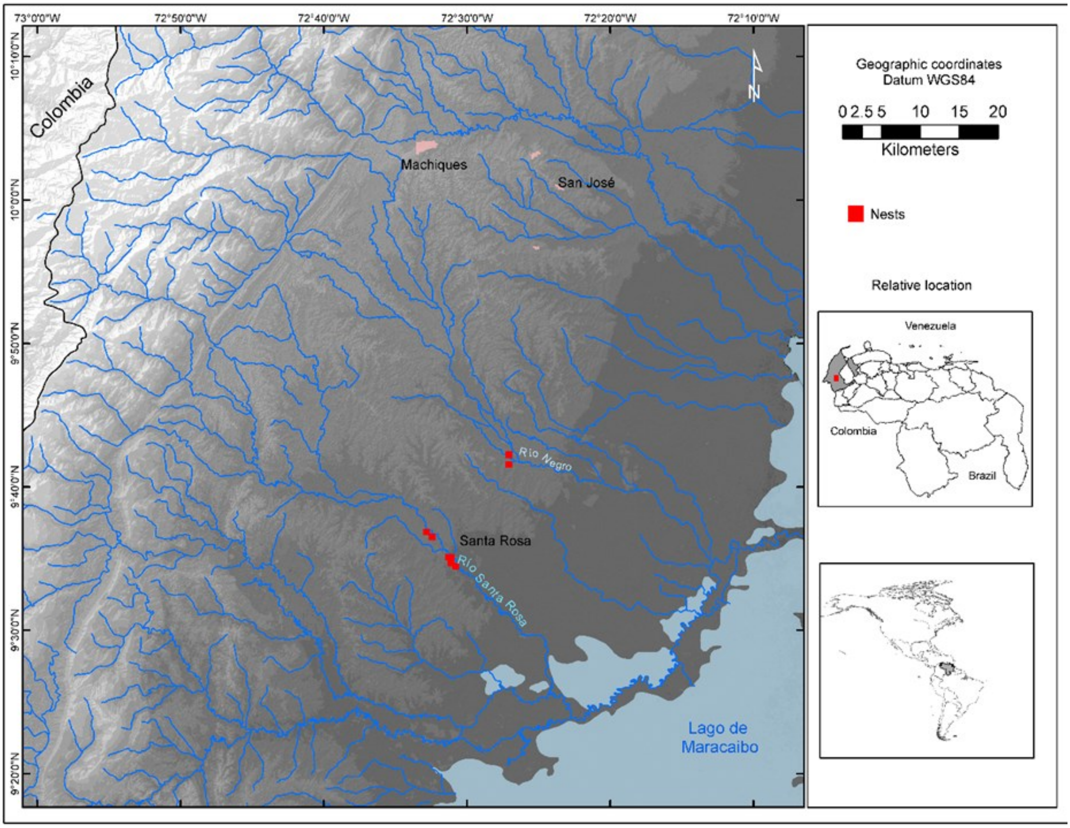

Figure 1: Relative geographical situation of Venezuela and study area. The red squares indicate the location of each nest in both rivers (Río Santa Rosa and Río Negro).

the sand in the incubators at 2, 10 and 20 cm from the surface (Fig. 2). The humidity of the boxes was maintained by adding water at least once a week when the sand in the incubators was visually dry, using a sprayer for this. Since it was not possible to control the internal temperature of the incubators, this artificial incubation method was considered as semi-controlled. Some clutches were divided in half to ensure at least three replicates at each depth.

Hatching success and hatchlings biometry

A total of eight nests of *C. acutus* were collected, six were found in the Río Santa Rosa and two in Río Negro. Of these clutches, 227 eggs were placed in 12 incubators corresponding to the different levels of DAI (Table 1). Since all the eggs were subjected to artificial incubation, so viability was not considered. Hatching success was determined in percentage terms for each level of artificial incubation treatments, according to the following formula:

$$Hatching\ succes\ (\%) = \frac{Number\ of\ hatched\ eggs}{Number\ of\ total\ eggs} \times 100$$

After hatching, biometric measurements of the neonates were taken. The total length (TL in cm) and standard length (SL in cm) were measured with a digital

