SRAS Final Report

Influence of three factors on the detectability and counting of crocodiles (genus *Crocodylus*) in the Yucatan Peninsula, Mexico

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ABSTRACT

In Mexico, populations of both *Crocodylus moreletii* and *C. acutus* declined sharply between the 1960s and the 1990s. To implement effective and appropriate conservation measures, the status of these species had to be accurately assessed. The most commonly used estimation technique for crocodile counting is Visual Nocturnal Detection (VND). In the 1980s, the first methods of estimating abundance using this technique were simplistic and took no account of variability factors. Models were then developed in the 1990s to take better account of these variability factors, such as the various Recapture-Mark models. From the 2010s, additional statistical models were developed to take into account variability in both ecological processes and detectability and counting of crocodiles. Currently, these models need to be improved in order to provide better estimates of abundance, particularly for detectability and counting variability. The goal of this study is to improve the accuracy of abundance estimates. The factors tested in this study are moon phase luminosity, crocodiles' nocturnal activity patterns and observer experience. The study took place on the Mexican peninsula of Yucatan, in the states of Quintana Roo and Campeche. The crocodiles were counted at night, following the renown counting technique described above. The influence of these factors on the detectability and counting variability was then analyzed using a generalized linear mixed statistical model designed for this study. The results show that the nocturnal activity patterns factor influences the detectability and counting of crocodiles. These results are extremely important for the development of more accurate and precise population estimates for *C. moreletii* and *C. acutus*. Both species face numerous threats, such as anthropogenic activities with the development of Mexico's "Tren Maya" megaproject, the widespread hybridization of the two species within the country, and the isolation of non-hybrid populations. This is particularly the case for *C. moreletii*, for which Mexico represents 88% of its range.

INTRODUCTION

Crocodilians belong to the ancient order of reptiles known as Crocodylia. Species have maintained a similar morphology for around 250 million years and have shown adaptability and resilience in the past, surviving several mass extinctions of biodiversity (Lemos Barão-Nóbrega et al., 2022). There are currently 26 crocodilian species recognized by the Crocodile Specialist Group (Vliet et al., 2024), separated into three distinct families. The Crocodylidae family includes "true" crocodiles (genera *Crocodylus*, *Mecistops*, *Osteolaemus*), the Alligatoridae family includes genera *Alligator*, *Caiman*, *Paleosuchus* and *Melanosuchus*, and the Gavialidae family includes *Gavialis* and *Tomistoma* (Crocodile Specialist Group, 2024). Crocodilians are highly valued by human populations, and they are essential to the ecosystems they inhabit. Culturally, crocodilians have been an integral part of the mythology and beliefs of many civilizations around the world since the dawn of time (Sánchez Herrera et al., 2011). Commercially, crocodilians generate several million dollars a year through tourism, trophy hunting and trade in their meat and skin (Escobedo Galván, 2003). For this reason, overexploitation of crocodilians increased in the 20th century, particularly in Central America (Sánchez Herrera et al., 2011). Ecologically, crocodilian species are considered essential to the proper functioning of the wetland ecosystems they inhabit. They are considered to be keystone species, ecological indicator species and engineering species. This is the case with Morelet's crocodile (*Crocodylus moreletii*) and the American crocodile (*C. acutus*), both of which inhabit the wetland ecosystems of Mexico (Escobedo Galván, 2003; Sánchez Herrera et al., 2011; Somaweera et al., 2019).

In Mexico, between the 1940s and 1990s, Morelet's crocodile and the American crocodile were excessively exploited due to the highly sought-after attributes of their skins. The absence of regulation on their exploitation in the 1940s and 1950s led to over-hunting of wild populations. Although hunting and exploitation were banned in the 1970s, they continued to be more or less tolerated in some localised areas until the 1990s with the development of a vast commercial poaching network across the country. Wild populations of the two species were severely affected by this overexploitation, which led to major demographic imbalances, a drastic decline in their populations and even the extinction of some localised ones (Sanchez Herrera et al., 2011; Platt et al., 2019). Consequently, Morelet's crocodile and the American crocodile were included in Appendix I of CITES in 1975 and classified as Endangered in the IUCN Red List in 1982. As a result, international trade of wildlife has been restricted to exceptional situations such as conservation and research. In 1994, Morelet's crocodile and the American crocodile were classified in the IUCN Red List as Least Concern and Vulnerable, respectively. These classification remains to this day (Sánchez Herrera et al., 2011; Rainwater et al., 2022).

