EGG COLLECTION AS A CONSERVATION TOOL OF ORINOCO CROCODILE (*Crocodylus intermedius*) IN THE COJEDES RIVER SYSTEM, VENEZUELA

Ariel S. Espinosa-Blanco, Andrés E. Seijas and Omar Hernández

SUMMARY

Due to the reduced population size and constrained geographical distribution, Crocodylus intermedius *has been considered one of the most threatened crocodiles in the world. Frequency of flooding events as well as human and animal predation reduces the amount of eggs and slows the recovery of its populations. The potential for egg collection along the Cojedes River System (CRS) and their incubation under controlled conditions was evaluated. During the 2009 nesting season 13 nests were found along 20.7km of CRS river bank surveyed. Eight of them were collected for incubation and set in artificial nests away from the river bank; the remaining five were geo-referenced and left in their natural conditions and considered as controls. Hatching success in the five control nests was 36.4% (90 hatchlings were produced), while for those transferred it was 53.5% (197 hatchlings). In addition, 24 additional nests were confirmed in the river section surveyed by the presence of hatchling pods during the hatching season; therefore, at least 37 females nested in the river section surveyed. We estimate conservatively that ~50 nests could be collected every year in the entire CRS, which could guarantee, at least, the production of 1200 hatchlings. The collection of eggs and artificial incubation in locations near the main river is a successful strategy and could be implemented as part of a conservation program for the species, in which different sectors of society (fishermen, workers, and farm owners) should be involved.*

Introduction

The Orinoco crocodile *Crocodylus intermedius* is considered as a critically endangered species (Rodríguez and Rojas Suárez, 2008; Seijas *et al*., 2010a). The over exploitation of its skin for commercial purposes, which began in the 1920s and lasted to the end of 1950s, increase the extinction risk of this crocodile species. Despite the cessation of commercial hunting, the crocodile populations have not recovered; on the contrary, few isolated populations remain, some of which have declined in the last two decades (Seijas *et al*., 2010b, Espinosa-Blanco and Seijas, 2012, Moreno, 2012). In 1984, the Orinoco Crocodile was included in the list of the most endangered animal species by the IUCN-International Union for Conservation of Nature and Natural Resources (Thorbjarnarson and Hernández, 1992).

Although *C. intermedius* is legally protected since the 1970s, and despite the efforts made in Venezuela (declaration of protected areas, population promotion and restoration through liberation of individuals), the wild populations have not achieved the expected recovery (Mendoza and Seijas, 2007). Currently, natural factors such as predation of eggs and hatchlings, and loss of eggs due to unexpected flooding events (Thorbjarnarson, 1993; Jiménez-Oraá *et al*., 2007), along with poaching, accidental death in fishing nets, and habitat loss and degradation, have retarded the recovery of natural populations (Mendoza and Seijas, 2007; Seijas *et al*., 2010a) and have maintained the species at a high risk of extinction.

The collection of eggs from the wild, to be incubated under artificial conditions, has been a common and useful activity implemented for the management of different crocodilian species for commercial and conservation purposes (Blake and Loveridge, 1975; Chabreck, 1978; Joanen and McNease, 1981; Child, 1987; Hines and Abercrombie, 1987; Whitaker, 1987). In Venezuela, there have been efforts to collect eggs of *C. intermedius* for conservation purposes in the Capanaparo River and Hato El Frio, Apure State, and in the Manapire River, Guárico State (Thorbjarnarson and Arteaga, 1995; Jiménez-Oraá *et al*., 2007; Antelo, 2008). Additional works have evaluated the same procedure with two populations of the genus *Crocodylus* of Venezuela, one of them of *C. intermedius* in Guárico state, and the other one of *C. acutus* in Zulia State (Barros *et al*., 2010). All these efforts have produced hatchlings that have been reared in captivity (usually a year or more) and then released into the wild.