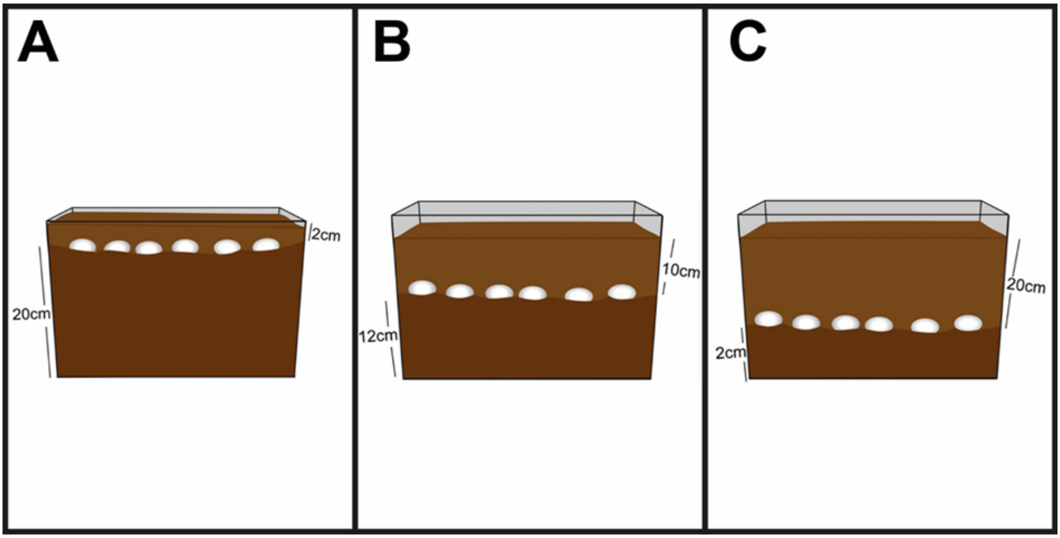

Figure 2: Design of the semi-controlled artificial incubation test at different depths.

caliper (± 0.01 mm), and the weight (W in g) using a balance (± 0.01 mm).

Statistical analysis

Hatching success data were analyzed by logistic regression, appropriate analysis for proportions data (CRAWLEY, 2013), to check if there were differences between DAI and between egg collection sites (Sites). In addition, the potential effect of clutch size (number of eggs) on hatching success was included in the models. In this analysis, the natural control level (natural nests) for DAI model was excluded, since all its values were zero, so the odds ratio values of the experimental levels would be inflated. It is important to note that DAI factors and sites could not be included in the same model (interaction or main effects), since the number of clutches was so low in the Río Negro site (see Table 1), that it could not be included in each DAI level; in such way each factor was analyzed separately.

Model comparison was performed using the analysis of deviance table with the sequential addition of the predictor variables and their interactions. Differences between models were tested with the likelihood ratio test, this approximately follows a chi-square distribution (MCCULLAGH & NELDER, 1989). Likewise, the corrected Akaike Information Criterion (AICc) for small sample size was used, with lower AIC values indicating a better model to fit the data.

To assess whether the biometric aspects TL, SL and W of the neonates of *C. acutus* differ between the two sites of origin of the eggs (Río Negro and Río Santa Rosa), the assumptions of normality were previously checked with the Lilliefors test (Kolmogorov-Smirnov) and homoscedasticity using the Levene test, and a *t*-test based on Monte Carlo permutations was used (TIBSHIRANI & EFRON, 1993). This analysis was complemented with a measure of effect size (Hedges's *g*) and its confi-

Table 1: Clutch size, depths of artificial incubation (DAI), and egg collection sites (Sites) for each experimental incubator.

Incub.	Nest ID*	Sites	DAI	Clutch size
1	SR-1A	SR	2 cm	21
2	SR-1B	SR	2 cm	21
3	SR-2A	SR	2 cm	20
4	RN-1A	RN	10 cm	15
5	RN-1B	RN	10 cm	15
6	SR-2B	SR	10 cm	16
7	SR-3A	SR	20 cm	17
8	RN-2	RN	20 cm	16
9	SR-3B	SR	20 cm	17
10	SR-4	SR	20 cm	18
11	SR-5	SR	20 cm	25
12	SR-6	SR	20 cm	26

*A and B correspond to half of clutches. SR: Río Santa Rosa, RN: Río Negro.

dence intervals, in order to quantify the size of the difference and to have an approximation to the biological importance of the differences (NAKAGAWA & CUTHILL, 2007). Additionally, the relationship between the biometric variables was evaluated using a Spearman correlation. All analyzes and graphs were performed in R (R CORE TEAM, 2021).

RESULTS

Hatching success

The incubation interval was from 93 to 98 days with the highest number of hatchings (80%), however hatching was maintained until day 103, when the last hatchlings were born. The nests left as natural control were totally lost, one of them was completely looted by inhabitants of the area and the other was lost due to flooding due to an increase in the level of the Río Santa Rosa towards the end of April 2008. Total hatching success was 74.89%, with an average of 74.01 ± 24.89%. The highest hatching percentage was reached at the 2 cm DAI level with an average of 90.32 ± 4.77 (85.71 - 95.24), followed by the 10 cm level (76.11 ± 10.05; 66.67 - 86.67), and the lowest percentage was obtained at 20 cm depth, where, in addition, a wide variation was observed (64.81 ± 32.40; 11.76 - 94.44) (Fig. 3).

The logistic regression models for the prediction of hatching success (hatching proportion) with the predictor variables DAI and Site only, were selected as the best models according to the AICc values. The inclusion of the clutch size variable (as main and interaction effects) did not significantly reduce the residual deviance (Table 2). Significant and positive effect of the 2 cm level ($P < 0.05$) was found on the hatching success, differing from the 10 cm and 20 cm levels, which did not differ between them ($P > 0.05$). The odds ratio value and its probability percentage for 2 cm depth, indicates that hatching success is 4.73 (82.53 %) times more likely compared to the other levels of DAI (Table 3, model DAI). The comparison between sites did not show a significant effect on the hatching success ($P > 0.05$; Table 3, model Sites).