The need to protect crocodilians is recognized as a priority issue by the scientific community, conservation organizations and some governmental institutions, as many threats continue to weigh on these species (Sánchez Herrera et al., 2011; Somaweera

et al., 2019). Morelet's crocodile and the American crocodile are victims of habitat loss due to anthropic activities, contamination of habitats by pollutants and microplastics, illegal hunting, climate change through rising temperatures, and hybridization between species in Mexico (Escobedo Galván, 2003; Sánchez Herrera et al., 2011; Somaweera et al., 2019). The process of hybridization is recognized as a recurrent phenomenon in nature. It is defined as the "reproduction between individuals of different species that can produce individuals reproductively isolated from either of the parental taxa" (Suarez-Atilano et al., 2023). Within the scientific community, biodiversity conservation and hybridization processes are the subject of constant debate, particularly as hybridization is thought to enhance species extinction. However, it has been shown that hybridization can also lead to new species with different and superior adaptations to those of the parent species. In Mexico, hybridization between Morelet's crocodile and the American crocodile was considered restricted to a specific area: the Yucatan Peninsula. Indeed, it is in the three states of the Yucatan Peninsula (Campeche, Quintana Roo and Yucatan) that the two species are mostly found in sympatry. Currently, the majority of Morelet's crocodile populations present in Mexico are hybrids and are therefore not protected by any conservation measures. In addition, populations of the parental species of Morelet's crocodile and the American crocodile in the country are rare and located in highly isolated areas. They also have extremely low genetic diversity, which indicates the potential disappearance of these species, particularly Morelet's crocodile in Mexico (Suarez-Atilano et al., 2023).

As crocodilians are long-lived species, long-term data collection and monitoring are essential to study population trends. However, the physical logistical constraints and human constraints associated with such studies have led to the development of several predictive methods to model population abundance (Joseph et al., 2009). Indirect methods have been developed for cryptic species and/or when direct methods, such as Mark Recapture, are ineffective. For crocodilians, the method described by Chabreck (1966), called Visual Nocturnal Detection, is the most widely used (Escobedo Galván, 2003). The population size is then estimated, without knowing the actual population size, using an equation called the Visible Fraction. This method was developed by Messel (1981) and King (1990) and their work has greatly helped to improve population estimates for crocodilian species. However, like direct methods, these equations are based on assumptions that are unlikely to be satisfied in the field (Balaguera-Reina et al., 2018). Furthermore, these estimation methods are overly simplistic and do not consider variability factors that can influence estimates, such as ecological processes and detection errors (Joseph et al., 2009).

In recent years, many scientists have been trying to develop and improve new models for estimating abundance, in order to take into account all factors involved in the variation of these estimates, particularly those involved in the detection and counting processes. These types of models, known as N-mixture models, enable abundance to be estimated from repeated count surveys at several different sites. They are generally made up of a series of hierarchical generalized linear models and they allow both "true" and "false" variations to be considered and to estimate relative abundance as accurately as possible (Joseph et al., 2009; Lemos Barão-Nóbrega et al., 2022).

The aim of this project is to study the factors of variability involved in the process of detecting and counting crocodile individuals, in order to improve the estimation of their abundance. Factors that appear to have a significant influence on crocodile detection, i.e., moon phase luminosity, nocturnal activity patterns of crocodiles and observer experience, will be studied using statistical N-mixture models especially created for this purpose.

MATERIAL AND METHODS

Study areas – Location

The first study area is in the eastern part of the Yalahau coastal lagoon (21° 26' 17.462" N and 87° 11' 9.063" W; **Figure 1**). Locally known as Yalikín, the study area is a brackish water hydrological network, made up of numerous branches and small channels, covering an area of around 1000 m². The vegetation surrounding the Yalikín hydrological system is mainly red mangrove (*Rhizophora mangle*), between 2 m and 7 m high, and to a lesser extent black mangrove (*Avicennia germinans*) and white mangrove (*Laguncularia racemosa*). Yalikín is fed both by freshwater from underground rivers from the inlands and by tides from the Gulf of Mexico (González-Sánchez et al., 2017).

The second study area is located 20 km from the village of Miguel Colorado (18° 46' 2.204" N and 90° 31' 21.133" W; **Figure 1**). Called Moku by the local communities, this freshwater lake is part of the MCA and has a surface area of 400 hectares and an average depth of 8.12 ± 3.39 m, with a high vegetation cover. The forest diversity is rich, with a majority of América's guayacán (*Guaiacim sanctum*) and *Lonchocarpus yucatanensis* trees, as well as various floral species such as *Bignonia diversifolia*. Unlike the two EMC cenotes, Lake Moku is not open to the public and requires an authorisation from the CEMC to access the site, as such it is slightly affected by anthropogenic disturbance (González-Sánchez et al., 2017).

Data collection - Visual Nocturnal Detection

The Visual Nocturnal Detection (VND) followed the methodology described by Chabreck (1966), used worldwide, and standardized by Sánchez Herrera et al. (2011) in Mexico, Guatemala and Belize. This method detects and counts the presence of crocodiles thanks to the red orange colour reflected by the tape-tum lucidum of their eyes, when illuminated by a bright light. The size of each individual detected is then estimated by the observer from the size of the eyes' reflection or from the size of the head, if visible. The size of the individual corresponds to the Total Length (TL), which is the length from the tip of the snout to the tip of the tail (Sánchez Herrera et al., 2011). The size of crocodiles was classified into the following categories: Yearling (TL <50 cm), Juvenile (TL ≥50 to 100 cm), Subadult (TL ≥100 to 150 cm), Adult (TL ≥ 150 cm) (Padilla et al., 2020). If it was not possible to determine the TL of the individual, it was classified in a category called Eyes Only (EO) (Sánchez Herrera et al.,

