"Estado actual de la población y evaluación de la temperatura en los nidos del cocodrilo americano (*Crocodylus acutus*, reptilia: Crocodylidae) en una laguna de la costa central de Oaxaca, México"

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Introduction

Of the 24 species of crocodiles in the world, the American crocodile (*Crocodylus acutus*) is one of the most widely distributed crocodile species across Latin America. It's present in 18 countries, from the United States to northern South America, including the Caribbean countries. (Thorbjarnarson, 2010). This species is subject to special protection in Mexico under the NOM-059-SEMARNAT-2010 (SEMARNAT, 2010) and considered as vulnerable in the Red List of Threatened Species by the IUCN (Ponce-Campos *et al*., 2012). The extensive commercial over-exploitation that occurred between the 1930s and 1970s was the main reason for the decline in their populations. Currently their main threats are the destruction of their habitat, in addition to the fact that in some areas they continue to be hunted illegally (Thorbjarnarson, 2010). However, being organisms with temperature-dependent sex determination (TSD), current global climate change could modify the temperatures of the nests in such species, and therefore, the traits of their offspring, which would lead to structural and survival problems of the populations (Charruau *et al*., 2017), producing strongly biased sexual proportions and finally leading to the extinction of the species (Girondot *et al*., 2004; Huey and Janzen, 2008; Escobedo-Galván *et al*., 2011).

Methods

With the purpose of knowing the current status of the population of the American crocodile (*Crocodylus acutus*) of the coastal lagoon known as "Palmasola" on the Central Coast of Oaxaca, 10 sampling periods of two days each were carried out from November 2017 to August 2018. Night tours were carried out with the help of a boat propelled with oars, to carry out counts using the night visual detection technique (DVN; Chabreck, 1966), during new moon nights. During the tours, in each sighting of a crocodile the separation from eye to eye of the organism was estimated, also the distance from the eye to the muzzle (whenever possible) to be able to determine the total length (TL). (Messel *et al*., 1981; Thorbjarnarson, 1989), this allowed the research to obtain the population structure by size, grouping the organisms into different classes, (for the purposes of this work, 5 size classes, and a single

class where the organism size could not be determined were considered), additionally, a note was obtained of the habitat where each organism was observed. In each sampling period, the depth of the lagoon was measured and a water sample was taken to see the salinity concentration. Likewise, data on the ecology of the nests were obtained, carrying out an intensive search around the lagoon through walking tours during the nesting season, from February to March 2018, following the methodology described by Cedillo-Leal *et al*. (2013). Some characteristics of the nesting area were taken: with the help of a measuring tape, the distance from the center of the nest to the nearest trees or shrubs (Dn-Ar1 and Dn-Ar2) was taken and the distance from the center of the nest to the body of water (Dn-Ag). The orientation-exposure was determined with the help of a compass and the internal measurements of the nests were taken, dividing the measurement into three parts: 1) the top, measured from the soil surface to the first egg; 2) the incubation chamber, measured from the first egg to the bottom of the nest (Cedillo-Leal *et al*. 2013); and 3) the total depth, measured from the surface to the bottom of the nest. Additionally, canopy coverage was estimated. Incubation temperatures were evaluated by introducing a thermal sensor (HOBO Pendant, model UA-001-08) in the center of the nest's incubation chamber. The thermal sensor was programmed to measure the temperature every 30 min throughout the incubation (embryonic development); the sensor was removed at the end of the same period. Temperatures were analyzed during the thermosensitive period, between 25 and 45 days of incubation, and throughout the embryonic period. The viability of the eggs was determined by observing the opaque band, where they were divided into fertile, infertile and embryonic death. The birth success was obtained.