Hatchlings biometry

Crocodylus acutus neonates from the Río Santa Rosa (SR) yielded the largest sizes (TL and SL) and total weight (W) compared to the neonates from Río Negro (RN). The latter presented a higher coefficient of variation (CV) in all the measures

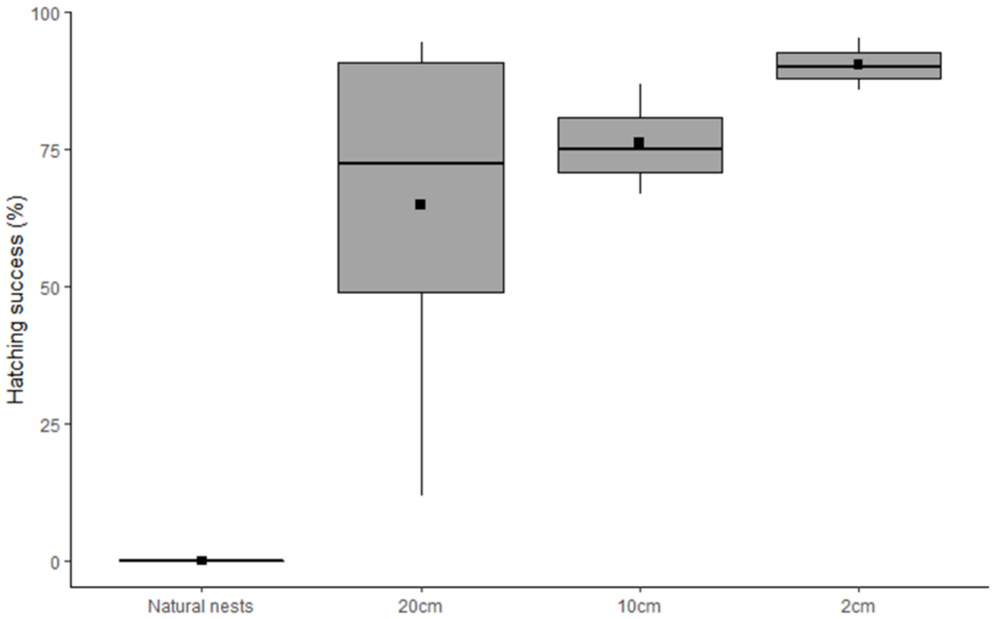

Figure 3: Hatching success of *Crocodylus acutus* clutches in natural environment and artificial incubation at different depths. The black squares inside the boxes indicate the mean.

Table 2: Analysis of deviance (likelihood ratio test) and corrected Akaike information criterion (AICc) for logistic regression models of proportions of hatching success (HS) of *C. acutus*. DAI: depths of artificial incubation, CS: clutch size, df: degrees of freedom, DR: deviance reduction, Pr: probability of a $\chi 2$ value exceeding the deviance reduction.

Predictors	Model	Residual deviance	df	DR	Pr > $\chi 2$	AICc
DAI	1: HS ~ DAI	47.137				**88.464**
CS	2: HS ~ DAI + CS	44.175	1	2.962	0.085	90.217
	3: HS ~ DAI*CS	44.118	2	0.057	0.972	105.246
Sites	4: HS ~ Sites	60.961				**98.622**
CS	5: HS ~ Sites + CS	57.435	1	3.526	0.060	98.762
	6: HS ~ Sites*CS	57.368	1	0.067	0.796	103.410

evaluated; likewise, the variable with the greatest dispersion regardless of the site was W (Table 4).

The relationship, distribution and correlation values between each pair of biometric variables for each site are shown in Fig. 4. In general, it is observed that these bivariate relationships are not linear. The shape of the distribution shows a strong separation between sites for TL and W, as well as asymmetric and bimodal distributions for each site (with the exception of

TL in RN and W in SR). Regardless of the site, Spearman correlation values between the three variables were significant. The correlations between TL-W and SL-W were very low and not significant (rho = 0.094 and 0.078 respectively; P > 0.05) for the neonates from RN. The highest correlation values were obtained for the relationship between TL and SL in RN (rho = 0.83; P < 0.001), and in SR for the relationship between TL and W (rho = 0.60; P < 0.001).

All biometric variables showed significant differences between the sites (P < 0.001). These differences showed high effect sizes (Hedges's g> 1). Variable W obtained the greatest effect of the difference between sites, followed by variable TL, which indicates that these two variables are more sensitive to the differences between sites (Table 5).

DISCUSSION

The results of this study suggest that the hatching success of *C. acutus* eggs is influenced by the depth at which the eggs are placed under semi-controlled conditions of artificial incubation. However, more studies are needed where the temperature and the type of substrate used in the incubators (among other variables), can be controlled. In addition, it is also shown that the size and weight of the newborns of this species, as well as the relationships between these biometric variables depend on the place of origin of the nests.

The number of nests found during the search and collection of eggs was low and may be related to the low population density recorded in these locations. VALERIS & BARROS (2011) reported a total of six nests in 77 beaches considered suitable for nesting, where the population density of individuals was 0.21 ind/km^2.

