CSG-SRAS Report

Croc Calls: How American and Morelet's Crocodile Vocalizations Differ in Structure

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Introduction

Out of the extant reptilians, crocodilians are the most vocal (Vergne *et al*. 2009), using vocalizations for social interactions such as courting and territorial defense (Vergne *et al*. 2012), as well as distress or threat calls (Boucher *et al*. 2020). The two species of crocodiles occurring in Belize, the American crocodile (*Crocodylus acutus*) and Morelet's crocodile (*C. moreletii*), have been hypothesized to interbreed based on genetic evidence and morphological traits of both species being exhibited in individuals (Ray *et al.* 2004). Although hybridization is a naturally occurring phenomenon, anthropogenic factors have escalated the rate at which hybridization occurs. Interbreeding is more prevalent now where species were separated in the past, as physical barriers, such as densely forested areas, have been removed. Hybridization is a major threat to *C. moreletii*, as it could eventually lead to the decline of the pure-bred populations and their displacement by hybrids (González-Trujillo *et al*. 2012).

With an increase in the use of technology in biology, new surveying methods are being implemented on a global scale. Acoustic monitoring uses sounds produced by a species to detect its presence by placing recorders in the field for an extended period of time. The recordings are then analyzed for any known sounds, such as specific calls made by an animal. There are many benefits to using Passive Acoustic Monitoring (PAM) for conservation, to estimate species presence and abundance, population density, and different community compositions, as well as monitoring spatial and temporal trends in species (Browning *et al*. 2017).

Some research on vocalization in *C. acutus* has been carried out (Boucher *et al*. 2020), but there is little known about the call structure of *C. moreletii*. This research aimed to provide a better understanding of vocalization structures in *C. acutus*, *C. moreletii* and hybrids of the two species, that could be used to: 1) understand differences in vocalizations between these species and hybrids; and, 2) possibly implement PAM distribution surveys in future research. In addition to looking at differences between species, differences between individuals were also examined.

Methodology

The study area was on the eastern coast of Belize, at five different sites (Fig. 1). Crocodiles were captured from a boat during surveys in August 2022. Ten (10) surveys were carried out, with some areas having multiple surveys (eg 3 surveys in Belize City).

Capture surveys were conducted following safe capture and examination practices developed by Sánchez Herrera *et al*. (2011). Spotlight surveys were carried out from a motorboat, and began 15-30 minutes after sunset. Crocodiles were spotted by shining a spotlight and looking for a red eyeshine. When a crocodile was spotted, it was captured by hand or with catchpole snares depending on size and brought on board the boat or onto land if an adult.

Vocalizations that occurred during capture and restraint were recorded for approximately 1-3 minutes using the AVR X application on an iPhone 11 with the settings set to; WAV file format, High encode quality, sample rate of 48,000 Hz, bitrate at 320 kbps and a Mono channel. The microphone was held approximately 1-2 m from larger sub-adult and adult crocodiles for safety, but smaller sub-adults, juveniles and hatchlings were handheld, and the microphone was placed about 0.5 m away (Boucher *et al*. 2020).

Morphometric data (head length, snout length, pre-orbital length, pre-orbital width, cranial width, maxillary width, premaxillary width, total length, snout-vent length, tail width, right hind foot length) and sex were recorded. Crocodiles

were also categorized on the basis of size [hatchling (<50 cm), juvenile (51-120 cm for *C. acutus*, 51-100 cm for *C. moreletii*), sub-adult (121-180 cm for *C. acutus*, 101-150 cm for *C. moreletii*), adult (181-240 cm for *C. acutus*, 151-200 cm for *C. moreletii*)] and possible species identification. If traits of both *C. acutus* and *C. moreletii* were present within one individual, it would be categorized as a hybrid. Photographs of nuchal scute and tail patterns were taken for possible future examination. We also examined individuals for health and presence of external parasites.



Figure 1. Locations (stars) where surveys were carried out.

The recordings were analyzed, and low-quality calls were filtered out and excluded from analyses. These low-quality files included any with heavy background sound interference and talking or movement noise. This was done by an aural and visual inspection of the spectrograms in RavenPro. To standardize analysis, only 7 calls were selected from each individual. The first usable 7 calls with clear spectrograms that contained little to no interference from undesired sounds in the file were selected for analysis.

