Biodiversity Monitoring in the Calakmul Biosphere Reserve 2014-2019: Results pertaining to prolonged drought, disappearing aguadas and the associated impact on fauna

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Aguada, herpetofauna, felid and ungulate analyses in collaboration with BSc thesis student Josh Daw, University of Hull and MSc thesis student Alex Didcott, University of Exeter

The Calakmul Biopshere Reserve (CBR) is an UNESCO World Heritage Site of Culture and Nature due to the forest of outstanding biodiversity that surrounds multiple ancient Maya ruins sites, including the city of Calakmul that contained up to 50,000 people during the height of its power between 250BC – 900AD. The tropical semi-deciduous forest in CBR is primarily medium forest where tree most trees grow to 20-30m in height due to limited rainfall and 20-40% of tree lose their leaves in dry season (Beletsky, 1999). Areas of forest close to Mayan Ruins are notably different with high densities of large fruiting trees (the result of Ancient Mayan agro-forestry) in comparison to other areas (Ross & Rangel, 2011). There is a steady increase in mean annual precipitation from the north to the south of the reserve that has a notable effect on tree species composition and forest structure. In addition, as there are no rivers or streams in the reserve, forest structure is also heavily affected by distance from the limited number of water bodies in the reserve known as aguadas.

Permanent water bodies are rare in CBR due to the geologic characteristics that cause rapid filtration of the rain (García-Gil *et al*. 2002). However, low-lying terrain allows the accumulation of water, and creation of temporary lakes, locally known as *aguadas*. These aguadas are filled by direct rainfall combined with water flowing across the forest floor during the peak of rainy season. As both water and leaf litter collect in these aguadas, the rotting leaf litter creates a mucus layer that stops the water filtering through the limestone karst. This system is entirely reliant on localized rainfall, and so changes to rainfall patterns can very quickly have a devastating effect on water distribution in the reserve. The prevalence of water in the a*guadas* of CBR has suffered alterations due to the effects of global warming (Reyna-Hurtado *et al*. 2010). For example, during the last 50 years Calakmul has endured a 16% reduction on the annual median precipitation values (Zuniga-Morales & Sima-Pantí 2015) followed by extreme fluctuations in the intensity and timing of rainfall over the last 10 years (Mardero et al., 2019). Changes to water availability in CBR have altered ranging patterns of ungulates such as peccary and tapir (Reyna-Hurtado et al., 2019) that are closely associated with water, which in turn is expected to affect ranging patterns of sympatric jaguar and puma.

Research Aims and Objectives

Operation Wallacea is a UK based NGO that specializes in biodiversity assessment and monitoring of protected areas using the expertise of university academics and students. The aim of this long-term project was therefore to assess the abundance, diversity and distribution of flora and fauna in the Calakmul Biosphere Reserve and monitor changes to this diversity over time. In addition, the project aimed to investigate habitat preferences of specific species to identify the most important sections areas of the Calakmul for these species and to help predict changes to the abundance and distribution of these species over time based on changes to their habitat from human impact and climate change. Moreover, data relating to the biological importance of Calakmul may be used to levee international funding to assist with the management of the reserve.

These broad project aims can be broken down into a series of specific objectives as follows:

1. To investigate the abundance, diversity and geographical distribution of flora and fauna in Calakmul reserve, specifically that of trees, birds, bats, herpetofauna (reptiles and amphibians), primates, felids and ungulates in relation to forest structure, distance to aguadas and remnants of Ancient Mayan agroforestry adjacent to ruin sites.
2. To utilise monitoring data to assess the impact climate change and the loss of aguada habitats on the abundance and ranging of fauna in the reserve
3. To create and manage a biodiversity database for Calakmul that may be used by all relevant parties to assist with management of the reserve
4. To trail aguada restoration methods that mimic natural manipulation of aguadas by ungulates and dry aguadas filled with vegetation to return to water bodies the following rainy season

Methods

Research Design

There is a notable precipitation gradient from the north to the south of the reserve Calakmul that has a significant effect on forest structure and tree species composition (Beletsky, 1999), and differences in the level of human impact to habitat from areas of forest that are continuous with, but outside of the reserve, the buffer zone of the reserve and the core zone of the reserve. To ensure that data collected were representative of the Calakmul area, it was therefore necessary to collect data in locations that represent each level of strata (Sutherland, 2006). Consequently, data collection will be carried out in 5 different locations with the Calakmul Biosphere Reserve (Figure 1). These camp locations have been chosen due to their accessibility during the wet season and because they cover the full geographical and vegetation range of the reserve. Each camp will contain four 2km long transect lines for data collection. Each transect line will be mapped using a GPS unit. Five sample sites will be located along each transect line at 400m intervals, giving rise to 100 sample sites across the 5 research camps in the reserve. Each sample site is a 20m x 20m area adjacent to the transect line that are marked, and the GPS location recorded. All research locations have a corresponding field camp apart from ZNS which is accessed by vehicle from the KM20 camp.

Data Collection

All data were collected from June-August each year by teams of students lead by university academics and local field guides. Aguada and habitat surveys were completed only once, but fauna surveys (birds, bats, butterflies, herpetofauna, primates and large mammals) require a minimum of four replicate surveys for each transect in each camp. Different survey teams rotate around the survey transects and aguadas according to a fixed schedule that allows each location sufficient time to rest between surveys. Minimizing human traffic in any given location in the reserve ensures a constant encounter rate of fauna throughout the field season.

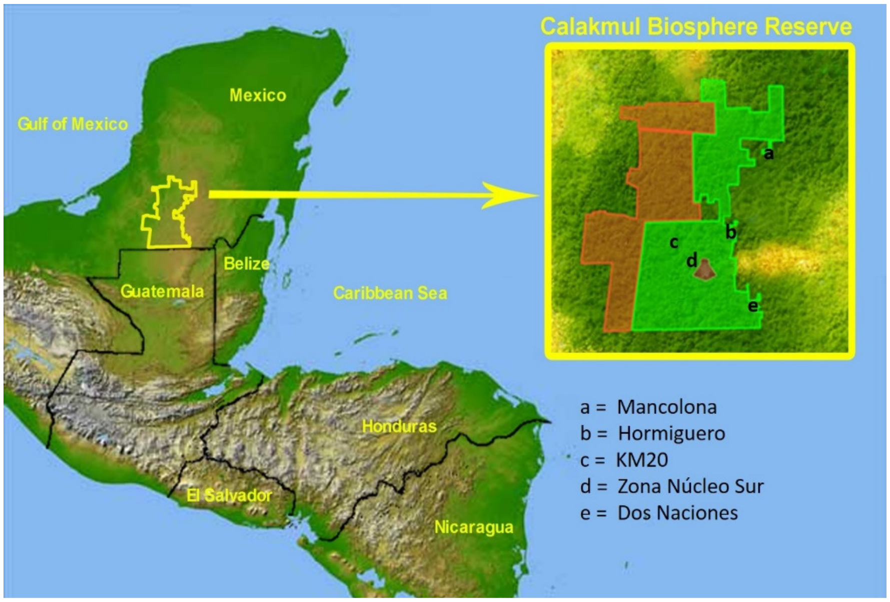


Figure 1: Location of Calakmul Biosphere Reserve and Operation Wallacea Research Locations with the reserve.

Aguadas

Twenty-five aguadas located in the 5 survey locations in CBR were monitored for water levels each year during the wet seasons. The maximum water level of each aguada was assessed based on changes to vegetation and water lines on trees. Current water levels were estimated based on 5 categories: Dry, Mud, 25%, 50%, 75% or 100%. For each aguada the type of water body (small pond, pond or lake), type of surrounding vegetation and predominate flora species, type and % coverage of vegetation in the aguada was recorded. In 2018 and 2019, aguada monitoring was expanded to 85 aguadas in the reserve as part of Jose Nobregas PhD research.

Habitat

Habitat surveys were conducted in each of 20m x 20m survey sites to investigate tree diversity and forest structure. On each transect the first plot will be located at 200m, the second at 600, the third at 1000m, the fourth at 1400m and the fifth at 1800m along the transect line. The number of saplings (trees with circumference <15cm and a minimum height of 2 metres) will be counted for each plot. For each tree in the plot with a circumference >15cm, the circumference at breast height (which will be converted to DBH), whether the tree is alive or dead, and the tree species, was recorded on datasheets. CBH was measured using 50m tape measures. The number of fallen trees and cut stumps in the plot was also be recorded.

Forest structure measurements include understorey vegetation, canopy cover and leaf litter depth. To measure understorey vegetation, the plot was bisected to produce the four quadrants. A 3m pole marked in 0.5m segments will be used to record the number of vegetation touches on the pole in each 0.5m segment up to a maximum of 10 touches, every 1m along these bisecting tapes. If one of the positions coincides with a tree, then each of the 0.5m segments were recorded as having vegetation touches. The openness of the canopy will be measured by taking a reading with a canopy scope (Hale & Brown, 2005) facing the largest opening in the canopy from the centre of each of the four quadrants and one from the centre of the overall 20m X 20m square. If any of these points is closer than 1m to a tree trunk, then the observation point should be moved slightly so that it is at least 1m from the nearest tree trunk. The perspex square has 25 dots engraved on the square. The observer should look upwards holding the square 20cm from the eye count the number of dots that coincide with gaps in the canopy to give a score out of 25. Leaf litter depth was recorded in each of the 4 quadrants and in the centre of the plot using a ruler to give 5 separate leaf litter measurements (mm) per plot.

