UICN-SSC SRAS Project 18/02 Summary Report “Population structure and genetics of the *Crocodylus moreletii* in the region of Calakmul (Campeche, Mexico)”

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Introduction

Although our knowledge of the Morelet’s crocodile (*Crocodylus moreletii*) life history across its distribution range has increased considerably over the last decades (e.g. see Sigler & Gallegos, 2017), aspects such as population size, structure and genetic diversity are still unknown in remote areas of the Selva Maya. The Calakmul region, situated in the southern portion of the Mexican Yucatan Peninsula, is no exception and little to no information is available on crocodiles inhabiting freshwater habitats across the region. In Calakmul, due to the geological characteristics that cause rapid filtration of the rain (García-Gil et al., 2002), aquatic habitat occurs in the form of semi-temporary natural ponds (aguadas) sustained by rainfall during the majority of the year, which occur at relatively low densities across the region (on average less than one non-dry waterbody per 535 hectares, García-Gil, 2000; Delgado-Martínez & Mendoza, 2020). The shape and size of these waterbodies vary seasonally in response to fluctuations in annual precipitation, and represent the only local source of standing surface water during most of the year (Reyna-Hurtado et al., 2010; Barão-Nóbrega, 2019).

Knowledge on population genetics in crocodylian species has increased significantly over the last decades. Hybridization between the American crocodile (*Crocodylus acutus*) and *C*. *moreletii* has been reported in coastal regions of sympatry and at inland sites outside the distribution range of *C. acutus*. As such, populations routinely identified as *C. moreletii* in Mexico are very likely to be admixed (Pacheco-Sierra et al., 2016; Pacheco-Sierra et al., 2018). Due to the geography of the region, reduced gene flow between localities may have led to a spatial population structure linked to family relationships.

This study aimed to provide baseline data on *C. moreletii* populations in Calakmul by 1) estimating local abundances in aguada habitats and total population sizes

across the region; 2) describe the population genetic structure using a genotyping by sequencing (ddRAD-Seq) approach to characterize the species genetic profile across the region.

Methods

Calakmul Biosphere Reserve (CBR) is located within the southern portion of the Yucatan Peninsula in Campeche, México (18°21.921' N, 089°53.220' W; Figure 19), and together with the adjacent state reserves Balam-Ku and Balam-Kin encompasses more than 1.2 million hectares of protected forest for which *C. moreletii* represents one of the main flagship species (Slater et al., 2020). CBR is part of the Selva Maya, which was home to the ancient Mayan civilization and covers 10.6 million hectares of forest across Mexico, Guatemala and Belize (Vester et al., 2007).

Candidate aguada habitats for *C. moreletii* surveys were identified using existing information (García-Gil, 2000), *Google Earth Pro* (Gorelick et al., 2017), and local knowledge by environmental authorities (*Comisión Nacional de Áreas Naturales Protegidas* - CONANP and *Pronatura Península Yucatán*), local guides and community representatives to define a total of 50 survey locations spread across the CBR and its surroundings.

As abundance estimation methods which are in common use for many crocodylians including *C. moreletii* (Cedeño-Vázquez et al., 2006; Tellez et al., 2017), we used a Minimum Population Size (MPS) approach as well as the King's Visible Fraction Method (VFM). MPS quantifies the number of recorded individuals to obtain baseline data (Sánchez-Herrera et al., 2011). VFM makes use of repeated counts per site, estimating the percentage of the total population observed during a single count (the visible fraction; King et al., 1990; Balaguera-Reina et al., 2018). Aside of these traditionally applied methods, we also used a binomial *N*-mixture modelling approach (Royle, 2004; Kéry et al., 2005; Kéry & Royle, 2016) and explored three alternative statistical distributions: Poisson, Negative Binomial (NB), and Zero-Inflated Poisson (ZIP).