In this paper, we evaluate the potential of implementing the strategy of egg collection in the Cojedes River System (CRS), which contains the largest breeding population

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Ariel S. Espinosa-Blanco. Biologist, Universidad Pedagógica y Tecnológica de Colombia. M. Sc. in Wildlife Management, Universidad Nacional de los Llanos "Ezequiel Zamora" (UNELLEZ), Venezuela. Professor, Universidad de Los Andes, Venezuela. Address: Laboratorio de Ecología Animal A, Facultad de Ciencias, ULA. Mérida. 5101, Venezuela. e-mail: arielbiologo@gmail.com

Andrés E. Seijas. Biologist, Universidad Central de Venezuela (UCV). M.Sc. and Ph.D., University of Florida, USA. Professor, UNELLEZ, Venezuela.

Omar Hernández. Biologist, UCV, Venezuela. Director, Fundación para el Desarrollo de las Ciencias Físicas, Matemáticas y Naturales (FUDECI), Venezuela.

COLECTA DE HUEVOS COMO HERRAMIENTA DE CONSERVACIÓN DEL CAIMÁN DEL ORINOCO (*Crocodylus intermedius*) EN EL SISTEMA DEL RÍO COJEDES, VENEZUELA

Ariel S. Espinosa-Blanco, Andrés E. Seijas y Omar Hernández

RESUMEN

Dada su reducida población y su limitada distribución geográfica, Crocodylus intermedius *ha sido considerado como uno de los cocodrilos más amenazados del mundo. Las inundaciones frecuentes y la depredación humana y animal reduce la cantidad de huevos y hace lenta la recuperación de sus poblaciones. Se evaluó el potencial para la colecta de huevos a lo largo del Sistema del Río Cojedes (SRC) y su incubación bajo condiciones controladas. Durante la temporada de desove en el 2009 se hallaron 13 nidos a lo largo de 20.7km de orillas del SRC inspeccionadas. Se colectaron ocho de ellos para su incubación en nidos artificiales apartados de la orilla y los cinco restantes fueron georeferenciados y dejados como controles en condiciones naturales. En los nidos control el éxito de eclosión fue del 36,4% (90 neonatos), mientras que en los transferidos fue de 53,5% (197 neonatos). Además, se confirmó la existencia de otros 24 nidos por la presencia de grupos de neonatos durante la temporada de eclosión, por lo que al menos 37 hembras nidificaron en la sección de río explorada. Estimamos, conservadoramente, que huevos de ~50 nidos podrían ser recolectados cada año en todo en SRC, lo que garantizaría, al menos, la producción de 1200 eclosiones. La colecta de huevos e incubación artificial en sitios cercanos al río es una estrategia exitosa y podría implementarse como parte del programa de conservación para la especie, en el que diferentes sectores de la sociedad (pescadores, trabajadores, hacendados) deberían estar involucrados.*

COLETA DE OVOS COMO FERRAMENTA DE CONSERVAÇÃO DO CROCODILO DO ORINOCO (*Crocodylus intermedius*) NO SISTEMA DO RIO COJEDES, VENEZUELA

Ariel Espinosa-Blanco, Andrés E. Seijas e Omar Hernández

RESUMO

Devido a sua reduzida população e sua limitada distribuição geográfica, o Crocodylus intermedius *tem sido considerado como um dos crocodilos mais ameaçados do mundo. As inundações frequentes e a depredação humana e animal reduz a quantidade de ovos e deixa lenta a recuperação de suas populações. Avaliou-se o potencial para a coleta de ovos ao longo do Sistema do Rio Cojedes (SRC) e sua incubação sob condições controladas. Durante a temporada de desove 2009 foram achados 13 ninhos ao longo de 20.7km de margens do SRC inspecionadas. Foram coletados oito deles para incubação em ninhos artificiais afastados das margens e os cinco restantes foram georreferenciados e deixados como controle em condições naturais. Nos ninhos controle a taxa de eclosão foi de 36,4% (90 neonatos), enquanto que nos transferidos foi de 53,5% (197 neonatos). Além disso, foi confirmada a existência de outros 24 ninhos pela presença de pegadas de neonatos??? durante a temporada de desove, pelo menos 37 fêmeas desovaram no trecho de rio explorado. Estimamos, conservadoramente, que ovos de ~50 ninhos poderiam ser coletados cada ano em todo em SRC, o que garantiria, ao menos, a produção de 1.200 eclosões. A coleta de ovos e incubação artificial em locais próximos ao rio é uma estratégia exitosa e poderia ser impulsada como parte de um programa de conservação para a espécie, em que diferentes setores da sociedade (pescadores, trabalhadores, fazendeiros) deveriam estar envolvidos.*

of the species (Seijas and Chávez, 2002). A methodological approach to collect and incubate under controlled conditions Orinoco Crocodile eggs is also presented, with the purpose of supporting the conservation program of *C. intermedius* in Venezuela.