Amphibians and Reptiles

Herpetofauna were collected using Visual encounter surveys (VES) along the forest transects between 8.30am-12.30pm (to monitor diurnal species) and between 7.30pm-11.30pm (to monitor nocturnal species). The duration of the survey and total distance travelled was recorded for each survey in order to calculate relative abundance of species that incorporates survey effort. During the VES, all possible microhabitats were searched, including leaf litter, tree trunks, decayed logs, fallen palm leaves and bromeliads. Due to the cryptic nature of anurans the disturbance of this vegetation using a probe is the most systematic method of detection. This was achieved by methodically probing through the area directly in front of the observers, including up to approximately 2m on either side of the trail. To identify herpetofauna during night transects instead of probing through leaf litter, torches were used to catch the reflection of light from the eyes.

Data collected in and around aguadas involved two methods. Diurnal and nocturnal timed searches were conducted for amphibians, snakes and lizards. Nocturnal crocodile count surveys will be conducted based on counts of eye shine reflected from torches. Each count will involve walking around the perimeter of the aguada searching for eyes with lights from torches. The process will be repeated several times until final numbers recorded become consistent across separate counts.

For each animal observed the species was identified using field guides (Lee, 2000; Kholer, 2008; Mandujuano et al., 2010; Cedeño-Vásquez et al., 2010; Colston et al., 2015), the GPS location and distance travelled along the transect were recorded along with the time, weather conditions and habitat type. Wherever possible, the animal was captured in order to mark for recapture (scale clipping of reptiles only) and to record additional information before releasing the animal in the same location as capture. For each animal captured the sex, age (adult or juvenile), weight (g), the length of the animal (SVL), length of the head, and length of tail (were relevant) and colouration (camouflage or aposematic) were recorded. In addition, the animal was photographed in situ (back, head and side).

Birds

Bird data were collected using point counts and mist netting. The point count surveys will be completed between 05:30am and 09:00am. If it is raining heavily or there are strong winds the survey should be cancelled. On all surveys the weather conditions at the time of the point count should be recorded. Point counts of birds (by sight or call) will be conducted at 10 different points along the transect a 200m intervals. No settling down period should be allowed with counts starting immediately. Then over the next 10 minutes for each species the following details should be recorded: species, number of individuals, whether the bird(s) was seen or heard, and the approximate distance of the bird from the observer (recorded at 5m intervals). Bird species will be identifies using field guides (e.g. Howel & Webb, 1995). Recording of bird calls during point counts was generally done directly due to the experience of local ornithologists in identifying calls, but students accompanying arnothilogists also recorded al call using a directional microphone and recording device for subsequent playback in camp for comparison with our bird vocalization library where necessary.

Bats

Bat mist nets surveys were run 6 nights per week at each research camp using a suitable existing clearing along each of the four 2km sample routes close to one of the habitat plots with enough space to erect five 6m long mist nets 2.5 meter high. The location of each mist net site was marked with flagging tape and the GPS location recorded to ensure that replicate surveys are conducted in the same location. Mist netting was conducted between 6:00pm and 1am, but as data collection may be affected by rain, the exact opening and closing time of the nets were recorded each session. The nets were checked every 15 to 20 minutes during the first 3 hours of sampling and every 30 minutes for the last three. All the bats were extracted from the nets following standardized protocols so as to minimize the stress and will be kept in a capture bags for 30mins, maximum. This time varied depending on the size of the bat and the sex; pregnant females were immediately measured and released. Bats were weighed, sexed, and then reproductive status, the length of the forearm, feet and leg were measured. Bat species were identified using relevant field guides (e.g. Reid, 2009).

<u>Primates, Felids and Ungulates</u>

Primates and large terrestrial mammals were surveyed along line transects (that were placed without any pre-determined knowledge of the distribution of the animals: Peres, 1999). Primates were surveyed using distance sampling (Buckland et al., 2001) as they are conspicuous, and the species present in the reserve do not shy away from observers. These data were collected by walking the entire length of the transect line in small groups of 4-5 observers walking quietly and slowly (500-1,000 m/hr) starting at 6.30am, when the primates are most active and are easiest to detect. Each time a primate was encountered, the species, whether the animals was seen or heard, number of individuals (visual sightings only), perpendicular distance from the individual to the transect line, habitat, time, distance travelled along the transect line and weather conditions were recorded.

The distance sampling method is only suitable when animals are relatively easy to detect and is therefore unsuitable for monitoring elusive species or species that naturally live at low densities such as jaguar. Thus, felids and unuglates were monitored along transects using Track Encounter Rate (TER) that may subsequently be analysed to compare TER across locations or conditions (Reyna-Hurtado & Tanner, 2005; 2007) or used for occupancy modelling (Mackenzie, 2006). These data were collected at the same time as the primate surveys. For each track encountered the following data were recorded: species, length and width of track, approximate age of track (days) and leaf litter depth.

Data Analyses

Mean relative abundance of birds was calculated based on the sum of calls recorded per point, per transect averaged across transects for each research locations. Mean relative abundance of bats was calculated based on the total number of captures per netting effort averaged across transects for each research locations. Mean relative abundances of herpetofauna and primates were calculated based on the number of individuals encountered per km walked averaged across transects per research location. Track encounter rates (TER) of felids and ungulates were calculated by dividing the total number of tracks/signs of each species per transect by the total distance surveyed. Mean TER for each research location was calculated by averaging TER across the four transects. Habitat preferences and the importance of aguadas for flagship species jaguar, tapir and spider monkeys were made possible by linking each animal record to the nearest habitat plot along the transects resulting in a set of corresponding mean habitat variables and distance to aguada for each animal record.

Statistical analysis was conducted within the R platform (2013) (version 3.4.2), using the MuMIn R package. The two forms of statistical analysis used were model simplification (MS) and multi-model inference (MMI). MS involved starting with a maximal model (all possible terms) and using analysis of variance (ANOVA) to find the minimal adequate model (only significant terms). MMI involved ranking all potential models using Akaike Information Criterion (AIC) values and measuring information loss (delta AIC) between models to determine which model best fit the data (Akaike 1973). The top model set was determined using a threshold of delta AIC less than two (dAIC<2), as the rule of thumb proposed by Burnham & Anderson (2003) states that models with dAIC<2 have "substantial support". MS on GzLMs with Poisson error structures tested jaguar, tapir and spider monkey records associated with each habitat were significantly affected by mean habitat variables of the plot and distance to nearest aguada with water. MMI was then run on GzLMs including to test main effects of the significant habitat factors and distance to nearest aguada with water.

Aguada water levels
Aguada water level analysis investigated the impact of drought on water availability in CBR, by conducting MMI on generalised linear models (GzLMs) with a Poisson error structure, including the interaction effect of year and zone on aguada water levels. This analysis determined whether there was a relationship between year and aguada water levels and whether this relationship varied by zone.

Felid and ungulate abundance and distribution
Species relative abundance analysis investigated the impact of drought on large mammal species' abundances, by running MS on GLMs including the three-way interaction between year, zone, and proportion of aguadas with water in each zone on each species' track encounter rate/km (TER). This analysis provided insight into whether species' distribution changed over time, varied across the reserve, and was driven by water availability.

Results

Aguada water levels

Dry was the modal water level across all aguadas (2014-2019), however, the modal water levels varied over time: 50-75% full in 2014, mud in 2015 and dry from 2016-2019 (Figure 2; *Appendix Table 1*); and varied across the reserve: dry in the Buffer North, Buffer Centre and Core South zones, mud in the Core Centre zone and 50-70% full in the Buffer South zone. Changes in aguada water levels were significantly associated with year, but varied between the zones – a negative relationship was found between water level and year in the Buffer north, Buffer centre zone, and Core south zone, whereas a weak positive relationship was found in the Core centre zone and a stronger positive relationship was found in the Buffer South zone (GzLM MMI: dAIC<2 threshold, ±95% confidence intervals do not overlap zero; Table 1).