With RavenPro, the calls were isolated, and the fundamental frequencies of the calls were analyzed in 0.005 second segments to measure beginning, end, first quartile, and max frequencies, as well as total duration and duration of the first quartile. The slope of the first quartile [Slope 1, calculated by $(F_{1/4} - F_{max})/D_{1/4}$] was determined, where $F_{1/4}$ is the frequency at the first quartile, F_{max} is the maximum frequency and $D_{1/4}$ is the duration of the first quartile. The slope of the remaining three quartiles [Slope 2, calculated by $(F_{end} - F_{1/4})/(DT - D_{1/4})$] was also determined, where F_{end} is the end frequency, and DT is the total duration (Boucher *et al*. 2020).

For each species and size class, averages and standard deviations were calculated for Slopes 1 and 2, as well as total duration, first quartile duration, maximum frequency and first quartile frequency. Individual crocodile vocalizations were also compared within species and size class.

Results

Vocalizations were recorded from 21 crocodiles (ranging in size from 32.4 to 110.7 cm) across all surveys, from which data for 7 crocodiles were analyzed: two juvenile and one sub-adult *C. moreletii*, and two hatchling and two juvenile *C. acutus*. Unfortunately, only one third of the crocodiles could be used due to low quality recordings.

Notwithstanding the low sample sizes involved, the following were concluded:

- The sub-adult *C. moreletii* had a lower total duration than the juvenile *C. acutus* group (0.13 s vs 0.21 s).
- Slope 1 for the juvenile *C. moreletii* group was more negative than that of the juvenile *C. acutus* group.
- Slope 2 for the sub-adult C. *moreletii* was more negative than that of the hatchling and juvenile *C. acutus* groups.
- Visual comparisons of the data reveal no clear distinction between age class or species, however, there were considerable differences between individual crocodiles.

For the k-means clustering analysis, the optimal number of clusters was determined to be two, which allowed for all 7 points to be grouped into a cluster. Cluster 1 was more widespread, whereas Cluster 2 was more compact (Fig. 2). Both clusters contained individuals of both species and contained different size classes. The average size of crocodiles in Cluster 1 was similar to the average size for crocodiles in Cluster 2 (66.27 vs 71.28 cm). There was no clear pattern found in species or size class.