Materials and Methods

Study area

The study was conducted in two continuous segments of the CRS: Caño de Agua-Confluencia Sarare (CA-CS; 6.5km) and Merecure-Caño Amarillo (M-CAM; 14.2km in the Central Llanos of Venezuela (Figure 1). The waters run through remnants of ancient tropical dry forests that characterized the region in the past. There are two clearly defined seasons in the area: the rainy season extends from May to October and the dry season from December to March.

Nests searching

Nests searching began on February 11, 2009. We looked for nests on sandy beaches on the river banks, following the procedures described by Pooley (1991), which consisted of observing the beaches to detect the characteristic tracks left by nesting females. In these active beaches a fine stick of wood was inserted in the sand. We dug where we noticed that the stick was introduced with relative easiness, as in these places there is a high probability of finding a crocodile nest. The coordinates (GPS, Garmin Etrex) of the sites where each nest was found were recorded.

Artificial incubation

Eggs from eight nests were collected for incubation. Once a nest was located, the eggs were carefully uncovered. Before handling them, a pencil mark was made to indicate their original orientation. Then, keeping that orientation, the eggs were transferred, one by one, to a polystyrene box, following the recommendations of Joanen and McNease (1977) and Hutton and Webb (1992) to avoid brusque turns, bumps and rough handling that could cause death of the embryo.

The box where the eggs were placed had been previously conditioned with a 10cm layer of moist sand taken from the natural nest. The extraction process took place under shadow, to avoid the overheating of the eggs by direct sunlight. Once all the eggs were transferred to the box, they were covered with a layer of wet sand, in order to avoid dehydration while being taken to the location where they were to be incubated.

A remaining group of five nests were left on their original settings to monitor hatching success in natural conditions. In these cases, the surface layer of the nesting material was removed just to confirm the presence of the eggs, but these were not counted or handled, so as to ensure the least possible disruption.

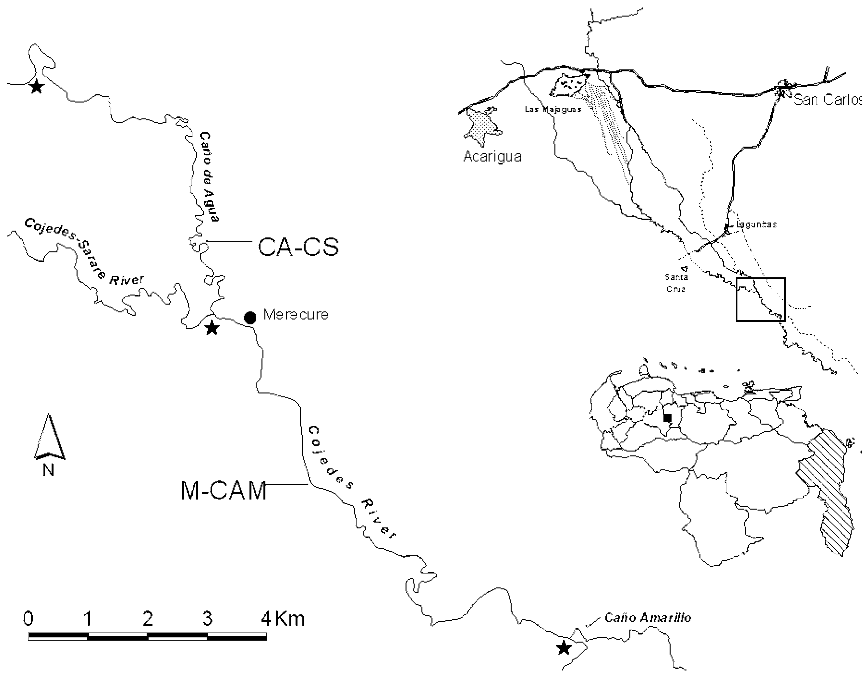

Figure 1. Geographical location and segment studied of the Cojedes River System, Venezuela. Stars indicate endpoints of segments.