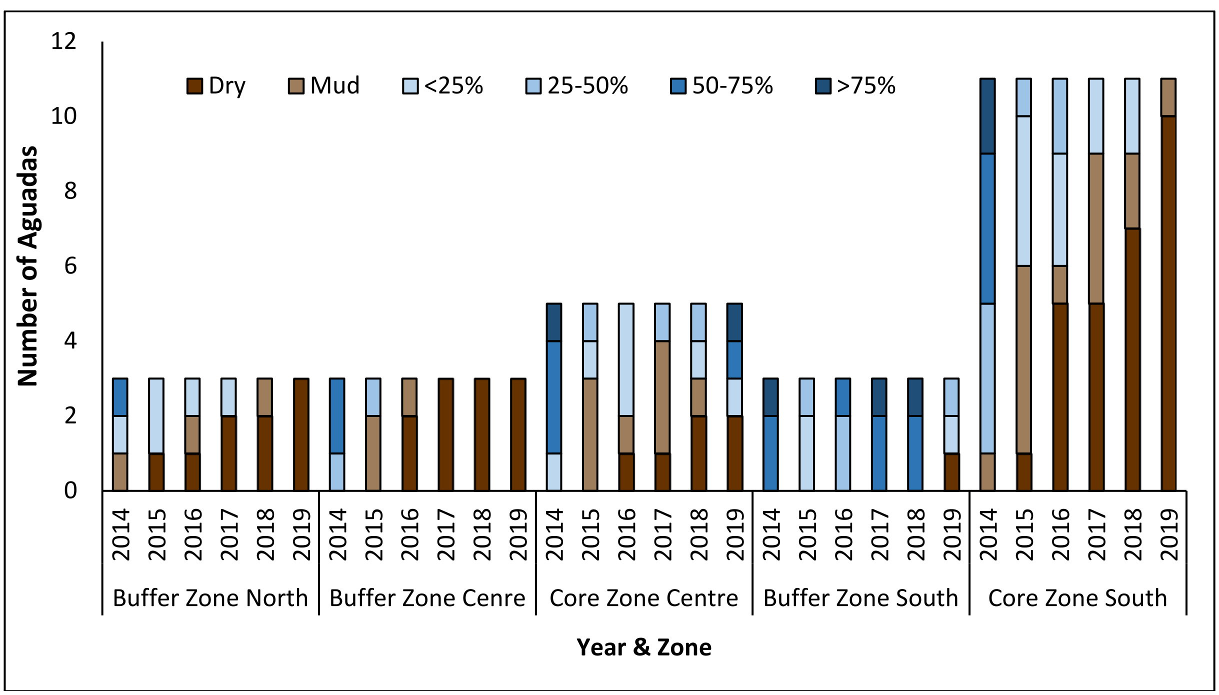
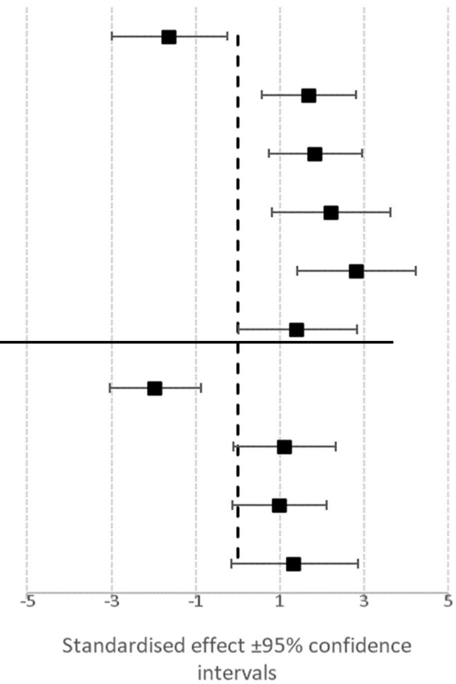


Figure 1. Categorical aguada water levels in CBR, split by year and zone. The bars show the proportion of aguadas in each water level category – Dry= dark brown, Mud = light brown, <25% full = light blue, 25%-50% = mid blue, 50%-75% = dark blue, >75% = black.

Table 1. Output from Multi-model Inference of models including the interaction between year and zone on aguada water levels. Plot shows averaged standardised effect sizes from the top model set (dAIC<2), error bars indicate ±95% confidence intervals – significant variables above the line. Relative importance (RI) is the proportion of models in the top set that include each variable. Whether the ±95% confidence intervals span zero indicates the importance of the factor.

Aguada water level variables	Effect Size	Relative Importance
Buffer Centre zone (Intercept)	**-1.62**	1
Interaction between year and Core Centre zone	**1.70**	1
Interaction between year and Buffer South zone	**1.85**	1
Core Centre zone	**2.23**	1
Buffer South zone	**2.83**	1
Core South zone	**1.43**	1
Year	**-1.95**	1
Interaction between year and Buffer North zone	1.12	1
Interaction between year and Core South zone	1.00	1
Buffer North zone	1.35	1



Changes to mean relative abundance and species richness of fauna 2014-2019

Mean relative abundance of birds showed a slight decline over time (Figure 2a) but bird species richness has remained constant (Figure 2b). Mean relative abundance of bats has fluctuated over time what appeared to be a declining population followed by a notable increase in 2018 which coincides with increased rainfall and fruit production (Figure 3a). Bat species richness, however, has steadily declined over time (Figure 3b). Herpetofauna abundance, particularly amphibians as declined significantly during drought with slight increases in 2016 that concided with a localized storm in KM20 and in 2018 that coincide with increased rainfall throughout the reserve (Figure 4a). As with bats, herpetofauna species richness has steadily declined over time (Figure 4b). Primate abundance appears to be relatively unaffected by drought with a constant mean relative abundance of howler monkeys and a fluctuating, but otherwise relatively stable population of spider monkeys (Figure 5). The three felid species in CBR that are heavy enough to reliably leave tracks on transects (ocelot, jaguar and puma), all show a slightly declining population (Figure 6). Mean relative abundance of ungulates has fluctuated over time and does not show clear population trends (Figure 7)

