Integrated assessment of chemical pollution and its impacts on wild populations of *Crocodylus moreletii* in the Mexican Yucatan Peninsula

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Purposes

The proposed study will measure levels of metals and trace elements (V, Cr, Mn, Co, Cu, Zn, Se, As, Rb, Sr, Mo, Ag, Cd, Sn, Sb, Cs, Ba, Hg, Tl, Pb, and Bi) and POPs (e.g., PCBs, dichlorodiphenyltrichloroethane (DDTs), poly-brominated diphenyl ethers (PBDEs), and perfluoroalkyl substances (PFASs) in blood plasma, caudal scutes, and claw samples of 11 wild *C. moreletii* populations within the Yucatan Peninsula in Mexico (YP; n = 10 per site).

Simultaneously, we will also reveal transcriptome (~10,000 transcripts) and metabolome profiles (hundreds of lipids, carbohydrates, amino acids, and hormones) in these samples. Bioinformatic approaches will be applied to determine the biomarkers and the biological pathways/networks affected by contaminants **[1]**.

Materials and Methods

Our proposed plan is being conducted in two teams: Fieldwork in Mexico and Lab work in Japan. For the fieldwork in Mexico, crocodile capture and collection of samples will be executed following our well-established protocols, targeting sub-adults and reproductive adults (> 86 cm total length) **[2, 3]**. Blood samples (whole and plasma) will be drawn from all captured individuals prior to their body size measurement and then caudal scutes and claws will be obtained **[3]**. To determine health condition, blood smears will be prepared for blood cell counts. Five fieldwork campaigns for sample collection have been conducted and one more campaign was envisioned from June – November 2022. A total of 47 crocodiles were surveyed before May 2022. For the sixth campaign 3 to 4 sites were targeted. It is expected to conclude the fieldwork by November 2022;

however, if the number of samples is below 70%, one more campaign will be conducted in 2023 to achieve the number of samples expected, as well as to conclude the blood counts in Mexico.

Samples were preserved in refrigeration and transcriptome samples were preserved in RNAlater® solution (Invitrogen™) until their arrival at the laboratory of El Colegio de la Frontera Sur (ECOSUR). Samples will be shipped to the host researcher's laboratory at Ehime University, Japan.

For the Lab work in Japan, blood samples, claws and scutes will be digested in a microwave system, and metals and trace elements will be determined by HG-AAS, ICP-MS, and CV-AAS **[4]**. POPs in blood plasma and scutes will be determined by GC-MS **[1]**. Transcriptome profiles in soft tissues of caudal scutes will be determined by next-generation RNA sequencing (RNA-seq) using Illumina HiSeq 2500 system **[1].** Metabolites will be determined in blood plasma by LC coupled with Q-TOF-MS **[5-7]**. Differences in contaminant concentrations between populations will be tested through left-censored data analyses **[8]**, followed by post-hoc analyses. Relationships between contaminant levels, metabolites and transcriptome profiles will be evaluated using class coinertia analysis (CIA) and factor analysis for multiple testing (FAMT) **[1]**. For mRNAs and metabolites that exhibit relationships with contaminant levels, enrichment analysis of transcription factors, pathways, networks, and diseases will be performed using databases (KEGG, Reactome and DiseaseComps) and bioinformatics analysis tools (DAVID, STRING, Cytoscape and TfactS).

Results

Before May 2022, 47 individuals were captured with a ventral length of 66.41 ± 4.76 cm (mean ± SE) and a weight of 12.21 ± 1.85 kg with a sex ratio of 1.3:1 (Female:Male). From June – December 2022, 26 crocodiles were sampled and 182 samples of blood, scutes, RNA Later samples and claws were obtained. So far, we have surveyed 73 crocodiles and the first batch of 203 samples (n = 30) was received in Japan in October 2022 (Fig. 1). Overall statistics of the captured individuals per site are presented in figure 2.

The relative index of body condition (ICC: Índice de Condición Corporal" in Spanish), was calculated for each individual using the observed weight, divided by the theorical weight predicted by the estimated model with empirical data ($W = (8.4x10^{-7}) * TL^{3.276}$; Fig. 3).