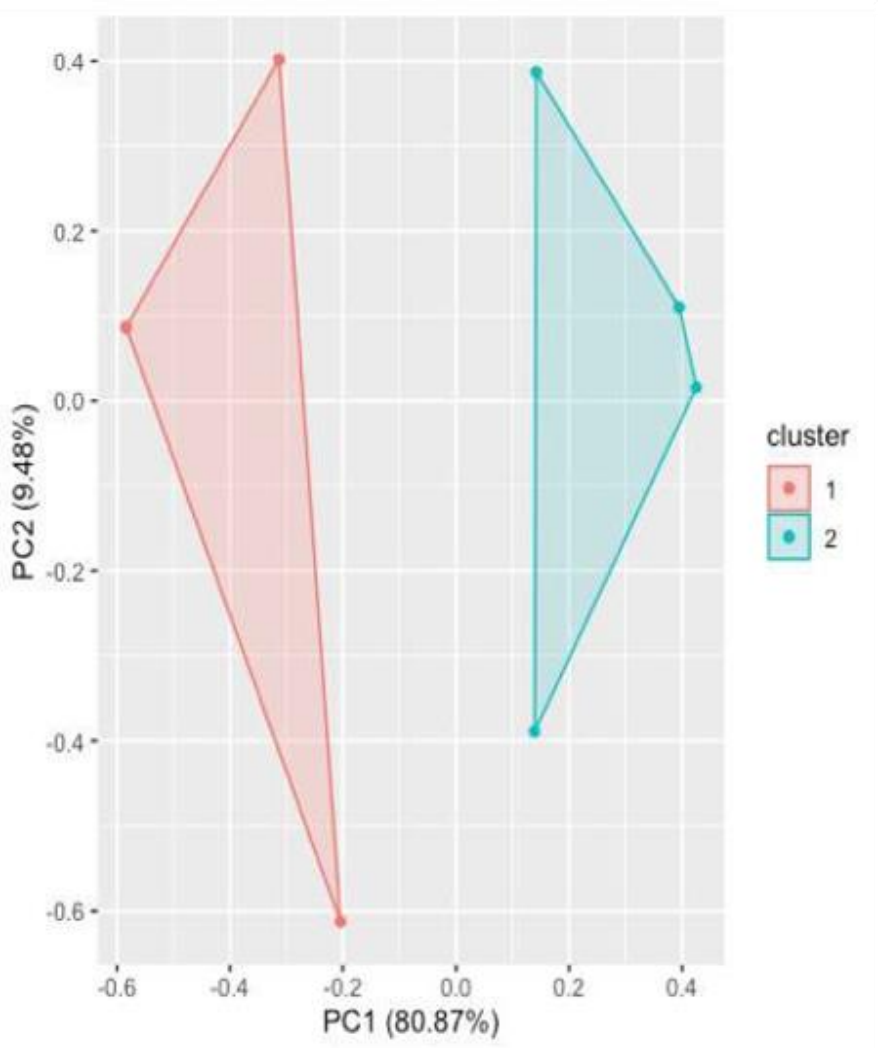


Figure 2. Results of K-means clustering analysis, with two clusters (1 and 2). Percentages are the proportion of how the variance in the data is explained by one of the principal components.

To determine if there is at least one statistically significant difference between individual crocodiles, an ANOVA test (with Welch's correction), using a confidence interval of 95%, was carried out. There was a statistical difference ($p<0.05$) between all vocalization variables.

However, although there was a difference between the variables, the ANOVA test has certain conditions that are expected to be met, including that data are random/independent and normally distributed, and that there is homogeneity in the variance. Welch's correction was used to eliminate the issue with unequal variances, however the Shapiro-Wilk test was used to determine whether the data were normally distributed. The only variables that had no evidence for non-normality were Slope 1 and Total Duration, meaning that the p-values calculated for the other variables cannot be trusted because the data did not meet the assumptions for the ANOVA test.

Slope 1 and Total Duration were further subjected to a Games-Howell Post-Hoc test for further interpretation. A Games-Howell Post-Hoc test is a follow-up test to determine where the statistical differences were found after running the ANOVA test. This test uses multiple comparisons that adjust the p-value to lower the risk of a false positive result. There is a 95% confidence interval, so the difference is statistically significant if the p-value is less than 0.05.

The post-hoc test for Total Duration indicated there were no statistical differences between:

- Morelet's Juvenile 1 and Morelet's Sub-adult or American Hatchling 2.
- Morelet's Juvenile 2 and American Juvenile 1 or American Hatchling 1.
- Morelet's Sub-adult and American Hatchling 2.
- American Hatchling 1 and American Juvenile 2.

The post-hoc test on Slope 1 indicated there were no statistical differences between:

- Morelet's Juvenile 2 and Morelet's Sub-adult or American Juvenile 1 or American Hatchling 2.
- Morelet's Sub-adult and American Juvenile 1 or American Hatchling 2.
- American Juvenile 1 and American Hatchling 2.
- American Hatchling 1 and American Juvenile 2.

Conclusions

Call structures in the 7 crocodiles analyzed varied between individuals. There was no clear pattern found between size class or species. However, this may have been due to a small sample size. It is possible that an expansion of the sample size may reveal patterns between size/age class, or species. Unfortunately, the hybrid crocodiles that were captured were not included in analysis due to low quality recordings.

When visually inspecting the spectrograms of all of 21 individuals, there were some observations that stood out. The slope pattern of the individuals differed, where some had increases within the slope (visually looks like a hill), others had only negative slopes. Those vocalizations with a portion having a positive slope were found in both species and sometimes occurred multiple times within the same individual.

There was also one individual whose call was not similar to any of the other individuals in that it had a long section with an increasing slope before the "standard" downward slope. The embellishment of the vocalization had a majority positive slope, which is different from the standard vocalizations.

It is clear that individuals can exhibit very different vocalization structures. There was a lot of variation between individuals within the same size class and species. For example, the two *C. acutus* hatchlings had no overlap within their values for five out of the seven variables visualized in box and whiskers plots. In addition to a visual comparison using boxplots, there were statistically different values in the variables analyzed with the Games-Howell post-hoc test. This suggests that these vocalizations are distinct from one another, although they are of the same species and size/age class.

It was previously expected that vocalizations made by crocodiles would be stereotypical and have little variation between individuals because they are reptiles, which are generally considered to have simple social and communication systems. However, these data show that there is great variation between individuals, so these vocalizations are more complex than previously thought.

Although this research is limited regarding species and size/age class, it could still be helpful when implementing PAM for conservation management purposes. It is known that American crocodiles inhabit areas with salt water frequently, and some call types are specific to certain age classes, like mating calls, so PAM could be used for population monitoring.

The study would have benefitted from being continued over a longer period of time, with better recording equipment and a larger sample size of species and size class, in order to make analysis more robust. In addition, the calls analyzed during this study were specifically distress calls. It would be beneficial for this study to be replicated and incorporate courting calls as well.

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