The boxes with eggs were transported (first by boat and then by car) to a cattle ranch located 4 to 5km from the nesting site. The insulating boxes were introduced into holes dug in the ground at the distance of 50cm each. These holes were deep enough to contain the box completely, with the upper layer of eggs at a depth of 30cm below ground level, simulating the natural conditions of the nest. The eggs inside the boxes were completely covered with original substrate of the nest (sand). Water was added (if necessary) to increase the humidity of the incubation substrate. The boxes were not filled to the brim, leaving an air chamber when the lid was put in place. Then, the boxes were covered with sand to form a small mound above the ground surface. In some of the boxes, a datalogger (Hobo®, Onset Corporation) was set and programmed to record temperature and relative humidity every 90min. Another datalogger was placed in the shade outside the box, to record the external temperature. The described incubation procedure is simple, inexpensive, and is suitable for effective incubation far away from the collection sites.

Hatching success in natural and artificial nests

On April 30, May 7 and 8, nocturnal spot-light surveys were conducted to locate groups of hatchlings (pods). The Orinoco Crocodile hatchlings remain in compact and well-defined groups during first weeks of life (Seijas and Chávez, 2002). The presence of a pod facilitated the search of the place where the nest was originally located. The number of hatchlings in each pod was used to estimate both the number of nests and hatching success. To estimate the number of nests that hatchlings in a single pod came from, we used the average number of hatchlings per pod (26.0 ±13.9) found by Seijas and Chávez (2002). That is, if a hatchlings group had more than 40 hatchlings (average number plus SD); it was assumed that they came from more than one nest. The average number of eggs in the collected nests was used as reference value to calculate the hatching success. In the artificial nests the hatchlings per nest were counted to estimate hatching success. We compared the hatching success of the artificially incubated nests with the control nests using the Kruskall-Wallis test.

Results

A total of 13 nests of Orinoco crocodile were located during the 2009 nesting season. Eight of them (Nests 1-8; 368 eggs) were transferred, and the remaining five (Nests 9-13) were left in natural conditions as control group. The average of eggs per collected nest was 49.5 ±7.0 (36-59) eggs. Seven of the collected nests were incubated following the procedure described above and the eighth one was placed at 30cm of depth directly at the excavation site (without box) and covered with wet sand, in the most similar conditions to the natural nest.

The hatching success of eggs that were transferred (Table I) was 51.9% (191 hatchlings produced, ranging from 7.5% (Nest 7) to 92.9% (Nest 4). The incubation temperature in artificial nests remained around 30ºC during the first month after collection, then it fluctuated and reached a maximum

TABLE I
NUMBER OF NESTS OF ORINOCO CROCODILE STUDIED IN THE COJEDES RIVER SYSTEM, VENEZUELA

Nest	Number of eggs	Incubated eggs	Hatchlings produced	Hatching success
Artificial incubation				
1	59	57	11	19.3
2	48	46	34	73.9
3	45	45	30	66.7
4	36	28	26	92.9
5	51	51	40	78.4
6	51	48	7	14.6
7	50	40	3	7.5
8	56	53	40	75.5
Totals	396	368	191	51.9
Control nests (natural incubation)				
9	49.5	0	0	0
10	49.5	0	0	0
11	49.5	0	32	64.6
12	49.5	0	23	46.5
13	49.5	0	35	70.7
Totals	248	0	90	36.3

The number of eggs of control nest was not counted. To calculate hatching success, the average number of eggs in collected nests was used.