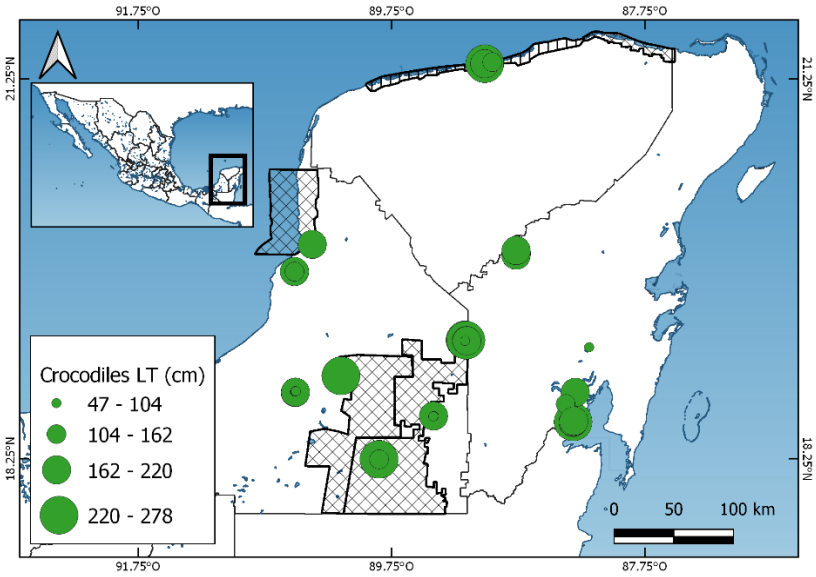

Figure 1. Location and size of individuals collected until December 2022.

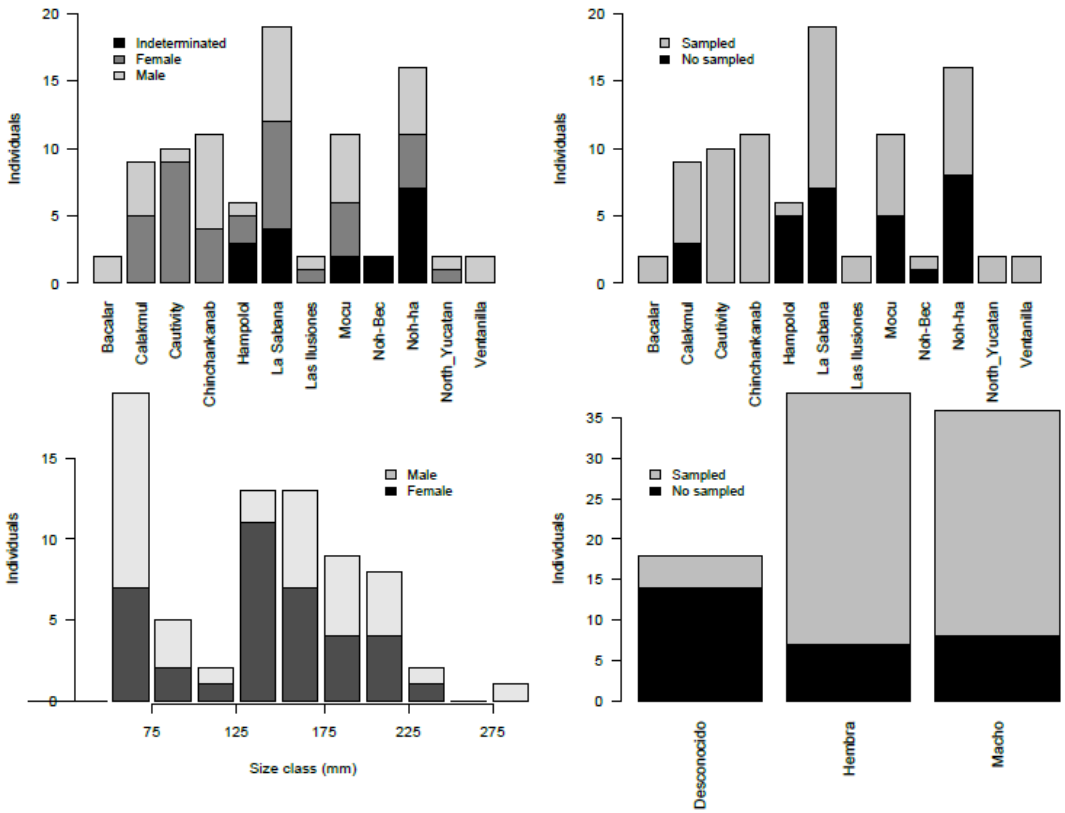

Figure 2. Overall statistics of individuals collected until December 2022.