The artificial incubation time recorded in this work is higher than that indicated for other localities, in which the incubation period lasts between 70 and 90 days (THORBJARNARSON, 1988; 1989; CASAS-ANDREU, 2003; HERNÁNDEZ–HURTADO *et al*., 2006; GONZÁLEZ-DESALES *et al*., 2016; GÓMEZ -GONZÁLEZ *et al*., 2017; BARRAGÁN *et al*., 2021). Although it was not possible to keep a record of the internal temperature of the

Table 3: Logistic regression models for depths of artificial incubation (DAI) and collection sites (Sites) on hatching success (proportion) in clutches of *C. acutus*. The reference levels are 20 cm for DAI and RN for Sites.

Model	Predictors	Odds ratios	CI	Odds ratios (%)	*P*
DAI	(Intercept)	1.97	1.36 – 2.92	66.39	**<0.001**
	Treatment [10 cm]	1.61	0.76 – 3.63	61.70	0.229
	Treatment [2 cm]	4.73	2.00 – 13.09	82.53	**0.001**
Sites	(Intercept)	3.18	1.67 – 6.57	0.76	**0.001**
	Site [SR]	0.92	0.42 – 1.92	0.48	0.834

Table 4: Descriptive statistics of the biometric variables of *C. acutus* neonates, according to their place of origin.

Sites	Variable	mean	sd	CV	range
Río Negro (n= 39)	TL (cm)	25.19	0.99	3.91	4.1 (22.5-26.6)
	SL (cm)	12.91	0.64	4.99	2.6 (11-13.6)
	W (g)	53.26	6.94	13.04	31.7 (38.2-69.9)
Santa Rosa (n= 98)	TL (cm)	26.86	0.82	3.05	4.7 (23.5-28.2)
	SL (cm)	13.66	0.57	4.17	5 (10.5-15.5)
	W (g)	67.99	7.31	10.75	33 (50-83)

incubators, this result could be due to low temperatures in the incubation room during the experiment, leading to a longer hatching time; reciprocally, it has been mentioned that a shorter incubation time (<80 days) is evidence of high temperatures and low humidity in nesting habitats (Casas-Andreu, 2003; Gómez-González *et al*., 2017).

The total and average hatching success of *C. acutus* nests obtained by artificial incubation in this work is within the interval reported in studies carried out in natural environments. Our results are similar to the values reported in Quintana Roo, Jalisco and Oaxaca in Mexico, where the hatching percentages were 72.3, 74.1 and 75.1 respectively (Casas-Andreu, 2003; Charruau *et al*., 2010; Cedillo-Leal *et al*., 2013) and higher than the percentages registered in Florida (Ogden, 1978; Mazzotti, 1989) and in the Monte Cabaniguán Fauna Refuge in Cuba (Rodríguez, 2009; Tabet, 2009) in which values lower than 60% were recorded. On the other hand, hatching success averages greater than 80% have been registered in some studies carried out in natural environments (Cupul-Magaña *et al*., 2004; Cupul-Magaña & Aranda-Mena, 2005; Balaguera-Reina *et al*., 2015; Barragán *et al*., 2021); however, due to the fact that the viability of the eggs was not estimated in this work, the hatching success could be underestimated, for example, Casas-Andreu (2003) refers that the viability can vary between 36 and 100%.

The aforementioned reflects the importance of including artificial incubation within crocodile species conservation programs. This makes more sense when considering the total loss of nests left in the middle as a natural control, caused by flooding and poaching of clutches. Flood associated with heavy rains is one of the main factors that cause the loss of nests, since excess humidity directly affects the viability of the eggs, due to the reduction in the oxygen diffusion capacity, causing the death of the embryo (Lutz & Dunbar-Cooper, 1984; Mazzotti *et al*., 1988; Casas-