Using a genotyping by sequencing (ddRAD-Seq) approach, we evaluated genetic structuring and presence of individuals exhibiting genomic admixture between *C. acutus* and *C. moreletii* in Calakmul using four mitochondrial and approximately

5000 nuclear DNA markers (SNPs) from 95 crocodile samples. For more specific information on sample collection, DNA extraction methods in the laboratory, SNP discovery and genotyping methods and also further details on the bioinformatic analyses employed, please see Barão-Nóbrega (2021).

Results

Population size estimates through MPS and VFM revealed a median of 0.4 (min – max: 0 – 67; Q1 – Q3: 0 – 3) and 0.95 (0 – 159; 0 – 8) non-hatchling *C. moreletii* for each aguada, respectively.The ZIP based *N*-mixture model shown overall superior confidence over Poisson and NB (Figure 1), and revealed a median of 5 ± 2 individuals (min = 0; max = 116 ± 15; Q1 = 0.9 ± 0.6; Q3 = 23 ± 4). Extrapolating these inferences across all waterbodies in the study area yielded in an estimated ~12,000 *C. moreletii* present (Table 1).

Our results show that putative *C. acutus*-specific alleles were detected in only 5-10% of our samples (and generally at low frequencies for nuclear markers), distributed amongst a small number of locations (Figure 2). Observed admixture proportions suggest a scenario of ancient admixture and/or incomplete lineage sorting rather than contemporary hybridisation. Our data also reveals some genetic differentiation and geographic structuring within Calakmul (F_{ST} range: 0.05–0.16).

Table 1. Estimates of total population size of *Crocodylus moreletii* occurring in the region of Calakmul based on five different estimation approaches. MPS – crocodile count data alone (without considering detection probability); VFM – King's visible fraction method; MPS^{GLM} – Generalized linear model considering the relation between MPS and waterbody perimeter; VFM^{GLM} – Generalized linear model considering the relation between VFM and waterbody perimeter; ZIP – Zero Inflated Poisson *N*-mixture approach.

Approach	Total population size
MPS	9,941 (6,651 – 13,230)
VFM	15,184 (14,014 – 16,355)
MPS^{GLM}	4,273 (4,112 – 4,440)
VFM^{GLM}	12,177 (11,543 – 12,846)
N-Mixture ZIP	11,681 (2,010 – 19,653)