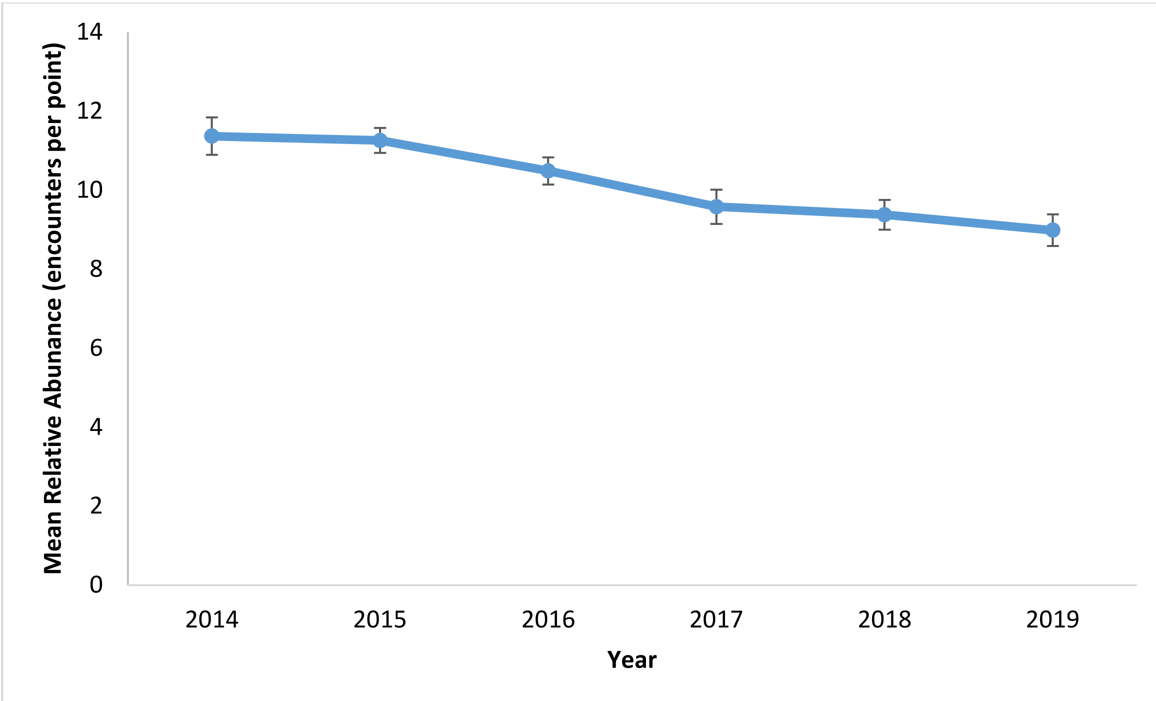


Figure 2a: Mean relative abundance of birds from point counts 2014-2019

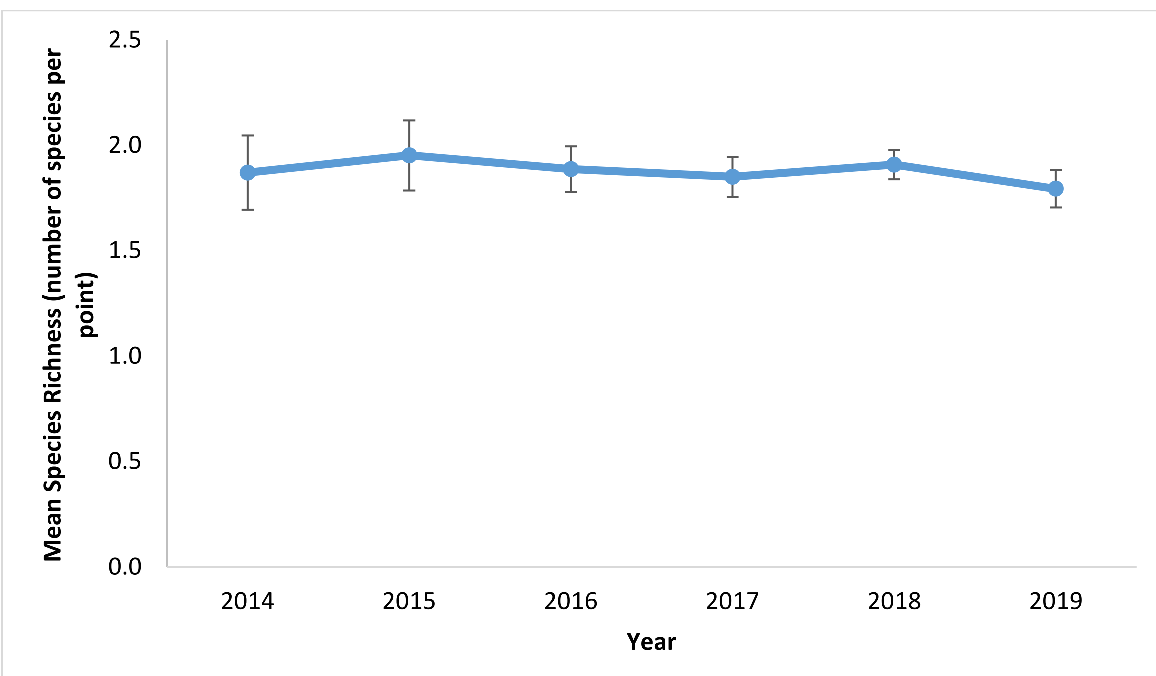


Figure 2b: Mean species richness of birds from point counts 2014-2019

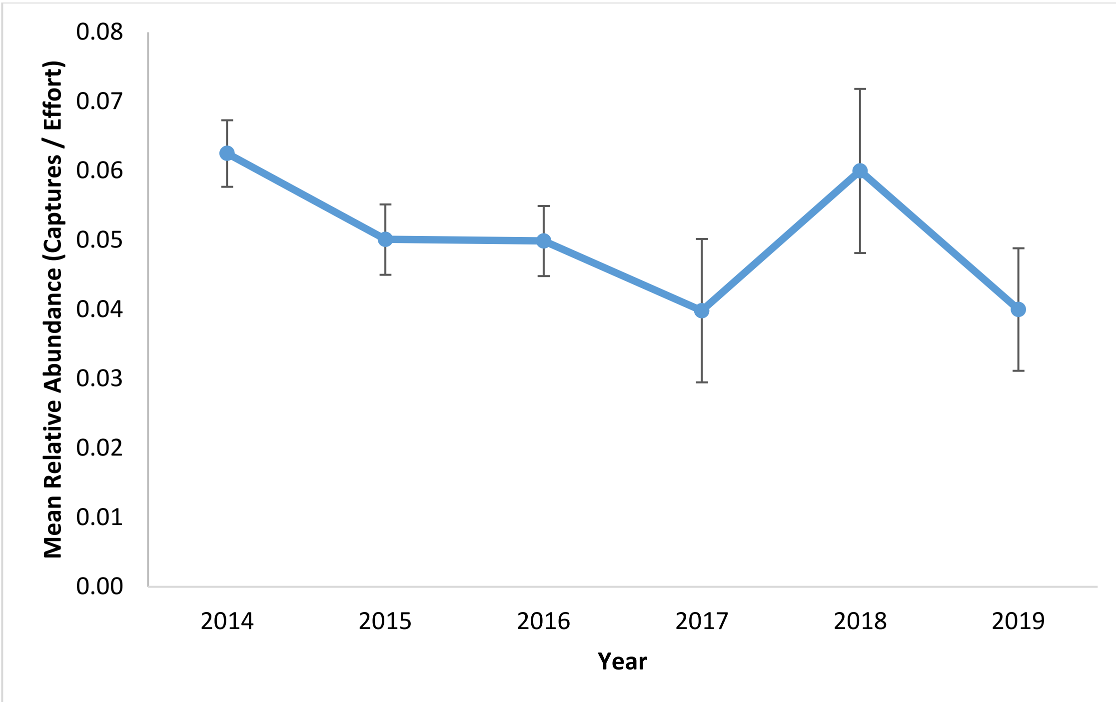


Figure 3a: Mean relative abundance of bats from mist net surveys 2014-2019

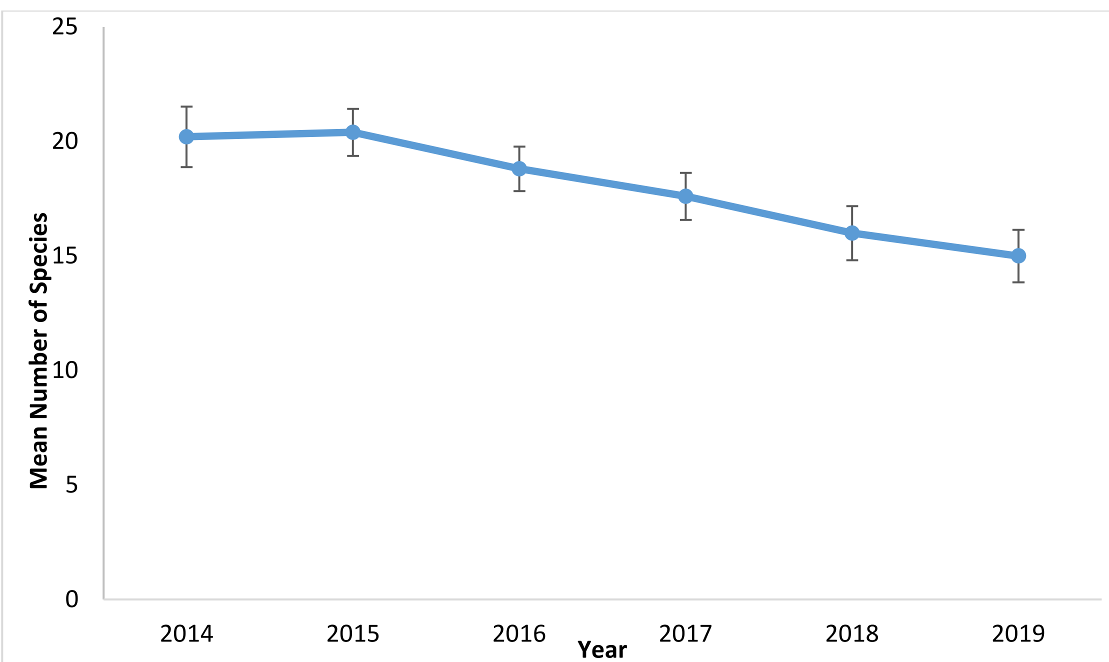


Figure 3b: Mean species richness of bats from mist net surveys 2014-2019

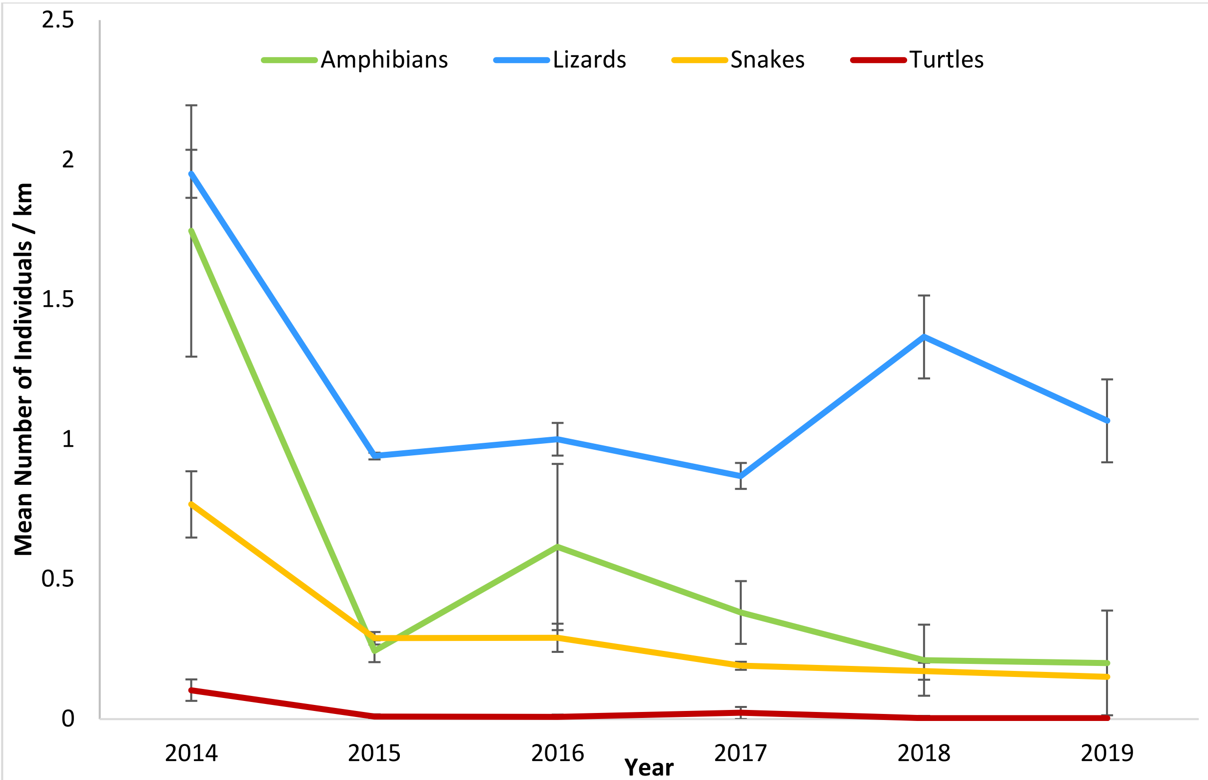


Figure 4a: Mean relative abundance of herpetofauna from transects surveys 2014-2019