2011). In order to keep the assumption that populations were closed, data were collected four times over a two-month period, between February and March 2024 at the Yalikín site and between May and June 2024 at the Moku site. For each site, two surveys were carried out during a full moon, and two during a new moon, in order to take into account the luminosity of the moon as a variable in the probability of detection. Each survey included three successive VNDs, the first of which took place 2 h after sunset, the second 2 h later and the third 2 h later, i.e., 4 h and 6 h after sunset, respectively. These times were chosen to ensure sufficient darkness to detect crocodile eye reflections, and to take into account the nocturnal activity patterns of *C. moreletii* and *C. acutus* as a variable in the probability of detection (Cedeño-Vázquez et al., 2006). The transect chosen for the VNDs were kept constant (**Figure 2**) and were conducted using a 4.3 m long aluminium flat-bottomed boat with a 15 hp motor (and oars when necessary) at a maximum speed of 5 km/h (or 2.5 NM). The crew of four people aboard the boat had each a specific role: two were in charge of conducting the VNDs, one was steering the boat, and the other was taking water temperature and salinity measurements (on the Yalikín site, with a Hanna model HI98319 seawater salinity tester). The team member in charge of the VNDs used a 600-1000 lumen headlamp (Fernández et al., 2015) and has a different level of experience to take the observer's experience into account as a variable in the probability of detection. For each presence detected, the number of individuals observed, their size class, the date, the time, and the GPS coordinates were recorded by the observers.

RESULTS

Abundance – Yalikín

The calculated visible fractions are 0.28 and 0.35 for the Messel and King equations, which means that 28% and 35% of the population were visible at site, respectively. The crocodile population size estimate (N) for the Yalikín hydrological system, based on Messel's visible fraction equation, is 17 individuals with a 95% CI = [2 to 32]. The crocodile population size estimate (N) for the Yalikín hydrological system, based on King's visible fraction equation, is 14 individuals with a 95% CI= [2 to 26].

Abundance – Moku

The calculated visible fractions are 0.72 and 0.64 for the Messel and King equations, which means that 72% and 64% of the population was visible at site, respectively. The crocodile population size estimate (N) of Lake Moku, based on Messel's visible fraction equation, is 47 individuals with a 95% CI = [36 to 58]. The crocodile population size estimate (N) of Lake Moku, based on King's visible fraction equation, is 53 individuals with a 95% CI = [40 to 65].

Abundance – Modelling

The selected GLMM model used throughout the study is as follows:

Encounter Rate ~ fix effects (Moon Phase + Hour of survey + Experience of observer), Random effect (Locality), Distribution family "Gamma" with link function "logarithm". It is written as followed in the R studio software: glmer(ER ~ Moon + Hour + Experience + (1|Locality), family = Gamma(link = "log")).

Two non-parametric bootstrap tests with 1,000 repetitions were performed on this model to determine the significance of the explanatory variables. The bootstrap test using the *pbnm()* function was found to be non-significant. However, the bootstrap test using the *PBmodcomp()* function was significant for one explanatory variable, which is the VND time (T (difference between the means ratio)= 7.505; P= 0.028). The p-values for each explanatory variable, sorted according to the type of bootstrap test, are presented in **Table 1**.

This explanatory variable comprises three different levels: 2 h after sunset, 4 h after sunset and 6 h after sunset. These three different levels were compared using a post-hoc test, using the *emmeans()* function. The test was found to be significant between 2 h after sunset and 6h after sunset (Z (Signal to noise ratio) = 2.827; P= 0.013) and can be seen in **Figure 3**. Observations made 2 h after sunset were greater than observations made 6 h after sunset, with a mean ER of 7.67 ± 6.86 (95% CI= [1.33; 44.3]) and 4.18 ± 3.73 (95% CI= [0.73; 24.0]), respectively. The graphical comparison of the three levels of the species' activity pattern can be seen in **Figure 4**.

The explanatory variable of the activity pattern of the species, and more particularly the observations made 2 hours after sunset, is significant. It was therefore decided to use the selected model on the data collected in previous years to assess the potential differences in ER values resulting from not taking this explanatory variable into account. This data collection, shared by the Mexican National Commission for Knowledge and Use of Biodiversity (CONABIO), includes ER values dating from 2012 to 2023. The Wilcoxon signed-rank test performed showed a significant difference between the ER values predicted by the model selected and the ER values calculated with the data collected in previous years (W (smaller of the rank totals) = 92, P= 0.009). The comparison between the ER values estimated from the selected model and the ER values calculated from the data collected in previous year can be seen in **Figure 5**.

CONCLUSIONS

In conclusion, this project aimed to improve the estimation of crocodile abundance by studying the factors of variability involved in the process of detecting and counting crocodile individuals. It was found in this study that the time of the VND, i.e., the crocodiles' nocturnal activity pattern, has a significant influence on the detection and counting of crocodiles ("false" variation).