Results and conclusions

A total of 1864 visual records of crocodiles were obtained, during 40 tours that were carried out in 20 nights of sampling. The estimated monthly population size varied between 15.2 and 84.81 individuals, a significant negative correlation was found ($r = -0.78$ $p = <0.05$), the deeper the lagoon was, the sighting of crocodiles decreased. The lagoon did not show any salinity level (0 ppm). Class II (juveniles) was the most abundant throughout the study (37.79%), followed by class III (subadults, 27.71%), organisms without being able to determine their total length (Just eyes, 15.50%), class I, IV and V (Young, adults and reproducers, 9.68%, 8.72% and 0.58%, respectively). A total of 27 nests were recorded, of which only 22 were sampled and in which thermal sensors were introduced during embryonic development to measure incubation temperatures. 55.55% of the nests were exposed to the north, 22.22% were exposed to the east and south (respectively), except to the west where there was no nest with this orientation. The distance from the nests to the shore of the lagoon had a minimum distance of 3.7 and a maximum of 24.1 meters. The distance to the closest tree or bush 1 (D-ar1) varied between 0.6 and 9.4 m, while the distance to the closest tree or bush 2 (D-ar2) had a variation between 2.2 and 11.9 m. The depth of the incubation chamber and the nest itself fluctuated between 15 and 40 cm, the depth of the top of the nest (from the first egg to the surface) varied between 15 and 45 cm and the total depth of the nest varied between 35 and 70 cm. The upper width of the nest varied between 28 and 42 cm and the

lower width varied between 22 and 35 cm. The tree cover fluctuated between 0 and 95%. A total of 737 eggs were counted, the number of eggs per nest fluctuated between 17 and 46 with an average of 30.7 ± 7.83 eggs. The fertility of the eggs in the nests had a variation between 70.59 and 97.67%, infertility had a variation between 0 and 29.03%; and embryonic death with a variation between 0 and 13.79%. The observed birth rate fluctuated between 28% and 100%. To determine the birth success, the proportion of the offspring that was fully formed at the end of the incubation period was considered, whether they were alive or dead. The temperature records of 10 nests were just considered because they were the only nests that presented embryo survival and the thermal sensors used in these were not affected by predation or any failure thereof, while the rest of the nests were affected by the floods that occurred at the site. The average incubation period lasted 77.6 ± 5.89 days. The temperature of the nests had a wide range of variation during the entire incubation period (28.06 to 38.04 °C). The average temperatures throughout the incubation fluctuated between 30.06 °C and 34.66 °C. Average temperatures during the thermo-sensitive period (25 and 45 days) fluctuated between 29.13 °C and 33.46 °C. Of the 10 nests considered for the temperature analysis, five of them had temperatures (> 34.5 °C) above the temperature range where a high degree of mortality is considered for the embryos, during different stages of the incubation period and one of them had a high birth rate (94.20%), none of the nests presented low temperatures (<28 °C), where it is considered mortal for the embryos. Following the suggestions of Charruau *et al*. (2017), the proportion of sexes in four nests production was 100% or mostly males, while in four nests the production was 50 and 50% for both sexes and in two of them, it favored females in a higher percentage (60 and 80% respectively).

The present work contributes to the knowledge of the current status of the *Crocodylus acutus* population in the "Palmasola" lagoon, and it is considered that the current status is stable and healthy; however, constant monitoring must be carried out in the lagoon and nearby bodies of water, it must be considered in future studies the collection of data that until now in the most recent studies, are not considered important, such as the depth of water bodies, and salinity, this last parameter has proven to be important in the distribution of the species. The present work reflects that the high temperatures caused by solar radiation have an important influence on the nests of the "Palmasola" Lagoon, especially in those nests without tree cover and those farther from the body of water; however, it must be considered and despite not having measured the humidity, the observations and evidence collected indicate that the lack of humidity was the factor that affected the survival and development of the embryo in the nests that did not suffer flooding. Although the temperatures seem to favor the production of males, the high temperatures recorded during the incubation period (> 36 °C), make the possibility of geographic variability in the TRT (Transitional range of temperature) exist and the TP (pivotal temperature) and consequently in the TSP (thermo-sensitive period), between populations of the Pacific coast and those of the Caribbean Sea and the Gulf slope. The high percentage of birth and survival that occurred during the last third of the incubation period casts doubt on the effect that high temperatures have on embryos, this would mean a finding and with more reason one could believe in the existence of geographic variability.