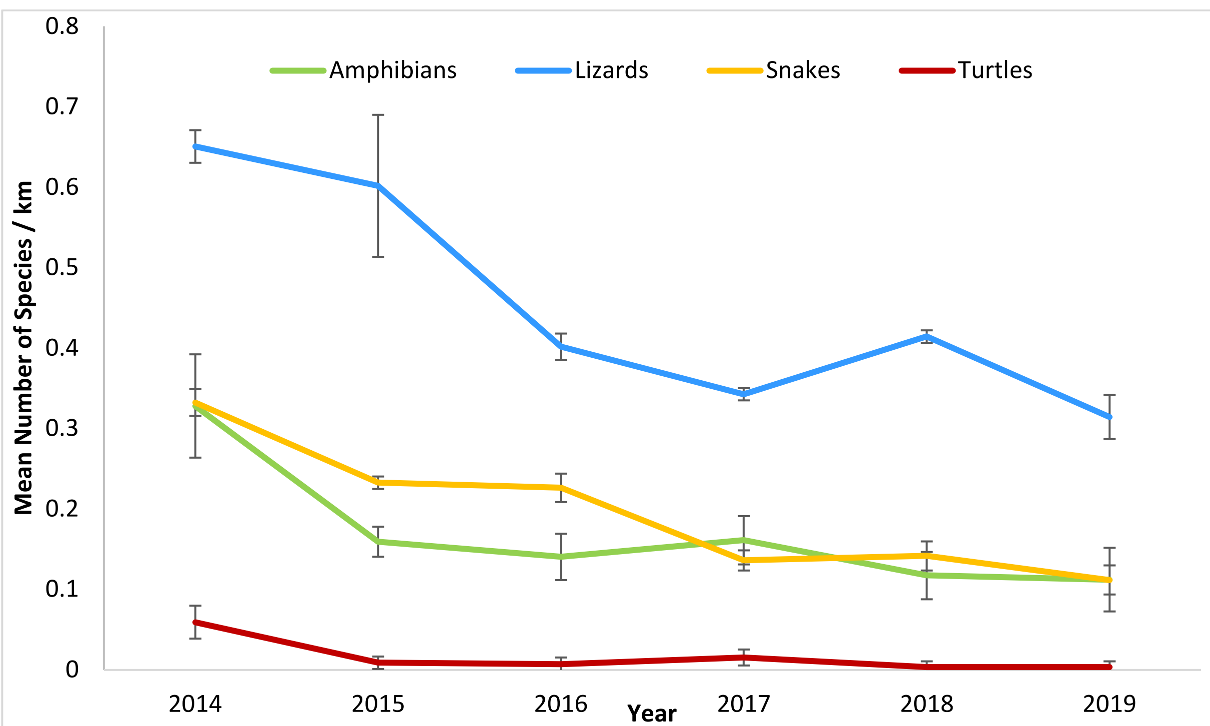


Figure 4b: Mean species richness of herpetofauna from transects surveys 2014-2019