It would also be extremely important to carry out genetic analyses of the population in Lake Moku and the Calakmul Biosphere Reserve. Indeed, the confirmation of a parental lineage of *C. moreletii* in this region could help the Mexican Government to make conservation decisions in its favour of this isolated species (Lemos Barão-Nóbrega et al., 2022), particularly in the face of a new threat in the Yucatan Peninsula with the construction of the national mega-project called "Tren Maya". Two stations with a capacity of 8000 passengers per day are being built within the Calakmul Biosphere Reserve and another station has been built near Lake Moku (Benítez & Alexander, 2019). This mega-project has been proved to damage the biodiversity and ecosystems of the country, in a negative way (Benítez & Alexander, 2019).

Finally, in view of the crocodile population present at Lake Moku, it would be possible to envision the development of a plan for the sustainable use of the species by the CEMC, as a touristic farm, for instance, in order to attract tourists and generate a new source of sustainable income for the local community (Clancy et al., 2024).

FUTURE/FOLLOW-UP WORK

This work will lead to the writing and publishing of a scientific article in peer-reviewed journal.

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I'd also like to thank my colleagues in the lab, Joana, Landy, César and Alberto, for the great times we've had together in the field, for the passionate discussions and for the invaluable advice we've shared during these five months of adventure.

ETHICAL STATEMENT

Authorizations for field research activities were issued by the Secretariat of the Environment and Natural Resources (SEMARNAT) of Mexico and by the National Commission for Protected Natural Areas (CONANP) of Mexico (Yalikín site: PARN/DGVS/06850/23; Moku site: SGPA/DGVS/03336/22). All field activities were carried out following the Guidelines for the use of live amphibians and reptiles for field and laboratory research, by Beaupre et al. (2004), and efforts were made to minimize discomfort and stress to individuals during handling.