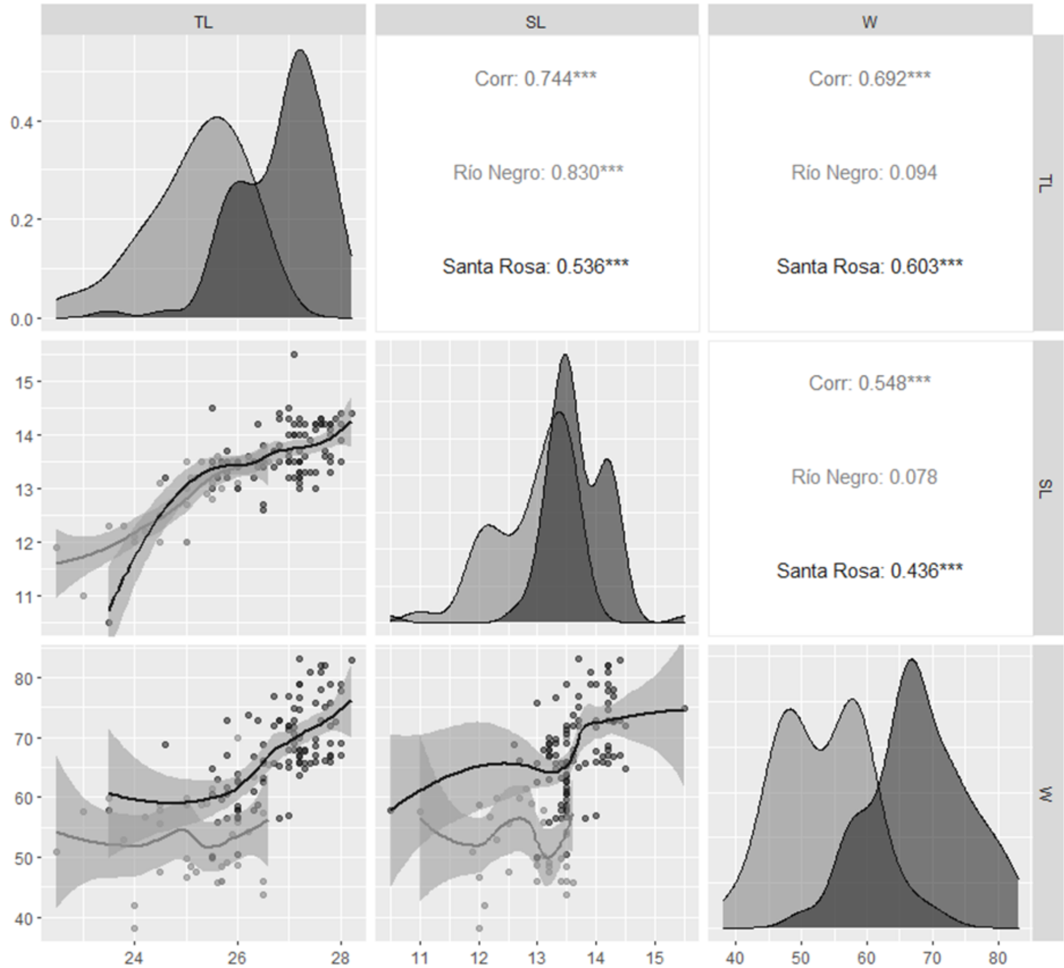

Figure 4: Relationship between the biometric measurements of *Crocodylus acutus* neonates at each collection site. The light gray and dark gray represent the Rio Negro and Rio Santa Rosa respectively. Correlation values were obtained using the Spearman method and the adjustment of the curves using the Loess smoothing function. TL: total length, SL: standard length, W: weight, Corr: Spearman total correlation, *** $P < 0.001$.

ANDREU, 2003; CHARRUAU *et al*., 2010; CEDILLO-LEAL *et al*., 2013). In addition, the looting of nests is one of the main anthropic factors that can have a great impact on the populations of *C. acutus* in the evaluated sites. Traditionally these eggs have been collected for consumption by local aboriginal people and peasants of the area; however, with the more recent emergence of terrestrial communication routes, the illegal trade in eggs was fostered (BARROS *et al*., 2010). GONZÁLEZ-DESALES *et al*. (2016) report a high percentage of looted nests (50%) between May and June 2014 in the La Encrucijada Biosphere Reserve (Chiapas, Mexico), the nests closest to hu-

Table 5: Permutation two sample t-test to evaluate differences in the biometric variables of *C. acutus* neonates, according to their place of origin.

Monte-Carlo permutation (10000) two Sample t-test				Bootstrap effect size (2000 replicates)	
Variable	**t statistic**	**Permutation *P*-value**	**Percentile CI (95%)**	**Hedges's *g***	**Bca CI (95%)**
TL	10.11	< 2.2e-16	1.25 – 2.10	1.90	1.34 – 2.31
SL	6.68	< 2.2e-16	0.50 – 1.00	1.26	0.67 – 1.63
W	10.79	< 2.2e-16	11.09 – 18.36	2.03	1.59 – 2.46

man settlements being the most likely to be looted.

When comparing hatching success with other studies of artificial incubation (controlled or semi-controlled) of *C. acutus* eggs, the total and average values obtained in this work are higher than those found by BARROS *et al*. (2010) who reported a hatching percentage of 65.6% in nests from the same area (Río Santa Rosa), and in a trial of homemade incubators in Chiapas (Mexico) an average hatching of 61.5% was reported (MANDUJANO, 2012). Likewise, these values are within the intervals reported for *C. acutus* in the "Reptilario Cipactli" (Jalisco, Mexico) evaluated during three incubation periods (HERNÁNDEZ-HURTADO *et al*., 2013), and lower than those registered in the La Tuna Carranza Aquaculture Center, located in the department of Tumbes (Peru), whose hatching percentages ranged between 84 and 100% (PÉREZ & ESCOBEDO-GALVÁN, 2005). These differences can be attributed, in addition to the intrinsic variability of the populations and reproducers, to the better or worse conditions in the control of variables during the artificial incubation process, mainly temperature and humidity (SEIJAS & GONZALEZ, 1994; BARROS *et al*., 2010; MANDUJANO, 2012).

The highest hatching percentage obtained at 2 cm DAI is probably due to the effect of optimal incubation temperatures (> 30 ºC) at this depth, which are the closest to those of the nests in their natural conditions (LUTZ & DUNBAR-COOPER, 1984; THORBJARNARSON, 1989; CASAS-ANDREU, 2003). HUTTON (1987), recorded strong variations in temperature (up to 14 ºC) at the depth of shallow eggs (13 and 17 cm) in nests of *C. niloticus*, reaching 35 ºC. However, the isolation and controlled heating system of the incubation room in this study would prevent these extreme fluctuations in temperature, thus favoring greater hatching at this depth. The lower hatching success at 20 cm DAI could be negatively affected by the combination of low temperatures and high humidity. Although the evaluation of the sex ratio in hatchlings is beyond the scope of this work, it could be assumed that this variable was probably also affected by the different depths of artificial incubation, since it is well known that the proportion of sex in crocodiles depends on temperature, both under artificial conditions and in natural environments (WEBB *et al.*, 1983; LANG & ANDREWS, 1994; AGUILAR-MIGUEL *et al.*, 1998; CHARRUAU, 2012; MANDUJANO, 2012). The lack of relationship between clutch size and hatching success is not surprising, as this has been reported for other crocodilian species (e.g., KHOSA *et al.*, 2012; MANDUJANO-CAMACHO *et al.*, 2018).

