HABITAT CHARACTERIZATION FOR THE PUERTO RICAN CRESTED TOAD (*Peltophryne [Bufo] lemur*) AT GUÁNICA STATE FOREST, PUERTO RICO

By

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Abstract

The endemic Puerto Rican Crested Toad (*Peltophryne [Bufo] lemur*) is endangered with extinction with only one remaining population located at Guánica State Forest. Habitat associated with toad observations was characterized in terms of vegetation type, vegetation composition, surface characteristics, average temperature and relative humidity as well as water characteristics at breeding ponds. Geographic Information System (GIS) information was collected to make predictive models of toad distribution. Fifty three percent (53%) of observed toads were found in the "rocks & cacti" vegetation association, mostly composed of xerophytic arboreus species and a bare rock surface. Twenty two percent (22%) of observations were related to evergreen association mainly composed of evergreen arboreus species with a leaf litter covered surface. GIS models predict a higher probability of toad observations at the "rocks & cacti" and "evergreen" vegetation near breeding ponds. Breeding ponds showed significant differences in terms of pH and salinity. The Puerto Rican Crested Toad (*P. lemur*) seems to exploit different habitats at the Guánica State Forest.

Resumen

El endémico Sapo Concho Puertorriqueño (*Peltophryne [Bufo] lemur*) está amenazado de extinción, restando sólo una población localizada en el Bosque Estatal de Guánica. Se caracterizó su hábitat en términos de tipo y composición de vegetación, características de superficie, temperatura y humedad relativa promedio, además de características acuáticas en las charcas de reproducción. Se utilizó un Sistema de Información Geográfica (SIG) para crear un modelo predictivo de distribución de individuos. Cincuenta y tres porciento (53%) de las observaciones de sapos ocurrió en la asociación vegetal “rocas y cactos”, compuesta mayormente por vegetación arbórea xerofítica y una superficie de roca expuesta. Veintidós porciento (22%) de observaciones ocurrió en la asociación “siempre verde”, compuesta mayormente por especies arbóreas siempre verdes con una superficie cubierta de hojarasca. El modelo creado mediante SIG predice una mayor probabilidad de observación de sapos en las vegetaciones de “rocas & cactos” y en la “siempre verde” cerca de las charcas de reproducción. Las charcas de reproducción muestran diferencias significativas en términos de pH y salinidad. Al parecer el Sapo Concho Puertorriqueño (*P. lemur*) puede utilizar diferentes tipos de hábitat dentro del Bosque Estatal de Guánica.

Acknowledgments

I want to thank Bob Johnson, Curator of Amphibians and Reptiles at the Metropolitan Toronto Zoo and AZA Puerto Rican Crested Toad SSP Coordinator, His support and confidence made possible the logistics of this project. His efforts in the conservation of *P. lemur* and its habitat have given the chance to establish new populations and give hopes to the recovery of the species.

Thanks to Mr. Miguel (Menqui) Canals, Guánica State Forest Manager Official, who keep a guarding eye and manages the conservation efforts at the remaining natural habitat of the species. His field knowledge and experience was invaluable for selecting study sites and finding *P. lemur* individuals.

Many thanks to graduate student Jan-Paul Zegarra, his assistance in the field proved invaluable for data collection. Also thanks to graduate student Gail Ross whose motivation for the conservation of *P. lemur* is an example for all.

I'm very grateful to professors Allen Lewis PhD, Angel Berríos PhD and specially Fernando Bird-Picó PhD, who gave me support and motivation to finish this endeavor.

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Introduction

Although amphibians represent the oldest group of terrestrial tetrapods (Pough et. al., 2001), they are actually among the most vulnerable vertebrates on earth. Since the end of the 1980's scientists have observed a worldwide decline of amphibian populations. Two examples of these include the Golden Toad (*Bufo pereglinensis*) from Costa Rica, extinct since 1989, and the Gastric-Brooding Frog (*Rheobatrachus silus*) from Australia, which disappeared in 1981. These cases have led to hypothesis of a worldwide crisis affecting amphibians. Mattoon (2000) summarizes the factors that could account for amphibian declines and refers the phenomenon as the "Monteverde Syndrome". These factors include: water pollution, which is associated with damage and malformation of eggs, tadpoles and adults; UV radiation, which affects the organisms at the molecular level; and climatic changes, which interfere with the reproductive cycles and water available to the organisms. In addition, infectious diseases unique to amphibian populations have been found. Chytrid fungi, saprogenic bacteria and iridoviruses are some of the most currently studied. Finally, the most widespread factor affecting amphibians includes habitat loss and destruction. Habitat modifications such as deforestation, wetlands drainage and human development (highways, dams, urban sprawl and expansion of agricultural lands) may be the direct cause of death for organisms that cannot cope with such changes.