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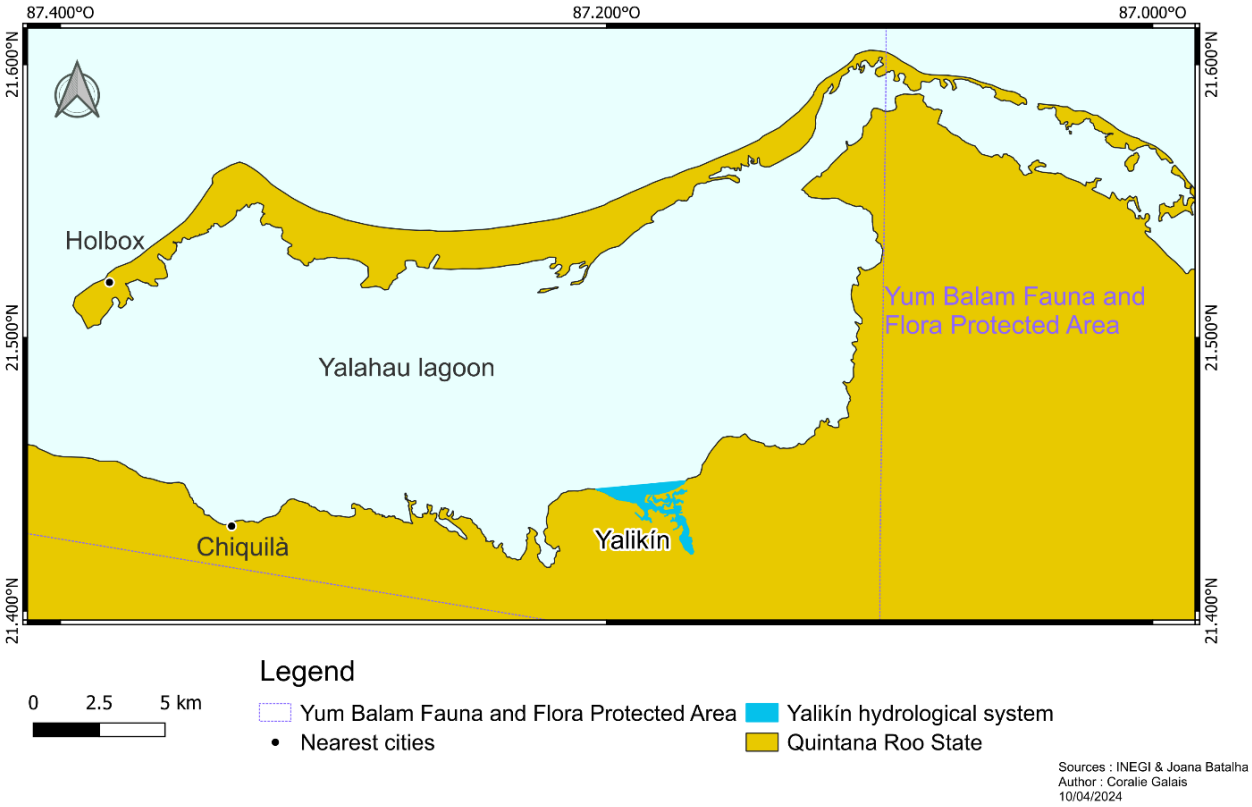
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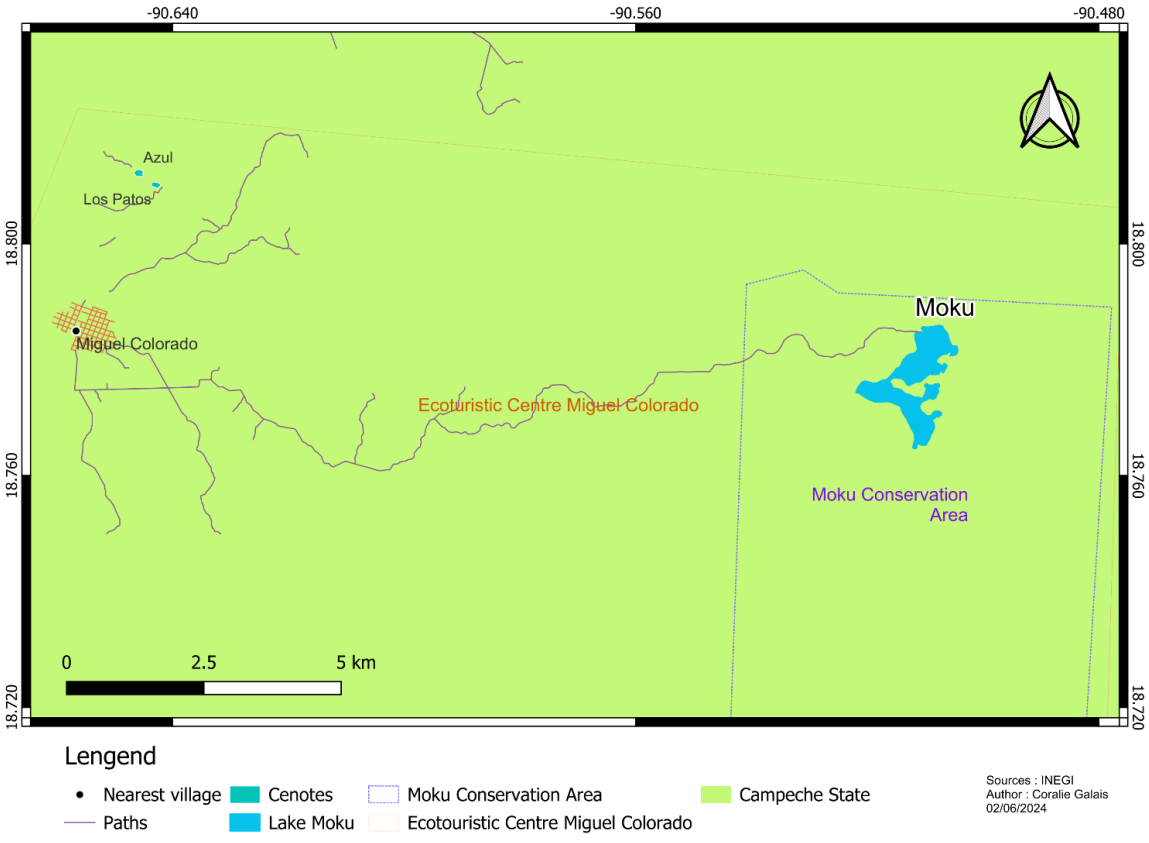
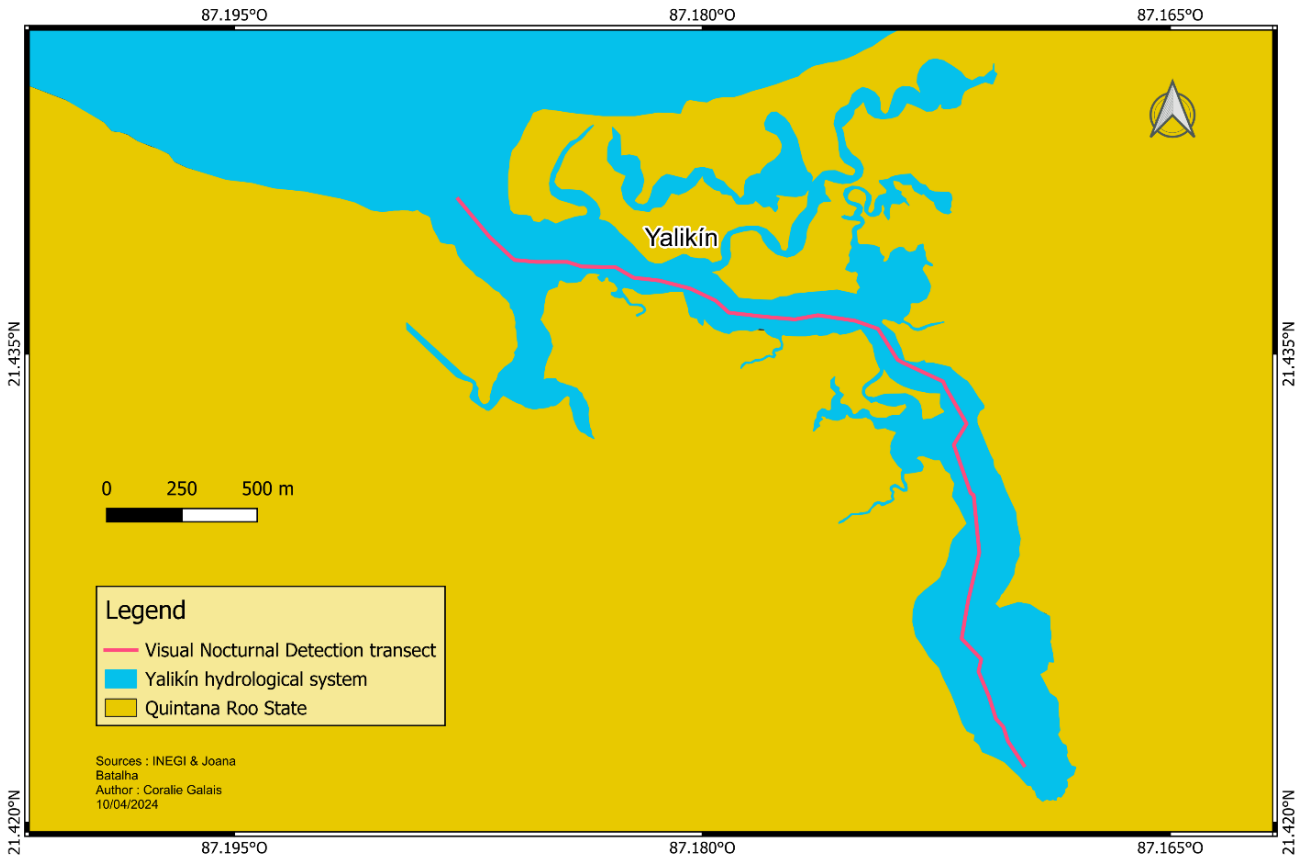