Regarding the biometric variables of the *C. acutus* neonates, these are within the average values registered in other regions (Florida, Mexico, Colombia, Peru), in which the average TL fluctuated between 24.0 and 28.2 cm, and W between 46.13 and 69.0 (BRANDT *et al.*, 1995; ÁLVAREZ DEL TORO, 2001; OSSA, 2002; CUPUL-MAGAÑA *et al.*, 2004; PÉREZ & ESCOBEDO-GALVÁN, 2005; SIGLER, 2010; GÓMEZ-GONZÁLEZ *et al.*, 2017). The values of standard length (SL) are rarely recorded for *C. acutus* neonates. The average SL values in neonates from the Río Santa Rosa coincide with the data indicated by OSSA (2002), who shows that this value can vary from 13.5 to 14.3 cm, while the Río Negro hatchlings were below this range. The differences in the biometric aspects between both sites could be associated with differences in the size of the females and in the physiological characteristics of the females for both sites (THORBJARNARSON, 1996; PÉREZ & ESCOBEDO, 2005). This is also supported by the absence of correlation between the two measures of length (TL and SL) with W for the neonates from Río Negro, which presented a very low weight and a high coefficient of variation.

This work is pioneering in demonstrating under experimental conditions of artificial incubation, the differential effect of depths on the hatching success of *C. acutus* eggs. The findings could be integrated into semi-controlled artificial incubation practices, which represents a viable and low-cost alternative to conservation programs based on assisted crocodile reproduction.

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REFERENCES

AGUILAR-MIGUEL, X.; HERRERA-MUÑOZ, J.; MERCHANT-LARIOS, H. & CASAS-ANDREU, G. (1998). Efecto de la temperatura de incubación sobre la actividad esteroidogénica en *Crocodylus acutus* y *C. moreletti*. *Revista de la Sociedad Mexicana de Historia Natural* 48: 95–103.

ÁLVAREZ DEL TORO, M. (2001). *Los crocodylia de México*. Instituto Mexicano de Recursos Naturales Renovables A.C., México.

Balaguera-Reina, S.A.; Venegas-Anaya, M.; Sanjur, O.I.; Lessios, H.A. & Densmore, L.D. (2015). Reproductive ecology and hatchling growth rates of the American crocodile (*Crocodylus acutus*) on Coiba Island, Panama. *South American Journal of Herpetology* 10: 10–22.

Barragán, R.; Grajales, J.G. & Martínez, E. (2021). Nest temperature assessment in an American crocodile (*Crocodylus acutus*) population on the central coast of Oaxaca, Mexico. *Journal of Thermal Biology* 99: 103012.

Barros, T.; Jiménez-Oraá, M.; Heredia, H. & Seijas, A. (2010). Artificial incubation of wild-collected eggs of American and Orinoco crocodiles (*Crocodylus acutus* and *C. intermedius*), Guárico and Zulia, Venezuela. *Conservation Evidence* 7: 111–115.

Brandt, L.A.; Mazzotti, F.J.; Wilcox, J.R.; Barker, P.D.; Hasty, G.L. & Wasilewsky, J. (1995). Status of The American Crcodile (*Crocodylus acutus*) at the Power Pant Site in Florida, USA. *Herpetological Natural History* 3: 29–36.

Casas-Andreu, G. (2003). Ecología de la anidación de *Crocodylus acutus* (Reptilia: Crococylidae) en la desembocadura del río Cuitzamala, Jalisco, México. *Acta Zoológica Mexicana (nueva serie)* 89: 111–128.

Cedillo-Leal, C.; García-Grajales, J.; Martínez-González, J.C.; Briones-Encinia, F. & Cienfuegos-Rivas, E. (2013). Aspectos ecológicos de la anidación de *Crocodylus acutus* (Reptilia: Crocodylidae) en dos localidades de la costa de Oaxaca, México. *Acta Zoológica Mexicana (nueva serie)* 29: 164–177.

Charruau, P. (2012). Microclimate of American crocodile nests in Banco Chinchorro biosphere reserve, Mexico: Effect on incubation length, embryos survival and hatchlings sex. *Journal of Thermal Biology* 37: 6–14.

Charruau, P.; Thorbjarnarson, J.B. & Hénaut, Y. (2010). Tropical cyclones and reproductive ecology of *Crocodylus acutus* Cuvier, 1807 (Reptilia: Crocodilia: Crocodylidae) on a Caribbean atoll in Mexico. *Journal of Natural History* 44: 741–761.

CITES (2021). The Checklist of CITES Species Website. Appendices I, II and III. Available at http://checklist.cites.org/. Retrieved on 09/13/2021.