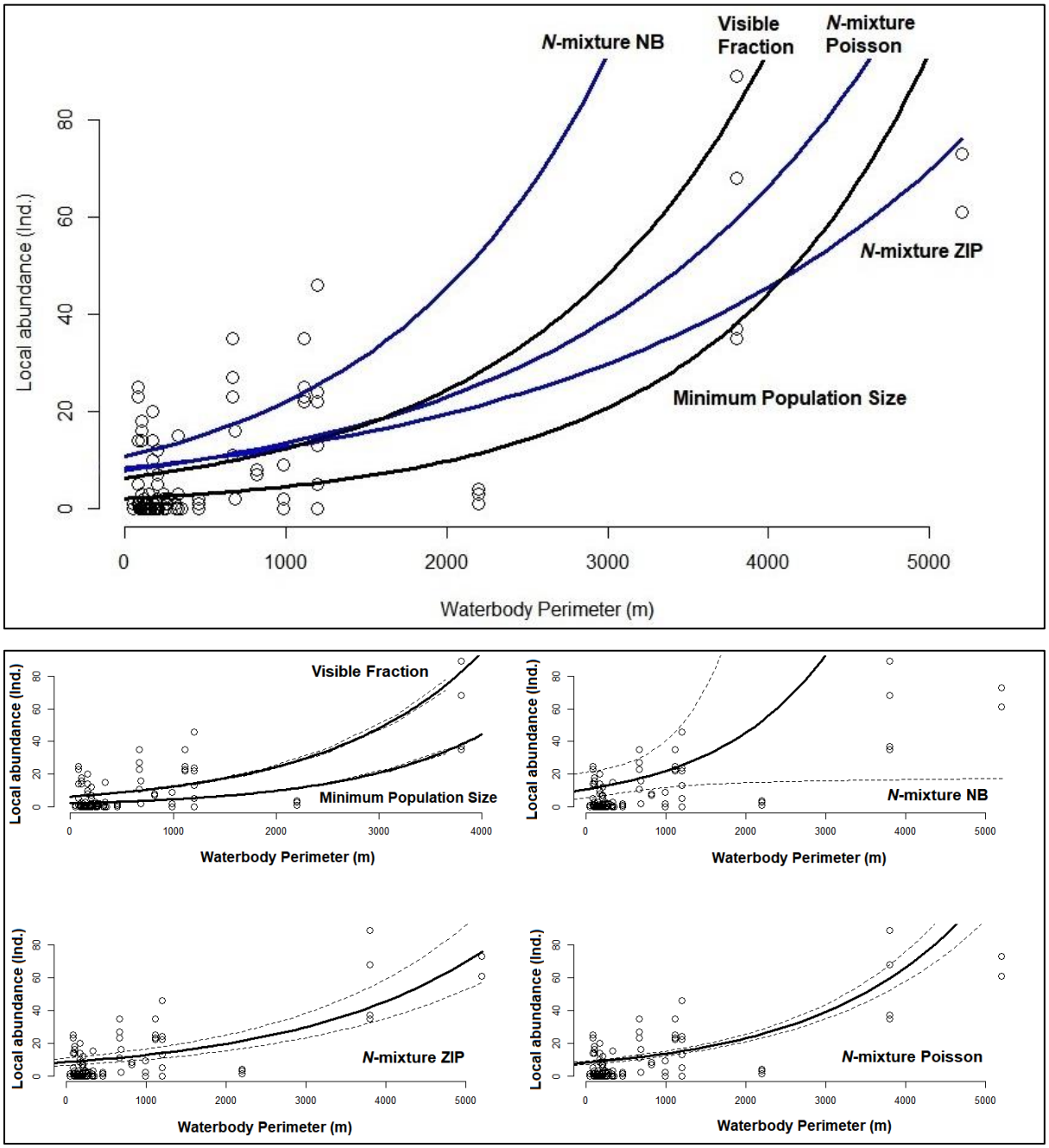


Figure 1. Generalized linear models between local *Crocodylus moreletii* abundance and waterbody perimeter in the region of Calakmul. Open circles represent all baseline crocodile count values (i.e. number crocodiles observed during the survey). Top figure – Black lines represent fitted values from estimations through King's visible fraction method and Minimum population size using only baseline count data, and dark blue lines represent fitted values from binomial *N*-mixture modelling using a Poisson, Negative Binomial and a Zero Inflated Poisson approach. Bottom figures – Dashed lines in graphs represent the upper and lower confidence intervals.