Figure 1. *Localisation of the study areas.* ***A.*** *The Yalikín hydrological system in the YBFFPA.* ***B.*** *Lake Moku in the MCA.*

A.



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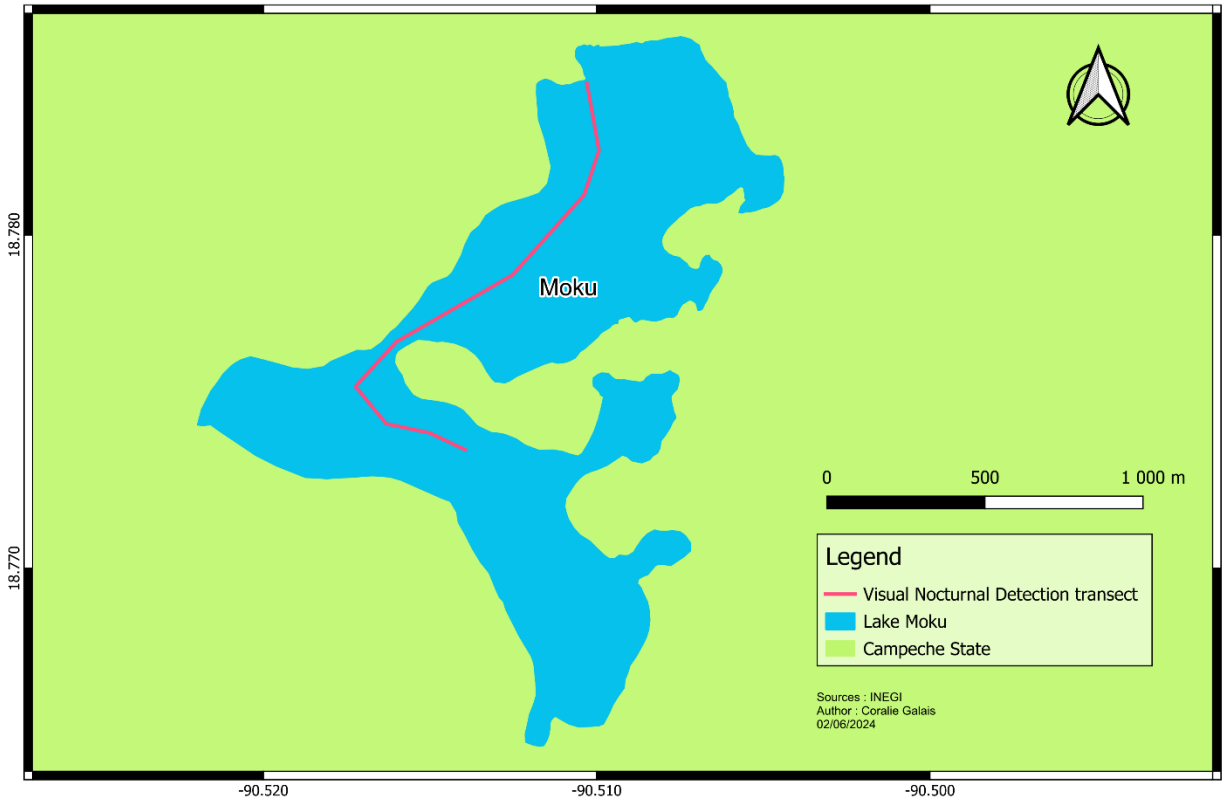


Figure 2. *Visual Nocturnal Detection transects chosen for the study sites.* ***A.*** *Transect of the Yalikín hydrological system in the YBFFPA.* ***B.*** *Transect of the Moku Lake in the MCA.*