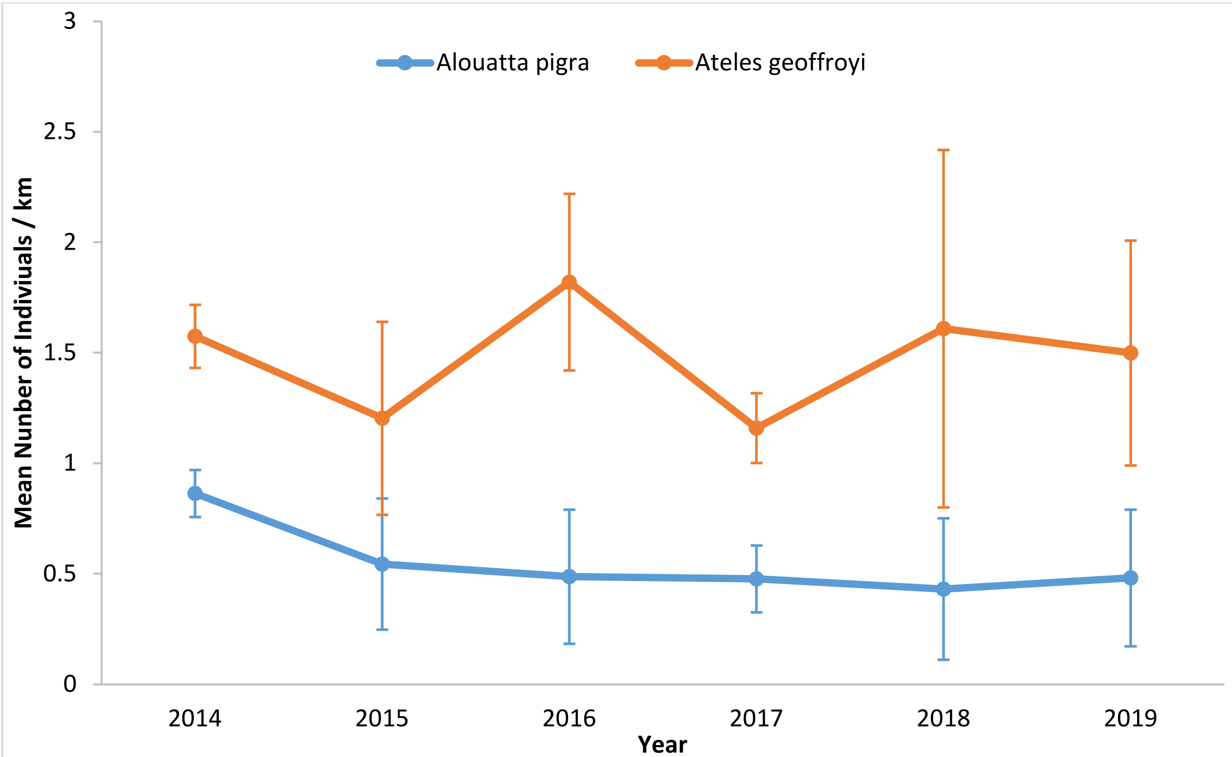


Figure 5: Mean relative abundance of primates from transects surveys 2014-2019

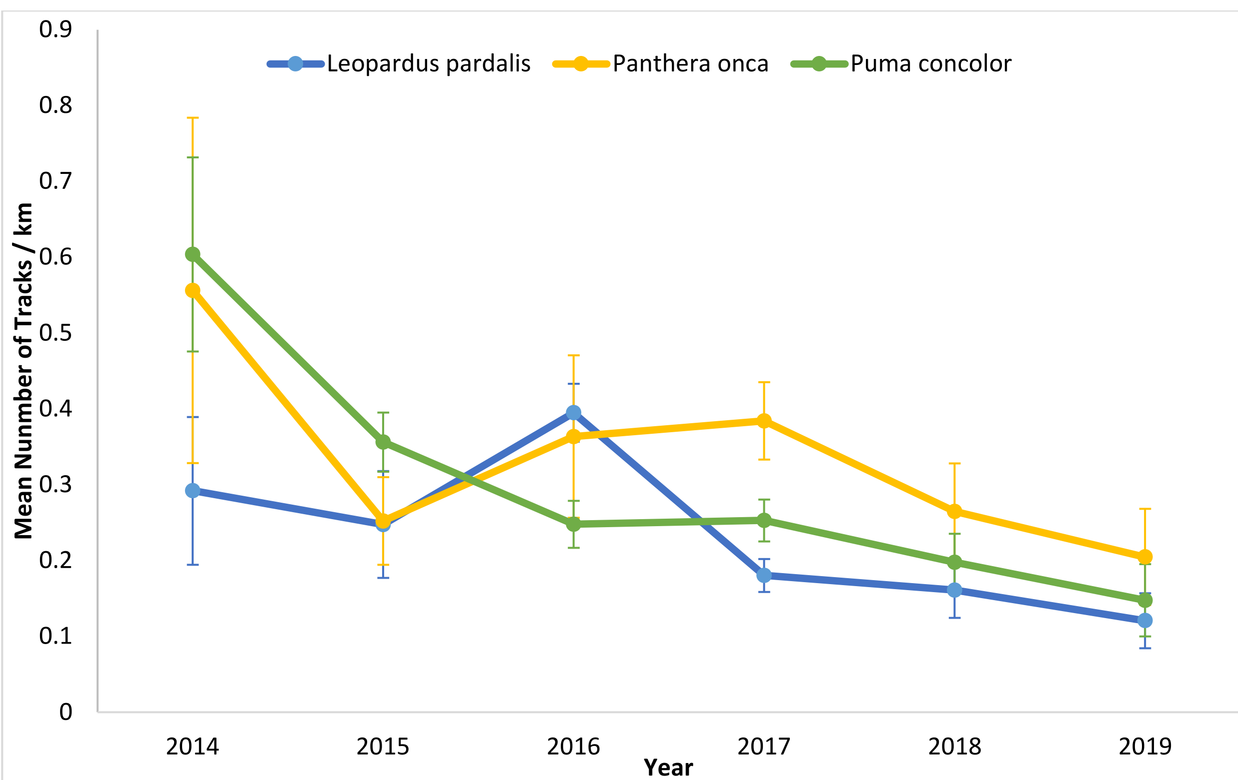


Figure 6: Mean relative abundance of felids from transects surveys 2014-2019

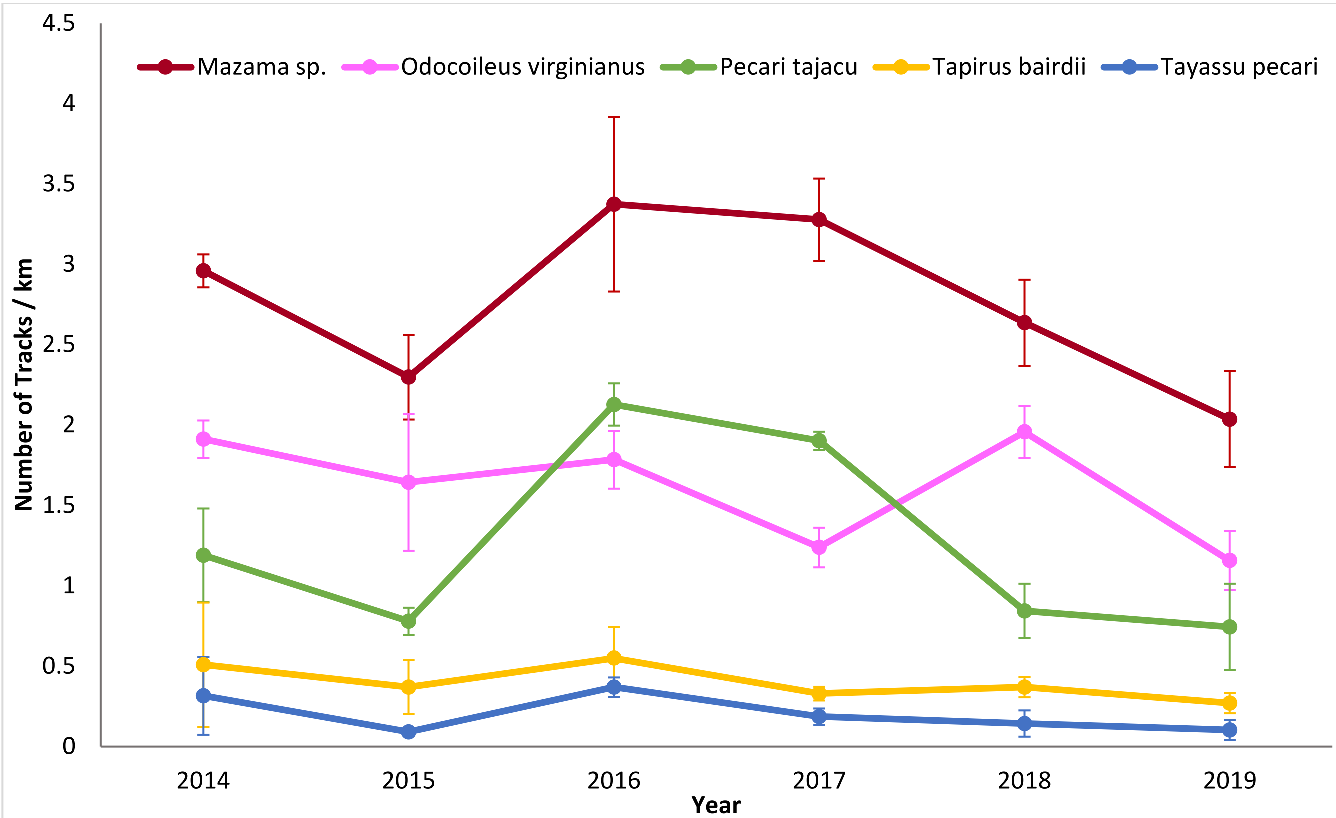


Figure 7: Mean relative abundance of ungulates from transects surveys 2014-2019

Herpetofauna diversity and aguadas

In total, 66 aguada surveys for herpetofauna were completed across 10 different aguadas in addition to monitoring of aguada water levels. Of these, 6 aguadas were classified as dry, and 4 were classified as wet. 7 aguadas were considered to have thick understorey vegetation, and 3 were considered to have no significant understorey vegetation. A total of 329 animals were counted, including 43 different species across 6 orders, out of a total of 89 species known to inhabit the survey area (Colston et al., 2015). In total, there were 26 species specific to wet aguadas, including 3 entire orders (Crocodylia, Testudines and Urodela), and 3 species specific to dry aguada. Additionally, there were 14 species specific to vegetated aguadas, and 10 species specific to aguadas without vegetation, including Bolitoglossa mexicana, the only discovered species of Urodela in the survey.

There was a significant association between herpetofauna density and water presence ($t = -5.7094$, $df = 3.1105$, p-value = 0.009652). This is supported by Figure 8, which shows a much larger abundance of herpetofauna at the water-filled aguadas than at those that were dry. At the order level, the only group for which water was a significant predictor of abundance was Anura ($t = -8.0635$, $df = 4.1252$, p-value = 0.001127). In the case of Crocodylia, Testudines and Urodela specimens were only discovered at wet aguadas. There were no significant differences in mean relative abundance of Serpentes ($t = -0.94262$, $df = 5.0374$, p-value = 0.3889) and Sauria ($t = -0.22203$, $df = 5.9626$, p-value = 0.8317) across wet and dry aguadas.

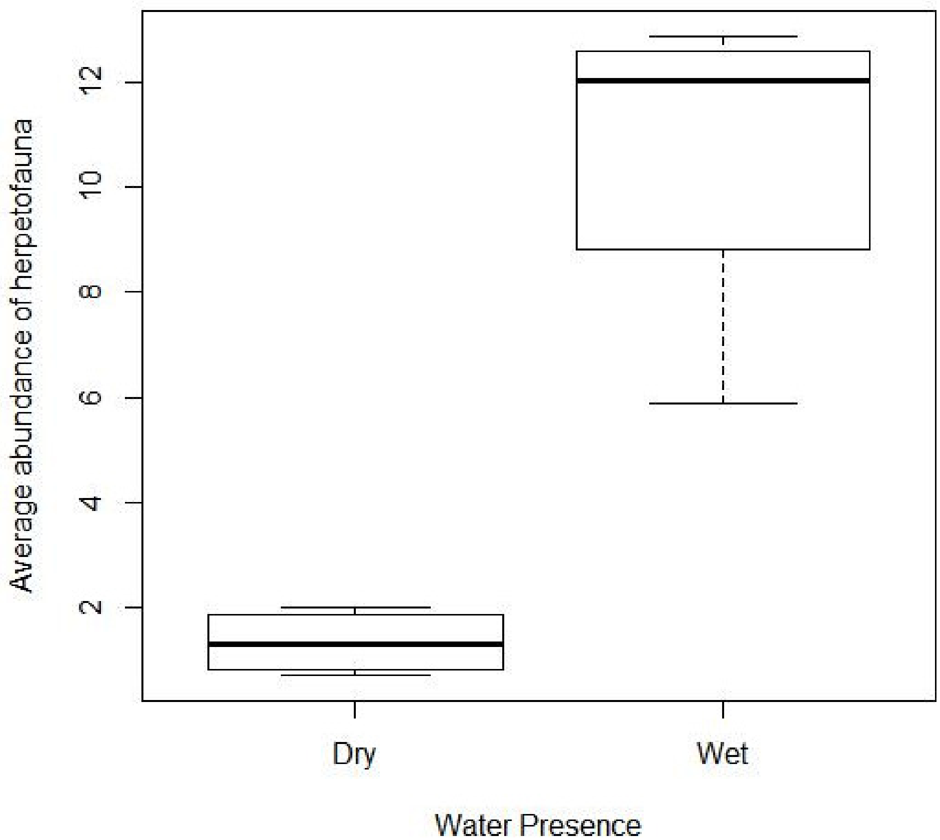


Figure 8. Box plot showing the mean relative abundance of herpetofauna within dry and wet aguadas.