In Puerto Rico habitat loss has been associated with the decline of several amphibian species. One of those species is the Puerto Rican Crested Toad (*Peltophryne* [*Bufo*] *lemur,* Cope 1868), the only toad endemic to Puerto Rico.

The Puerto Rican Crested Toad is a rare and cryptic amphibian for which little scientific research has been carried out. It was first described by Cope in 1868 as *Peltophryne lemur*, later its name was changed to *Peltophryne gutturosus* in 1876 and changed again in 1881 to *Bufo gutturosus*. It was named *Bufo lemur* by Stejneger on 1904. On 1981 Pregill brought back the genus *Peltophryne* based on synapomorphies of the skull, maxillary arch and rostrum shared by the endemic toads of the West Indies previously described as *Bufo*. Actually the genus *Peltophryne* (Anura: Bufonidae) could be used to describe 10 species of West Indian toads (also called the *Bufo peltocephalus* group) from the Virgin Islands, Cuba, Hispaniola and Puerto Rico (Pregill, 1981). Although monophyly of the group has been questioned (Graybeal & Cannatella, 1995), recent work based on osteological, morphological and DNA data has provided evidence on the relationship of West Indian toads inside the *Bufo peltocephalus* group (Pramuk, 2002). At present the issue of *Peltophryne* as synonymous to *Bufo* has not been fully clarified.

Individuals of *P. lemur* are mid-sized (64-120 mm, SVL); characterized by supraorbital crests and a long upturned snout. Their dorsal color is blackish with white or yellow marbling and a yellowish abdomen. Sexual dimorphism has been described for the species (Rivero et al., 1980) where females attain a larger size (120mm), have prominent cephalic crests and lack nuptial pads on the first fingers; males tend to be more yellowish in color and rarely attain a size larger than 85mm.

This species has historically been rare and reported only from the Virgin Islands and few locations in Puerto Rico (Figure 1). From 1868 to 1931 only 28 toads were collected by herpetologists. Since 1931 the species was considered extinct until 6 specimens were collected at Isabela in 1966 (García-Díaz, 1967). Today, the only reproductively active population known since 1984 (Moreno, 1985) is found at the Guánica State Forest, in the southwestern part of the Island. No toads have been seen in other historically known areas since 1991(Johnson, pers. comm.).

In Guánica, this species lives at low elevation, semi-arid area, with rocky outcrops of limestone that provide shelter for the species. Because of the xeric conditions of the area, this species only breeds when seasonal rains form temporary ponds. Population decline and habitat loss are the major factors that have led this species to be listed as threatened on 1987 (FWS, 1992).

Actions that can be implemented for the recovery of this species include the identification of habitat and microhabitat preferences. This information can be used to make better decisions regarding which habitats need to be preserved as well to identify suitable areas for reintroduction. The purpose of this project was to measure the characteristics of the habitat presently used by the Puerto Rican Crested Toad. Management and recovery programs could be improved with this information.