Therefore, an organism tending to an ICC > 1 presents poor body condition, while individuals tending to an ICC > 1 may present overweight. Individuals presenting an ICC near 1 are considered organisms with good body condition.

We observed 53.7% of the captured crocodiles were above their “optimal” weight, while 46.2% of them were below their “optimal” weight (Fig. 4).

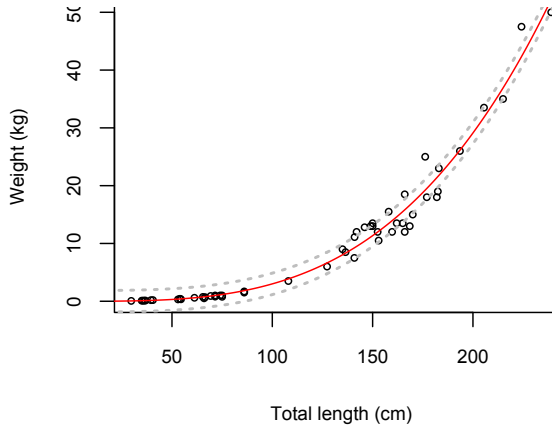

Figure 3. Exponential modeling estimated with empirical data $W = (8.4x10^{-7}) * TL^{3.276}$.

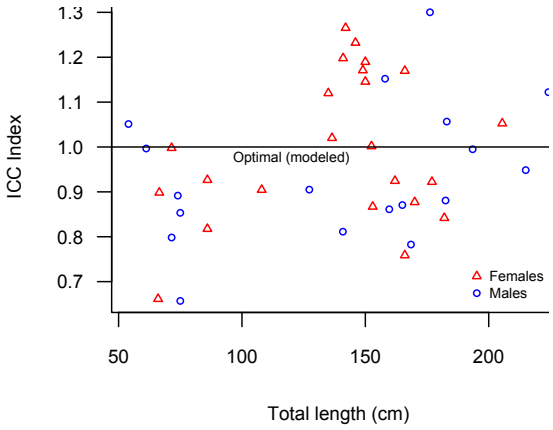

Figure 4. Ratio between observed ICC and modeled ICC with empirical data), Straight line represents the "Optimum" ICC.

We did not find significative differences in ICC between sexes (t = 1.1393, df = 37.554, p = 0.2618; Fig. 5), ICC females mean = 0.9967 and males = 0.9407.

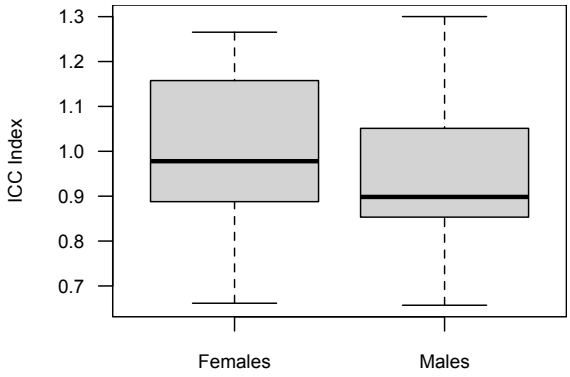

Figure 5. Median, first and third quantile, maximum and minimum of ICC index of captured crocodiles.

Future Challenges

1) We expected to obtain 110 individuals for this project. However, 73 crocodiles have been captured, which represents 66.4% of the total expected.

2) Based on the fieldwork results, we still need to conduct one more campaign at least with 3 to 4 sites targeted to reach the 37 crocodiles expected for the project in 2023.

3) After this last campaign in 2023, the second batch of samples will be prepared for exportation and shipped for the laboratory analyses in Japan.

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