The biodiversity of herpetofauna in aguadas was calculated using Simpson's diversity index:

$$D = 1 - \frac{\Sigma n(n-1)}{N(N-1)}$$

In which D is the diversity, n is the number of individuals of a particular species and N is the total number of individuals of all species. Herpetofauna biodiversity was associated with the presence of water within an aguada ($t = -4.1557$, $df = 7.976$, p-value $= 0.003205$). This suggests that the presence of water is an important factor in maintaining herpetofauna biodiversity, even though water was not found to be a significant predictor of abundance for all herptile groups.

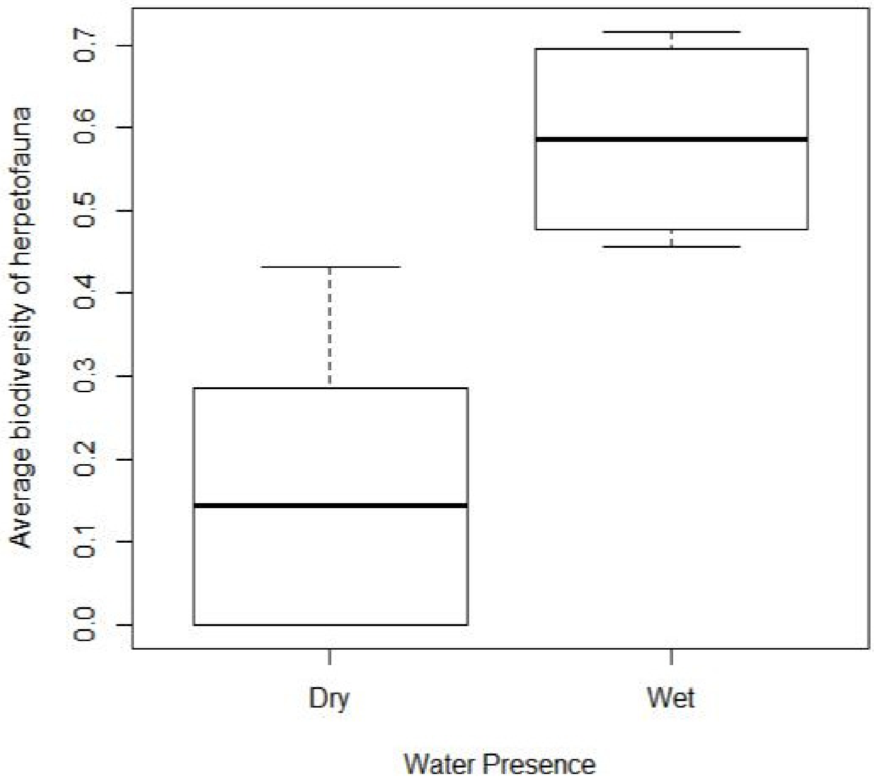


Figure 9. Boxplot showing the relative biodiversity of herpetofauna encountered within wet and dry aguadas.

Felid and ungulate distribution patterns

The overall relative abundances (RA) of ungulate species were found to be greater than felid species (Figure 3) – average TER: Brocket deer was 2.71/km, white-tailed deer 1.62/km, collared peccary 1.31/km, Baird's tapir 0.43/km and White-lipped peccary 0.21/km; whereas average TER of jaguar was 0.35/km, ocelot 0.25/km and puma 0.31/km. Additionally, total species' average TER varied over time, but decreased overall – 2014 was 1.04/km, 2015 0.75/km, 2016 1.14/km, 2017 0.97/km, 2018 0.82/km and 2019 0.67/km. Finally, total species' average TER varied by zone – lowest in the Buffer North (0.51/km) and Buffer Centre (0.57/km), higher in the Core Centre (0.96/km) and Buffer South (1.05/km) and highest in Core South zone (1.41/km).

Zone significantly affected the RA of several ungulate species: higher TERs of white-tailed deer, collared peccary and Baird's tapir were recorded in the Core South zones (p-values<0.05, Fig.10c, e & g). Brocket deer TER was higher in the Core Central and Southern zones than in the North (p-value<0.01, Fig.10b). Aguada water availability was found to positively correlate with white-lipped peccary TER (p-value<0.01, Fig.10h). Felid RA was significantly affected by water and zone: Ocelot TER was positively correlated with water availability and significantly higher in Core South (p-value<0.01, Fig.10a); jaguar TER was positively correlated with aguada water availability in the Core South (p-value<0.01, Fig. 10d); puma RA was affected by zone, water and year: TER was highest in the Core South zone, where year and water availability were negatively correlated, puma TER positively correlated with water availability and decreased over time in this zone (p-value<0.01, Fig.10f).

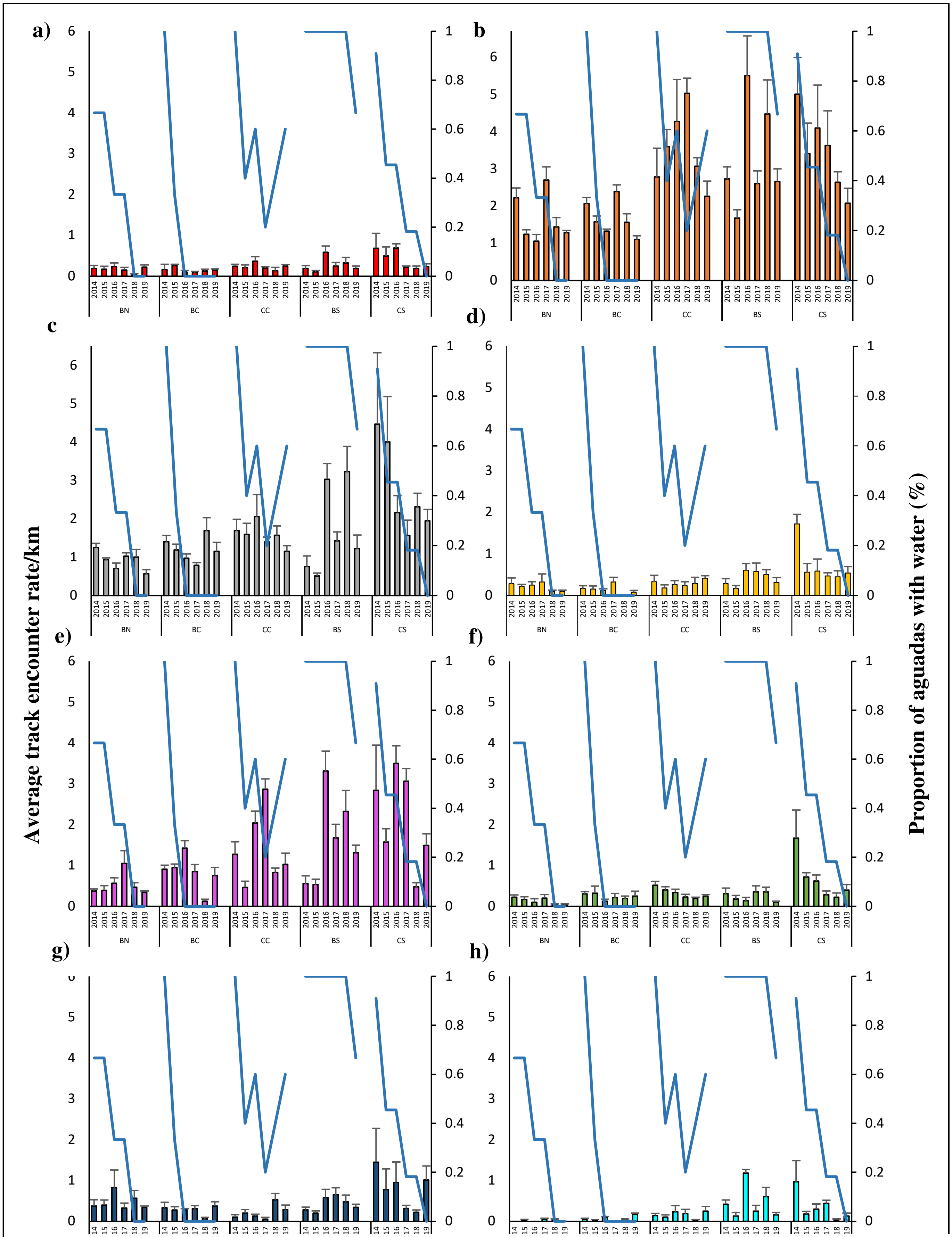


***Figure 10**. The effect year, zone and water availability on species track encounter rates/km (TER) – a) ocelot, b) Brocket deer spp. c) white-tailed deer, d) jaguar, e) collared peccary, f) puma, g) Baird's tapir, h) white-lipped peccary. The bars represent species' TERs, error bars are +1SE. The blue lines show the proportion of aguadas with water over time, split by zone.*

Habitat predictors of flagship species

Initial exploratory analysis found that jaguar track count negatively correlated with distance to nearest aguada with water (GzLMER: $X2(1)=46.88$, p-value<0.01) as did tapir track count (GzLMER: $X2(1)=57.04$, p-value<0.001) and spider monkey encounter rate (GzLMER: $X2(1)=16.23$, p-value<0.01). MS indicated that the model that best represented jaguar track count data included distance to aguada and IVI. GzLMER including IVI and distance to aguada showed that only distance to aguada is a significant predictor of jaguar track count ($p < 0.001$) with IVI having no significant effect ($p = 0.163$). MS indicated that best model to explain tapir track count only included distance to aguada with subsequent GzLMER showing that distance to aguada is a significant predictor of tapir track count ($p < 0.001$). MS for spider monkeys indicated that best model included distance to aguada, tree species richness, mean tree DBH and number of large trees (DBH>30cm). GzLMER including these variables showed that all variables were significant predictors of spider monkey abundance (all variables $p<0.001$).