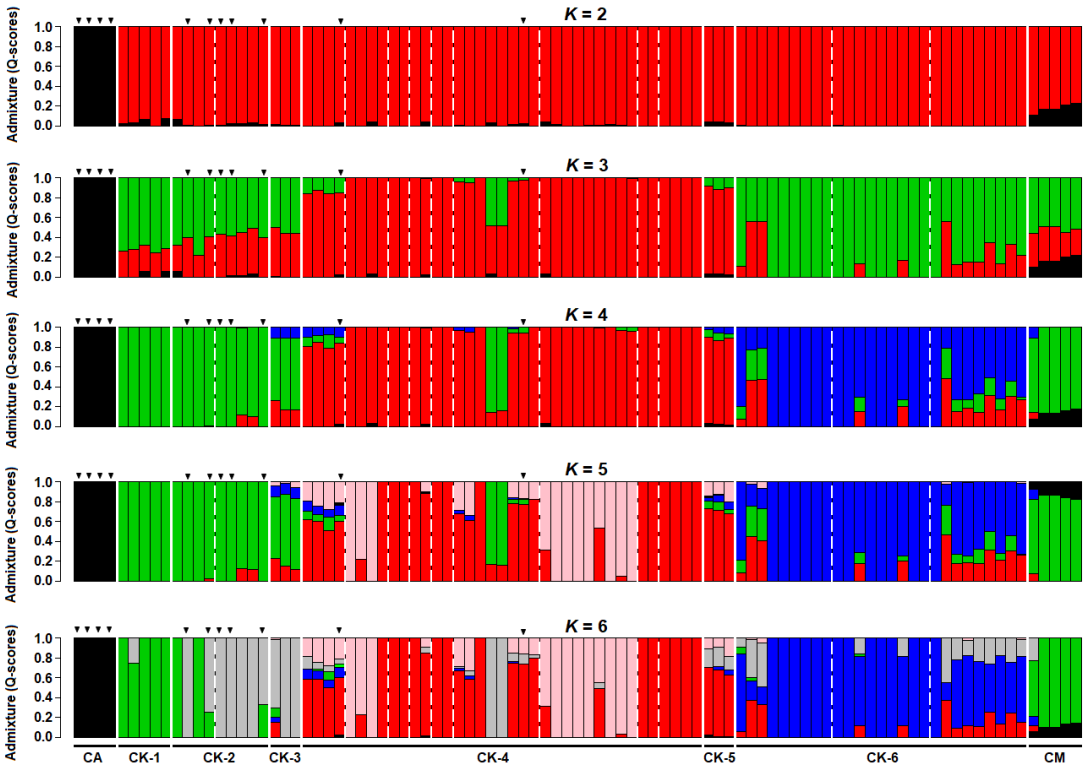


Figure 3. Genetic structure and ancestry proportions (Q-scores) from NGSadmix (Skotte et al., 2013) with *K* = 2 to 6, where solid lines are 95% credible intervals and different colours represent each admixture proportion. Each vertical-coloured bar corresponds to one crocodile sample. Dashed white lines delimitate different sample locations within each region of Calakmul (CK). Inverted black triangles represent samples with reads matching regions 2278-2872 and 9528-9622 of *Crocodylus acutus* mitochondrial genome. CA and CM represent, respectively, the external reference samples of mexican *C. acutus* (Cozumel and Banco Chinchorro, Quintana Roo) and *C. moreletii* (Altamira, Tamaulipas). Assignment results based on the CLUMPAK method indicated that the number of clusters that best explain the structure was *K* = 4.

Conclusions

This PhD project had two major aims: 2) Provide baseline data on *C. moreletii* populations in CBR by estimating local abundances in aguadas and total population sizes across the region; 3) Describe the population structure of *C. moreletii* in Calakmul (CBR and surrounding areas) by using a genotyping by sequencing (ddRAD-Seq) approach to characterize the species genetic profile across the region. In investigating these questions, the following key findings were made:

1) N-mixture models applied to spotlight count data have provided insightful estimates of crocodylian detection rates and local abundances in a dynamic environment, enabling insights into population responses to local environmental conditions by facilitating pooling of information of both small aguadas and large lakes and allowing comparisons between highly heterogeneous survey sites;

2) Extrapolating abundance estimates across Calakmul yielded in an estimated ~12,000 *C. moreletii* present, highlighting the region is an important global stronghold for this species;

3) Calakmul still harbours a demographically healthy population of non-admixed *Crocodylus moreletii*, unlike what has been reported in other parts of the Yucatan Peninsula where genetic pollution through increased hybridization with the American crocodile (*Crocodylus acutus*) is common;

4) *Crocodylus moreletii* shows a distinct genetic structure across Calakmul, with six main geographical regions where crocodile were sampled largely representing own genetic units;

5) The spatial population structure within each region is determined by, and reflected in extended family networks as revealed by parentage inferences.

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