Table 1. *Comparison of the results of one LRT (Likelihood Ratio Test) test and two bootstrap tests, comparing the influence of three explanatory variables on the detectability and counting of crocodiles. Test 1 and 2 correspond to the bootstrap tests using the pbnm() function and the PBmodcomp() function, respectively. (*) P-value < 0.05*

	LRT test		Bootstrap tests			
			Test 1		Test 2	
Explanatory variable	LRT statistic	P-value	Bootstrap Statistic	P-value	Bootstrap Statistic	P-value
Moon Phase	3.1938	0.073	3.1938	0.151	3.1938	0.111
Observer Experience	1.1225	0.289	1.1225	0.337	1.1225	0.316
Hours after sunset	7.505	0.023 *	7.505	0.068	7.505	0. 028 *

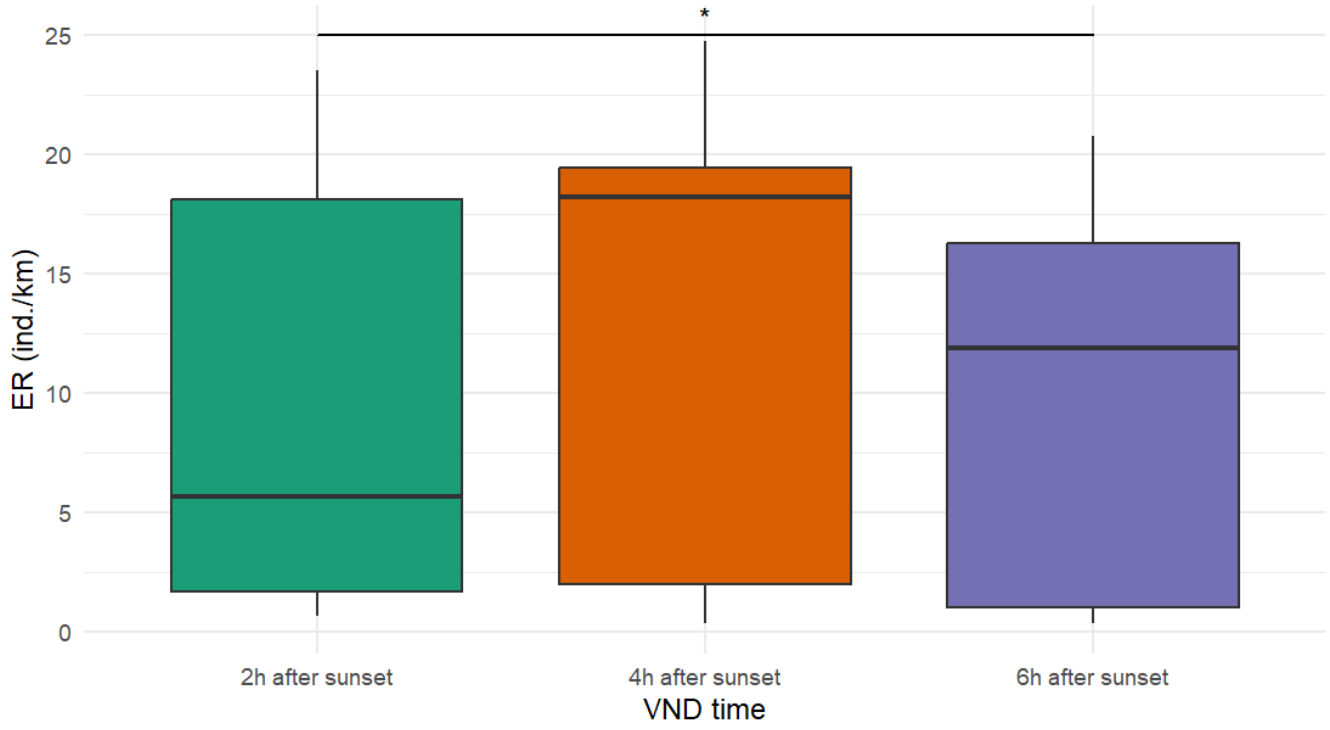


Figure 3. *Encounter rate (ind./km) of crocodiles calculated for all the surveys carried out during the study divided by the hour of the VND. Horizontal black line is the median. Tukey's test: (*) P-value < 0.05.*