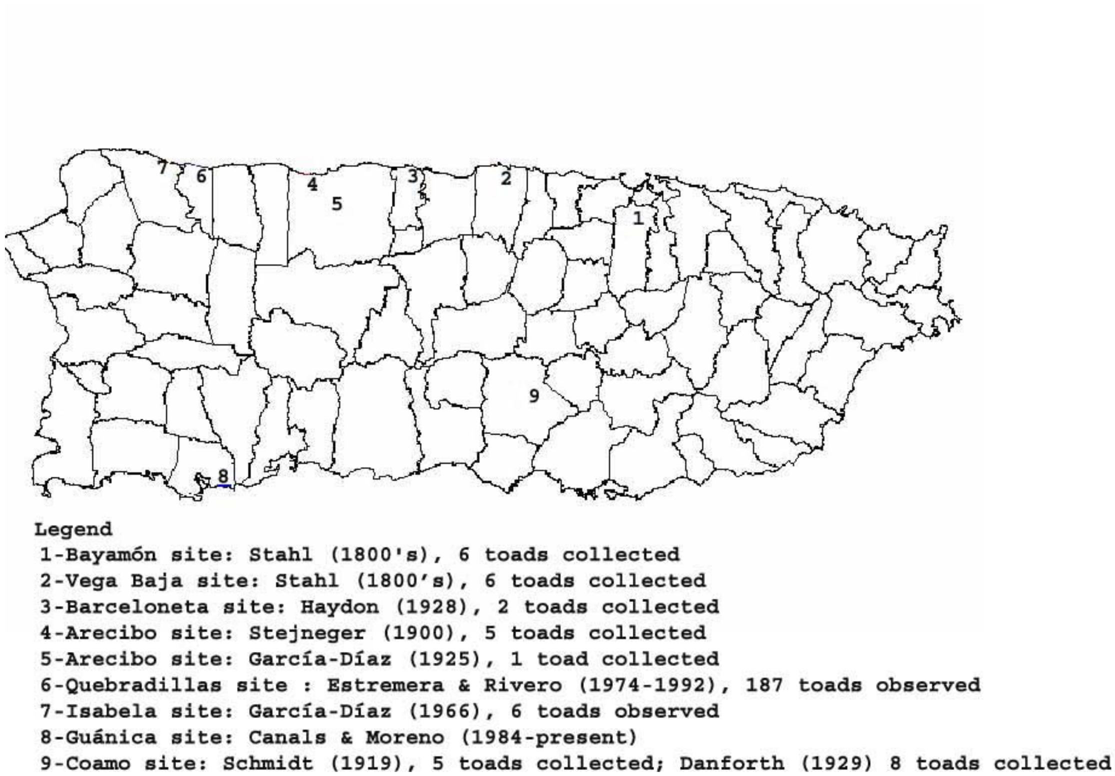

Figure 1. Location of *Peltophryne [Bufo] lemur* historic sightings*

* As in García-Díaz (1967), Grant (1932), Schmidt (1928) and Stejneger (1904)

Materials and Methods

The Guánica State Forest is classified as a Subtropical Dry Forest (Ewel and Whitmore, 1973) located in the southwest of Puerto Rico (17°57'N, 66°50' W). The forest comprises two sections that add up to an area of approximately 4000 ha with an elevation from sea level to approximately 228 m. Mean annual temperature is about 25°C with a high temperature of 27°C between August and October and a low temperature of 24°C in January. Average rainfall ranges between 791 mm to 861 mm with a dry season between January to March and a wet season between August and November. Annual evaporation is about 2074 mm with evapo-transpiration of 722 mm and a mean solar radiation of 4366 kcal/m^2/day (Lugo et al., 1978). The soil is classified as stony, shallow and dry Class VII by the USDA Soil Conservation Service (Carter, 1965) and composed mostly of tertiary limestone deposits of the Juana Diaz and Ponce Formations deposited through the Oligocene and Miocene (Conde-Costas and González, 1996).

Habitat characterization was defined as: Breeding and Non breeding habitat. Breeding habitat consists of the temporary ponds in which toads attend to courtship, deposit and fertilize their eggs and where the larvae complete metamorphosis. Non breeding habitat consists of all other areas in which toads look shelter and forage. To identify non breeding habitat night searches were conducted at the Guánica State Forest to look for *Peltophryne* activity. Searches were conducted twice a month during the dry seasons and after any raining event during the wet season. All searches for toad activity began at the breeding pond margins and extended along forest trails up to 1.5 km into the forest. Two investigators walked up the trail looking for activity at each side within 10 m from the center. All searches were made with equal intensity as conditions permitted. Any toads observed were captured and basic measurements were taken (snout-to-vent length/minimum head width) (Fellers et al., 1994). Each observation site was marked and identified with a global positioning system (GPS) (Hayek and McDiarmid, 1994). At each site a 10 m by 10 m quadrat was established. Twenty five 1 m x 1 m samples were taken at each quadrat. A description of vegetation type, composition and frequency, was recorded for each 1m x 1m sample. This description also included surface composition, canopy cover and horizontal obscurity at each sample. To evaluate toad habitat selection plots were paired to other equally sized quadrat placed randomly at 20 m from the first.