Crawley, M.J. (2013). *The R Book, 2nd Ed*. John Wiley & Sons, Ltd, Chichester, UK.

Cupul-Magaña, F.G. & Aranda-Mena, O.S. (2005). Éxito de eclosión del cocodrilo americano (*Crocodylus acutus*) y la tortuga golfina (*Lepidochelys olivacea*) en Puerto Vallarta, Jalisco, México. *Revista Electrónica de Veterinaria* 6: 1–7.

Cupul-Magaña, F.G.; Niz-Villaseñor, A.; Reyes-Suarez, A. & Rubio-Delgado, A. (2004). Historia natural del cocodrilo americano (*Crocodylus acutus*) en el estero Boca Negra, Jalisco, México: anidación y crecimiento de neonatos. *Ciencia y Mar* 8: 31–42.

Ernst, C.H.; Ross, F.D. & Ross, C.A. (1999). *Crocodylus acutus* (Cuvier) American crocodile. *Catalogue American Amphibians and Reptiles* 700: 1–17.

Espinosa-Blanco, A.S.; Seijas, A.E. & Hernández, O. (2013). Egg collection as a conservation tool of Orinoco Crocodile (*Crocodylus intermedius*) in the Cojedes River System, Venezuela. *Interciencia* 38: 358–363.

Gómez-González, J.J.; Narváez-Barandica, J.C.; Báez, L. & Patiño-Flórez, E. (2017). Ecología de la anidación de *Crocodylus acutus* (Reptilia: Crocodylidae) en Bahía Portete, La Guajira, Colombia. *Revista de Biología Tropical* 65: 211–228.

González-Desales, G.A.; Monroy-Vilchis, O.; Zarco-González, M.M. & Charruau, P. (2016). Nesting ecology of the American crocodile in La Encrucijada Biosphere Reserve, Mexico. *Amphibia-Reptilia* 37: 261–271.

Hernández-Hurtado, P.S.; Vega-Villasante, F. & Hernández-Hurtado, H. (2013). Éxito de eclosión por incubación artificial en nidos de *Crocodylus acutus* (Reptilia: Crocodylia) en cautiverio. *Cuadernos de Herpetología* 27: 71–75.

Hernández-Hurtado, H.; García, R. & Her-

NÁNDEZ-HURTADO, P. (2006). Los cocodrilos de la costa Pacífico occidental (Michoacán, Colima y Jalisco) de México, *In* M.C. JIMÉNEZ -QUIROZ & E. ESPINO-BAR (eds.) *Los recursos pesqueros y acuícolas de Jalisco, Colima y Michoacán*. Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación/ Instituto Nacional de la Pesca/ Centro Regional de Investigaciones Pesqueras–Manzanillo, Colima, México, pp. 375–388.

HUCHZERMEYER, F. (2003). *Crocodiles: biology, husbandry and diseases*. CABI Publishing, Wallingford, UK.

HUTTON, J.M. (1987). Incubation temperatures, sex ratios and sex determination in a population of Nile crocodiles (*Crocodylus niloticus*). *Journal of Zoology* 211: 143–155.

JIMÉNEZ-ORAÁ, M.; SEIJAS, A.E. & HEREDIA, H.J. (2007). Colecta de huevos como estrategia de conservación del caimán del Orinoco (*Crocodylus intermedius*) en el río Manapire, Guárico, Venezuela. *Biollania* 8: 36–42.

JOANEN, T. & MCNEASE, L. (2009). Artificial incubation of Alligator eggs and post hatching culture in controlled environmental chambers. *Proceedings of the annual meeting-World Mariculture Society* 8: 483–490.

KHOSA, P.; IMBAYARWO-CHIKOSI, V.E. & HAMANDISHE, V. (2012). Comparative analysis of hatching rates and clutch sizes of Nile crocodile (*Crocodylus niloticus*) eggs collected on- and off-farm in Zimbabwe. *Tropical Animal Health and Production* 44: 905–909.

LANG, J.W. & ANDREWS, H.V. (1994). Temperature-dependent sex determination in crocodilians. *Journal of Experimental Zoology* 270: 28–44.

LÓPEZ-LUNA, M.A.; HIDALGO-MIHART, M.G.; AGUIRRE-LEÓN, G.; GONZÁLEZ-RAMÓN, M. & RANGEL-MENDOZA, J.A. (2015). Effect of nesting environment on incubation temperature and hatching success of Morelet's crocodile (*Crocodylus moreletii*) in an urban lake of Southeastern Mexico. *Journal of Thermal Biology* 49: 66–73.

LUTZ, P.L. & DUNBAR-COOPER, A. (1984). The Nest Environment of the American Crocodile (*Crocodylus acutus*). *Copeia* 1984: 153–161.

MANDUJANO, H. (2012). Ensayando la incubadora de fabricación casera para cocodrilos (*Crocodylus acutus* y *Crocodylus moreletii*). *Ciencias Agropecuarias y Biociencias* 3: 18–20.

MANDUJANO-CAMACHO, H.; HÉNAUT, Y.; COCROFT, R.B. & VLIET, K.A. (2018). Duración y éxito de eclosión en diferentes tamaños de nidadas de *Crocodylus moreletii* (Crocodylia: Crocodilidae). *Hidrobiológica* 28: 157–162.