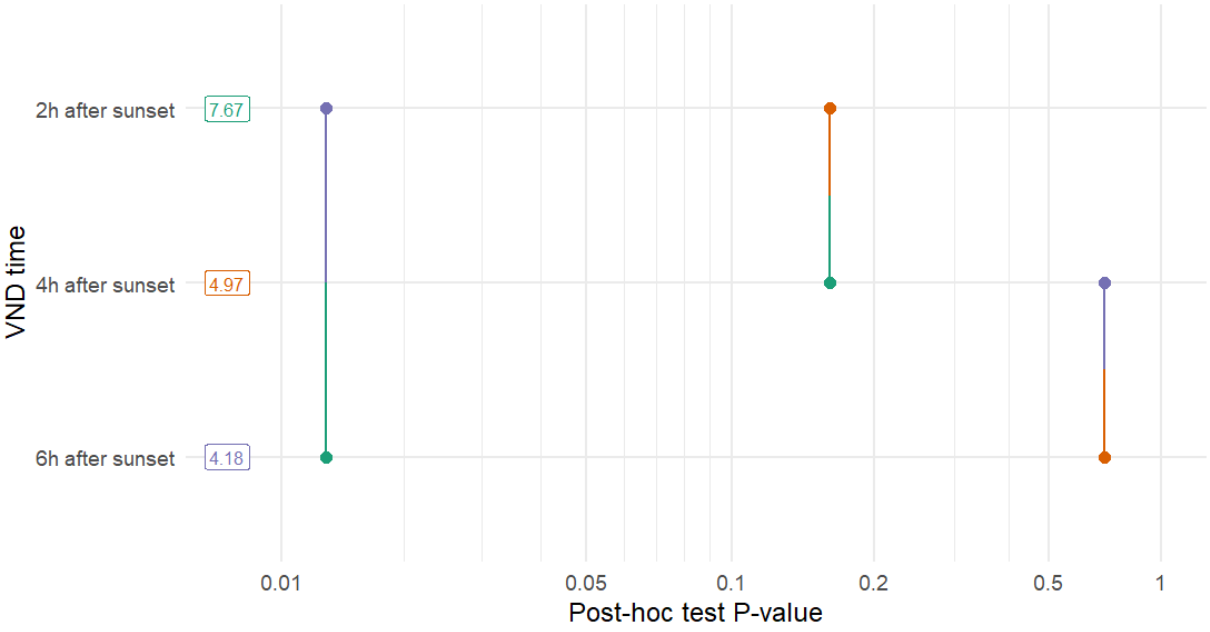


Figure 4. *Graphic representation of the Tukey's test comparison of the three levels of the hour of the VND explanatory variable. Levels are compared two by two. The position of the comparison segments reflects the significance of their difference, the confidence level being 95% (P-value < 0.05).*

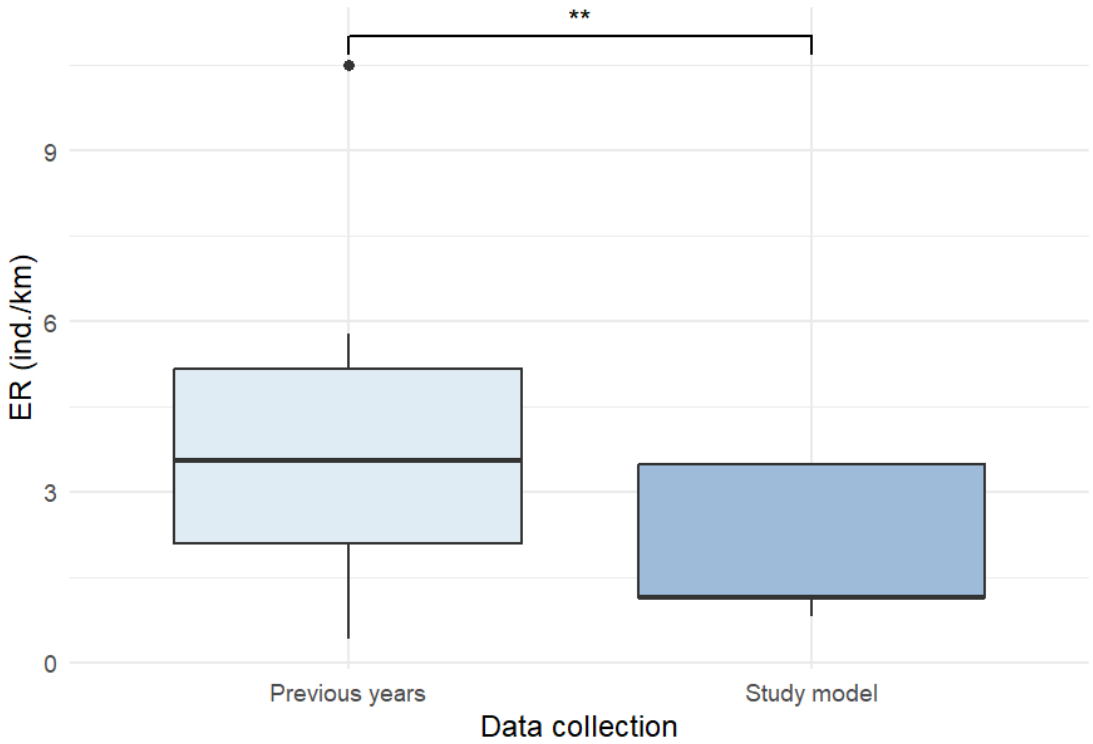


Figure 5. *Comparison of the encounter rates (ind./km) of crocodiles calculated using the data collected from previous years (between 2012 and 2023) and the encounter rates (ind./km) of these same years, calculated using the model selected in this study. Data collected include ERs of Yalikín and Moku sites. Horizontal black line is the median. Wilcoxon signed-rank's test: (**) P-value < 0.01.*