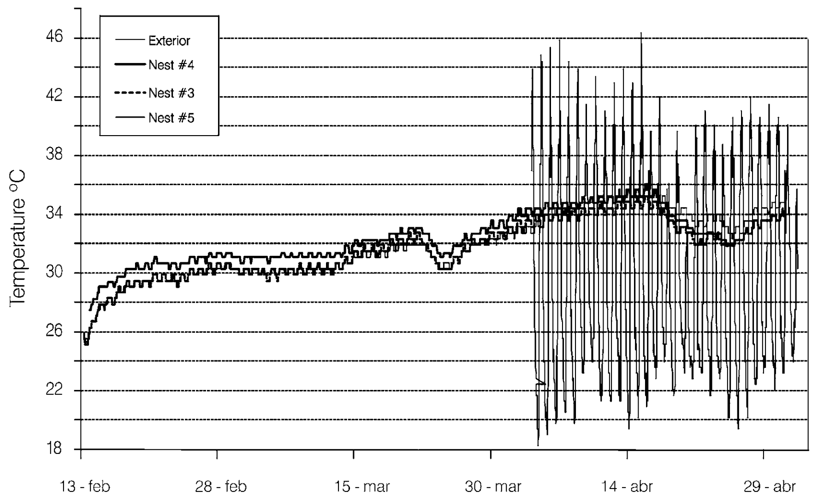

Figure 2. Fluctuation of nest temperature (Nests 3, 4 and 5) and exterior temperature during the last two weeks of incubation is also shown.

temperature between 35 and 36ºC between during April (Figure 2). Daily temperature within the nests fluctuated less than 1ºC, whereas external mean daily temperature was ~25ºC, with minimum and maximum temperatures of 18.2 and 46.4°C, respectively (Figure 2).

Of five nests in natural conditions, 60% (3 nests) produced hatchlings, with an overall hatching success of 36.4% (90 hatchlings produced from 247 estimated eggs). Nest 13 showed the highest hatching successes (70.7%), followed by Nest 11 (64.6%) and Nest 12 (46.5%). We could not determine the causes of the failures (predation or loss due to flooding) in two of the control nests that did not produce hatchlings. The number of offspring produced per nest was higher in those which were transferred (51.9%) than in the ones left as a control in the river bank, but the differences were not statistically significant. (Kruskal-Wallis test; X^2= 0.3; P= 0.4195).

Twenty-two pods were found along the surveyed river section, with a total of 665 hatchlings. The number of hatchlings per pod ranged from 6 to 65 (Figure 3). Groups with more than 40 individuals were assumed to contain hatchlings from two nests. This indicates that at least 27 nests produced hatchlings, with a mean of 24.6 individuals per nest. Taking into account eight nests that were collected, and two nests that did not produce hatchlings, we estimated at least 37 nesting females in the 20.7km of the Cojedes River System.

Discussion

Previous studies have mentioned the advantages of artificial incubation, such as to prevent predation (Joanen, 1969; Pooley, 1973; Chabreck, 1978), create the best conditions for embryonic development (Joanen and McNeese, 1977; Chabreck, 1978) and protect the egg from flooding (Jiménez-Oráa *et al.*, 2007).

Despite the small sample size, the present results showed a higher hatching success in artificially incubated eggs as compared to those in the nests left as control, in the wild. However, they were slightly lower than values reported by Jiménez-Oraá *et al.* (2007), Mercario *et al.* (2008) and Barros *et al.* (2010) in previous studies (61.9, 71.7 and 60.7%, respectively) with *C. intermedius* in Manapire River, Guárico State and *C. acutus* in the Negro, Santa Rosa and Santa Ana rivers, Zulia State. In general, the percentage of hatching success was well below the ideal (90% or more; Joanen and McNease, 1981).

It is possible that some of the embryos in the eggs that were transferred had been already dead at the time of collection, due to flooding of the river bank, caused by the heavy rains that had fallen in the area days before the collection, and by the management of floodgates of the Las Majaguas reservoir, located upstream of the studied sectors (Figure 1; Mendoza and Seijas, 2007). This could explain the relative low hatching success. Another possible reason could be the incubation techniques used, whose use is justified by its low cost and ease of implementation near the collection sites (Barros *et al.*, 2010). But this does not allow a rigorous control of incubation conditions. Temperature, for example, did not remain constant during the incubation period. However, this cannot explain the high mortality in some of the nests. Temperature variation in the three nests monitored by dataloggers was essentially identical and we assume that the other nests experienced similar temperatures. Constant temperature above 34ºC during incubation has been shown to produce a high embryo mortality in *Alligator mississippiensis* (Ferguson and Joanen, 1982) and has been cited as a mortality factor for embryos of other species as well (Hutton and Webb, 1992). Nest 8, which was incubated in the most similar conditions to the natural nest (incubated in a hole dug in the ground), presented the second highest hatching success (75.5%). This procedure could be even cheaper and easier to implement for conservation program in the Cojedes River System. This incubation procedure has been used with *Crocodylus siamensis* in Thailand (Hutton and Webb, 1992), and was also used simultaneously during a study with eggs of the Yellow-headed Sideneck Turtle in the same area (Hernández *et al.*, 2010) with satisfactory results.