Discussion

This study provides strong evidence that drought is severely and detrimentally impacting CBR's water sources, habitats, and species, by limiting water availability, altering abundance and diversity of fauna populations, and triggering the movement of felids and ungulates southwards towards more humid environments with greater water availability. Drought severely reduced the amount of water held by CBR's aguadas, but impacts varied by zone, supporting the prediction that worsening drought conditions would significantly reduce water availability, but that the severity would vary across CBR. Worst affected were the Buffer North, Buffer Centre and Core South zones which experienced similarly severe declines in water availability over the study period.

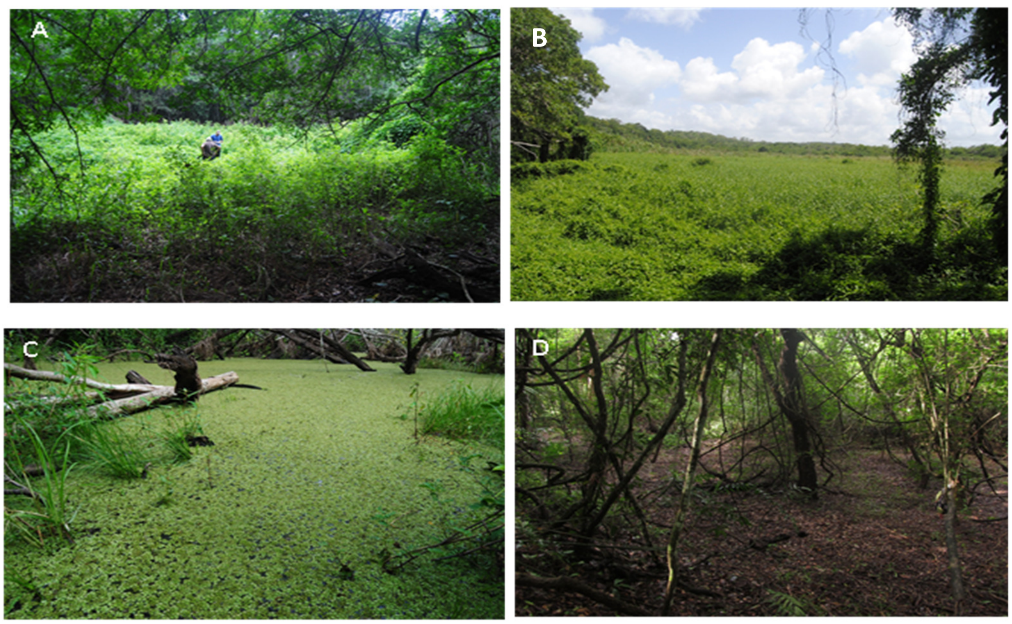
Of all the taxanomic groups monitored, birds appeared to be the least effected by disappearing aguadas with only a slight decline in abundance detected in latter years of monitoring and no decline in species richness. Bat abundance was similarly unaffected, but bat species richness did show a decline over time. Herpetofauna, especially amphibians showed a dramatic decline in abundance and steady decline in species richness since the onset of drought in 2015 (following the absence of heavy rains in September and October of 2014). Primate abundance fluctuated over time, but did not show indication of a declining population, which is most likely because although heavy rains required to fill aguadas did not happen during the drought periods, localized rainfall did periodically occur enabling fruit production at times when aguadas were dry. Jaguar, puma and ocelot relative abundance showed a declining population, whereas ungulate relative abundance has fluctuated considerably over time and shows no clear pattern.

Changes to mean TER of felids and ungulates across locations in CBR and over time were significantly affected by changes to water distribution which directly compliments earlier studies of ungulates and aguadas in Calakmul (Reyna-Hurtado et al., 2020). Analyses of habitat preferences of flagship species indicated that the only important predictor for jaguar and tapir was distance to aguada, which was also a significant predictor of spider monkeys in conjuction with other habitat variables. The association between aguada water levels and tapir population dynamics coincide with long-term camera trap studies of tapir at aguadas in Calakmul (Reyna-Hurtado et al., 2019) and the declining jaguar population in Calakmul reported here is also most likely due to the disappearance of aguadas.

The extreme drought conditions experienced by CBR from 2014 to 2017 prevented the aguadas from refilling with water despite precipitation levels increasing in some areas of the reserve in 2016 and the majority of the reserve in 2018. The growth of vegetation within these dry aguadas may be breaking down the impermeable membranes, preventing the aguadas from collecting future water. This zone's unexpected drought impacts highlight the need for intervention and aguada conservation efforts across the entire reserve, not only within the drier regions, to protect the fauna from detrimental drought impacts.

Subsequent Aguada Restoration Work

After several years of drought by 2019, many aguadas were overgrown with secondary vegetation that should not be in an aguada (Images A and B). In addition, the soil had completely dried up and has lost layer of putrifying litter that prevents water seepage. In these conditions, even though it rains, the aguadas have lost the ability to fill because the vegetation sucks all the water and continues to grow and areas without vegetation cannot keep the water because it filters through the fine soil and limestone karst substrate. Aguadas that only have aquatic vegetation, or that still have mud, do not require rehabilitation (Images C and D).



To continue conserving the fauna of the RBC, it is necessary to ensure that fauna has access to water. After several years of drought, many aguadas have secondary vegetation that should not be in an aguada. However, areas of aquatic and semi aquatic vegetation along the aguadas are very important as habitat for herpetofauna (reptiles and amphibians) and as food for various species of ungulates (e.g. tapir). When ungulates such as tapir and deer visit aguadas to feed on vegetation they trample vegetation and leaf litter into the mud which forms the protective layer that prevents filtration of water. Therefore, it is always necessary to maintain some areas of vegetation in and around aguada in conjunction with "beaches" of mud and trampled leaf litter ready to fill with water when the rains begin.

In the summer of 2019 OW and CONANP, trialled methods of aguada rehabilitation in aguadas that have spent more than 4 years without filling with water. Three manipulation methods were used. Secondary vegetation was cut with machetes and extracted from the aguada to create clean areas in conjunction with islands of natural aquatic and semi aquatic vegetation. Where the ground was completely dry, water trucks from the municipality were used to add water to the “beaches” and create mud. Sacks of leaf litter collected in the general area of the aguada were then poured on the “beaches” and trampled into the mud by teams of OW students. In the areas around the aguadas, canals were created to ensure that rainfall in the general area would flow into aguadas. Four aguadas received all 3 restoration methods. An additional 4 aguadas received either 1 or 2 of the restoration methods, but not all. Control aguadas (that received no manipulation) adjacent to each manipulated aguada were also included in the study.

Following the rains in September and October of 2019, all aguadas that received the full three methods of manipulation filled to the top (see images E and F). Control and aguadas and aguadas that only received a subset of the manipulation methods did not fill and remained full of secondary vegetation. Collectively these preliminary results indicate that aguadas can be restored without the need to introduce man-made substances such as pond liner, but that clearing of secondary vegetation, trampling of leaf litter into mud and creation of canals to ensure water flow into aguadas are all necessary elements of restoration.

Conclusion

Results of the Operation Wallacea monitoring project in CBR have shown a significant decline in availability of water in aguadas that does not necessarily correlate with rainfall once aguadas have been dry for long periods and have consequently filled with secondary vegetation. Loss of aguadas has resulted in declines in abundance and diversity of fauna with the worst affected being herpetofauna and felids. Felid and ungulate distribution patterns were found to change in response to disappearing aguadas with what appears to be migration to the southern parts of the reserve where the climate is more humid and more aguadas have retained water. Many felids and ungulates may have travelled further into Guatemala, but without corresponding monitoring data in adjacent southern reserves it is not possible to confirm this. In response to disappearing aguadas and the corresponding impact on fauna, aguada restoration methods were trialled during 2019. Results of these initial trials were promising as all fully manipulated aguadas filled to the top during the rains in September and October of 2019 whereas control aguadas and partially manipulated aguadas did not. Collectively the results of this study indicate the upmost importance of aguadas for the conservation of fauna in CBR and the need for mitigation methods of conservation to ensure a continued water supply for fauna in the reserve.

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