MAZZOTTI, F.J. (1989). Factors affecting the nesting success of the American crocodile, *Crocodylus acutus*, in Florida Bay. *Bulletin of Marine Science* 44: 220–228.

MAZZOTTI, F.J.; KUSHLAN, J.A. & DUNBAR-COOPER, A. (1988). Dessication and cryptic nest flooding as probable causes of egg mortality in the American crocodile, *Crocodylus acutus*, in Everglades National Park, Florida. *Florida Scientist* 51: 65–72.

MARNR (Ministerio del Ambiente y de los Recursos Naturales Renovables) (1997). Estación Puente Sobre el Lago. Maracaibo, Venezuela.

MCCULLAGH, P. & NELDER, J.A. (1989). *Generalized linear models, 2nd Ed*. Chapman and Hall, London, UK.

NAKAGAWA, S. & CUTHILL, I.C. (2007). Effect size, confidence interval and statistical significance: A practical guide for biologists. *Biological Reviews* 82: 591–605.

OGDEN, J.C. (1978). Status and Nesting Biology of the American Crocodile, *Crocodylus acutus*, (Reptilia, Crocodilidae) in Florida. *Journal of Herpetology* 12: 183–196.

OSSA, J.L. (2002). *Guía para el manejo y cría del caimán del Magdalena o caimán aguja Crocodylus acutus (Cuvier)*. Convenio Andrés Bello (Serie Ciencia y Tecnología, No 96), Bogotá, Colombia.

PÉREZ, A. (2001). Incubación artificial de huevos de baba (*Caiman crocodilus crocodilus*). *Zootec-*

nia Tropical 19: 151–164.

Pérez, O. & Escobedo-Galván, A.H. (2005). Notas sobre la reproducción en cautiverio de *Crocodylus acutus* (Cuvier, 1807) en el Perú. *Revista peruana de biología* 12: 479–481.

Pérez, O. & Escobedo, A.H. (2005). Observaciones biométricas de *Crocodylus acutus* (Cuvier, 1807) recién nacidos en cautiverio, Tumbes, *Revista peruana de biología* 12: 171–172.

Ponce-Campos P.; Thorbjarnarson J. & Velasco A. (2012). *Crocodylus acutus*. Available at https://www.iucnredlist.org/species/5659/3043244. Retrieved on 09/13/2021.

R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. Available at: http://www.R-project.org/. Retrieved on 07/15/2021.

República de Venezuela. (1996). Decreto 1496 (Lista de Especies en Peligro de Extinción en Venezuela). Gaceta Oficial de la República de Venezuela n° 6.062, Caracas.

Rodríguez, R.A. (2009). *Situación, estrategia de conservación y uso sostenible de Crocodylus acutus en Cuba*. Unpublished doctoral dissertation. Universidad de Alicante, España / Universidad de Pinar del Río, Cuba.

Seijas, A.E. & González, I. (1994). Incubación Artificial de Huevos de Caiman del Orinoco. *Revista Unellez de Ciencia y Tecnología* 12: 36–41.

Seijas, A.E.; Barros, T. & Babarro, R. (2015). Caimán de la Costa, *Crocodylus acutus*. *In* J.P. Rodríguez; A. García-Rawlins & F. Rojas-Suárez (eds.) *Libro Rojo de la Fauna Venezolana*. Provita y Fundación Empresas Polar, Venezuela, pp. 157–158.

Sigler, L. (2010). La historia natural del Cocodrilo Americano *Crocodylus acutus* en el Parque Nacional Cañón del Sumidero, Chiapas, México. *Revista Latinoamericana de Conservación* 1: 73–82.

Tabet, M.A. (2009): *Comportamiento del cocodrilo americano (Crocodylus acutus) en el Refugio de Fauna 'Monte cabaniguán', Cuba*. Unpublished doctoral dissertation. Universidad de Alicante, España / Universidad de Pinar del Río, Cuba.

Thorbjarnarson, J.B. (1988). The status and ecology of the American crocodile in Haiti. *Bulletin of the Florida Museum of Natural History, Biological Sciences* 33: 1–86.

Thorbjarnarson, J.B. (1989). Ecology of the American crocodile, *Crocodylus acutus*. *In* P.M. Hall (eds.) *Crocodiles, their ecology, management and conservation*. IUCN-The World Conservation Union Publications, Gland, Switzerland, pp. 228–258.

Thorbjarnarson, J.B. (1996). Reproductive Characteristics of the Order Crocodylia. *Herpetologica* 52: 8–24.

Tibshirani, R. J., & Efron, B. (1993). An introduction to the bootstrap. *Monographs on statistics and applied probability* 57: 1–436.

Valeris, C. & Barros, T. (2011). *The crocodiles of the Santa Rosa River, Venezuela: Population status, spatial distribution and reproductive aspects*. LAP LAMBERT Academic Publishing, Saarbrücken.

Webb, G.J.W.; Buckworth, R. & Manolis, S.C. (1983). *Crocodylus johnstoni* in the McKinlay River Area N. T, VI.* Nesting Biology. *Wildlife Research* 10: 607–637.