The identification of the vegetation association type was carried out according to Dugger (1979), who describes nine vegetation associations for the Guánica Forest based on species density and composition. Vegetation association types were scored as rocks and cacti, scrubland, caducifoleous (semi-evergreen) forest and evergreen forest.

To estimate the vegetation composition at each sample, only plants with stems above knee height (AKH) were considered. The proportion of all AKH plants was calculated and classified as arboreus, cacti, weedy, succulent or other. The frequency of all trees AKH was calculated by species. Additionally plants with stems above head height (AHH) were considered to describe the canopy cover at each sample site. Canopy cover was estimated using an ocular tube by which a proportion of the tree crown projection onto the ground surface was measured. The ocular tube consisted of a 15cm long by 3.75cm in diameter PVC pipe with a crosshair.

Surface composition was described as a proportion of leaf litter, rocks, vegetation, crevices, and logs. Horizontal obscurity (the visual obstruction caused by vegetation along the horizontal plane) was estimated using a Nudds table (Higgins et al., 1996) by which visual obstruction of the vegetation was assessed at 0.5m intervals above the ground.

A geographic information system (GIS) was used to create a predicting model of *P. lemur* distribution probabilities throughout its habitat. Three habitat variables were correlated to observation sites using a logistic regression model. The first variable was vegetation cover. Satellite images from Landsat were analyzed with IDRISI® software to define vegetation cover types based on Near Infrared Vegetation Index (NIVI) analysis and supervised classifications. Areas having more than 20% of vegetation cover were selected. Using IDRISI's "distance module" tool a distance model was created to measure distances between vegetation covered areas and *P. lemur* observation sites. The second variable considered in the analysis was distance from breeding areas. The breeding areas of Tamarindo, Aroma and Atolladora were identified by GIS and a distance model was created between these ponds and toad observation points. The third variable was the distance from dry ravines beds that serve as rain water runoff areas. Using digital elevation models the ravines were identified and a distance model was created with the IDRISI distance module. These three variables were correlated to the observation points in the logistic regression model. A final image was obtained from the regression model indicating areas with high probability of finding *P. lemur*.

A description of the toad breeding areas was also carried out. The breeding areas of *P. lemur* at Guánica State Forest consist of three temporary ponds formed after seasonal rains. The Tamarindo pond (17°57.260 N, 066°50.903 W) is the principal breeding area with the largest water retaining capacity. The other two ponds are Atolladora (17°57.423N, 066°51.251W) and Aroma (17°57.284 N, 066°51.048 W). Temperature, pH, conductivity and salinity were measured at the breeding ponds. Ten measurements were taken between the first 24-48 hrs of pond formation when egg lying and eclosion occurs. At each pond, measurements were taken from transects established at equally distant points. Pond characteristics were compared using a one way analysis of variance (ANOVA) with Minitab® statistical software

Results

Thirty two toads were captured and measured from March 2002 to September 2002. The proportion of males to females was of 29:3. Males averaged 64.33 mm snout-to-vent length (SVL) with an average minimum head width (MHW) of 19.7 mm. The average male weight was 17.92g. The females averaged 80.45 mm SVL with 25.13 mm MHW. The female's weight averaged 38.33 g. All observations were made at an average temperature of 26.59 °C (range 25.33-30.33 °C) with a mean relative humidity of 62.22 % (range 40-67.5%).

Non breeding habitat

A total of 24 plots were established related to four vegetation associations: rocks & cacti, scrubland, caducifoleous and evergreen forest. The rocks & cacti association encompassed 46% of the studied sites. Seventeen (17) toads were observed at the rocks & cacti accounting for 53% of observations. All toads observed in the rocks & cacti were males.

The predominant vegetation type (AKH) in the rocks & cacti was the arboreus (67%, Figure 2) which included bushes and trees like: *Melochia piramidale, Croton discolor, Thouinia striata portorricensis*, *Capparis flexuosa, Croton lucidus, Exosterma caribaeum*, *Bursera simaruba*, *Reynosia uncinata*, *Comocladea dodonea*, *Krameria ixina* and *Plumeria alba* (Figure 4). The second most predominant vegetation type in the rocks & cacti association was the xerophytic (25%) with two species: *Cephaloceres royenii* and *Melocactus intortus*. The other types of vegetation consisted of herbaceous plants (3%) and weeds (5%). Canopy cover in the rocks & cacti association was estimated in 15.34% and predominantly composed of the species described on Figure 5. Species composition percentage for the rocks and cacti is described on Table 1. Vegetation density at the rocks & cacti, estimated as horizontal obscurity, was calculated as 37.47%.