Only three of the five control nests produced hatchlings. The factor that probably explains the loss of these two nests was flooding. Navarro-Laurent (2007) reported that flooding explained the failure of seven (53.8%) of the thirteen *C. intermedius* nests known to exist in the CRS section that she surveyed. On the other hand, Seijas and Chávez (2002) and Seijas *et al.* (2010b) suggested that hatching success varies year to year depending on flooding. Hernández *et al.* (2010) observed that, due to flooding, the hatching success of the Yellow-headed Sideneck Turtle was 15.4%, which clearly demonstrates the important role of flooding in the reproductive success of species that inhabit the CRS.

We estimated a total of 37 nesting females during nest-

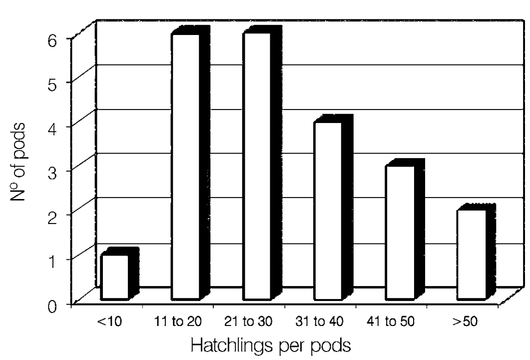

Figure 3. Frequency distribution of hatchlings per pod observed at the beginning of the hatching season. Pods with more than 40 individuals are presumed to contain individuals from more than one nest.

TABLE II
COMPARISON OF CLUTCH SIZE OF *C. intermedius* IN DIFFERENT STUDIES IN THE COJEDES RIVER SYSTEM AND IN OTHER WILD POPULATIONS IN VENEZUELA

Author	Eggs (average)	±SD	Number of nests	Locality
Ayarzagüena (1987)	43.3	9.8	4*	CRS
Thorbjarnarson and Hernández (1993)	38.6	9.9	34	Capanaparo
González-Fernández (1995)	42	---	1	CRS
Jiménez-Oráa *et al.* (2007)	43.9	9.7	22	Manapire
Navarro-Laurent (2007)	41	9.8	13	CRS
Antelo (2008)	41.2	9.6	43	El Frío
Present study	49.5	7.0	8	CRS

* Multiple nests were not taken into account.

ing season in the CRS. However, some nests may have failed completely and some pods may have remained undetected, particularly if they were composed of a few individuals. Nesting also occurs in other river sections, as it was shown in previous studies (Seijas and Chávez, 2002). Therefore, fifty is a conservative estimate of the number of females nesting in the CRS every year.

The average number of eggs per nest (49.5 ±7.0) found in the present study was higher than those of previous studies made in the CRS and other wild Orinoco crocodile populations in Venezuela (Table II). If only clutch sizes reported for the CRS are taken into account, we have an average of 44 eggs per nest. Therefore, the number of eggs which may be collected each year would be ~2200. Even with the relative low hatching success obtained during the study, a figure of around 1200 hatchlings could be produced yearly.

The incubation technique implemented in this study is classified as rustic and simple (Barros *et al*., 2010). In spite of this, it is of great utility in areas of difficult access, where handling and manipulation of nests is carried out near the reproductive habitat of the species. It also minimizes costs, since it is not necessary to build expensive structures**.** The collection of eggs for incubation in artificial nests can increase greatly the reproductive success of the Orinoco crocodile. This paper demonstrates the feasibility of implementing such a strategy in the CRS and we recommend to proceed with this strategy on a larger scale in the near future. The hatchlings produced can be used to reinforce their own population of *C. intermedius* in the CRS, which has been declining in the last 20 years (Seijas *et al*., 2010b; Espinosa-Blanco and Seijas, 2012) as well as other wild populations of the species. Additionally, this strategy would allow the participation of local communities, involving fishermen, workers and farm owners, as the most effective way to ensure success in the Orinoco crocodile conservation.

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