The most predominant surface characteristic (Figure 3) in the rocks & cacti association was bare rock (52%), followed by leaf litter (36%). Other surface characteristics found on the rocks & cacti were: green vegetation (6%), dirt (3%), crevices (2%) and logs (1%). The average temperature found in the rocks & cacti was of 27.72°C with an average relative humidity of 66.63%.

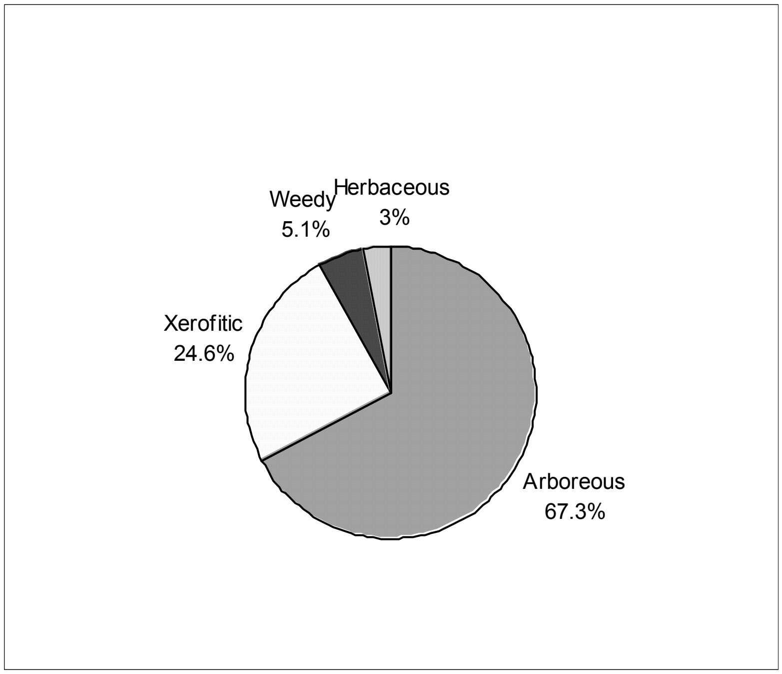

Figure 2 Vegetation Characteristics for Rocks &Cacti Association

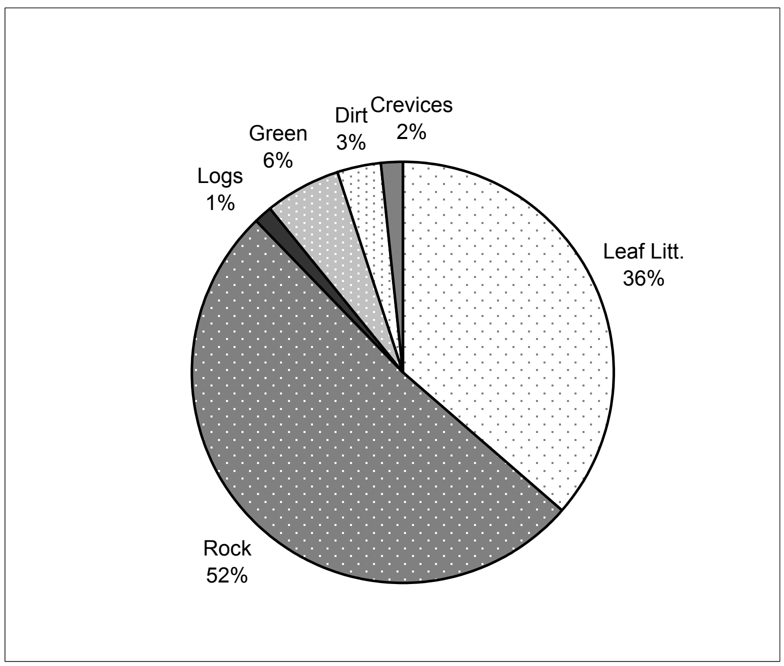

Figure 3 Surface Characteristics for Rocks & Cacti Association

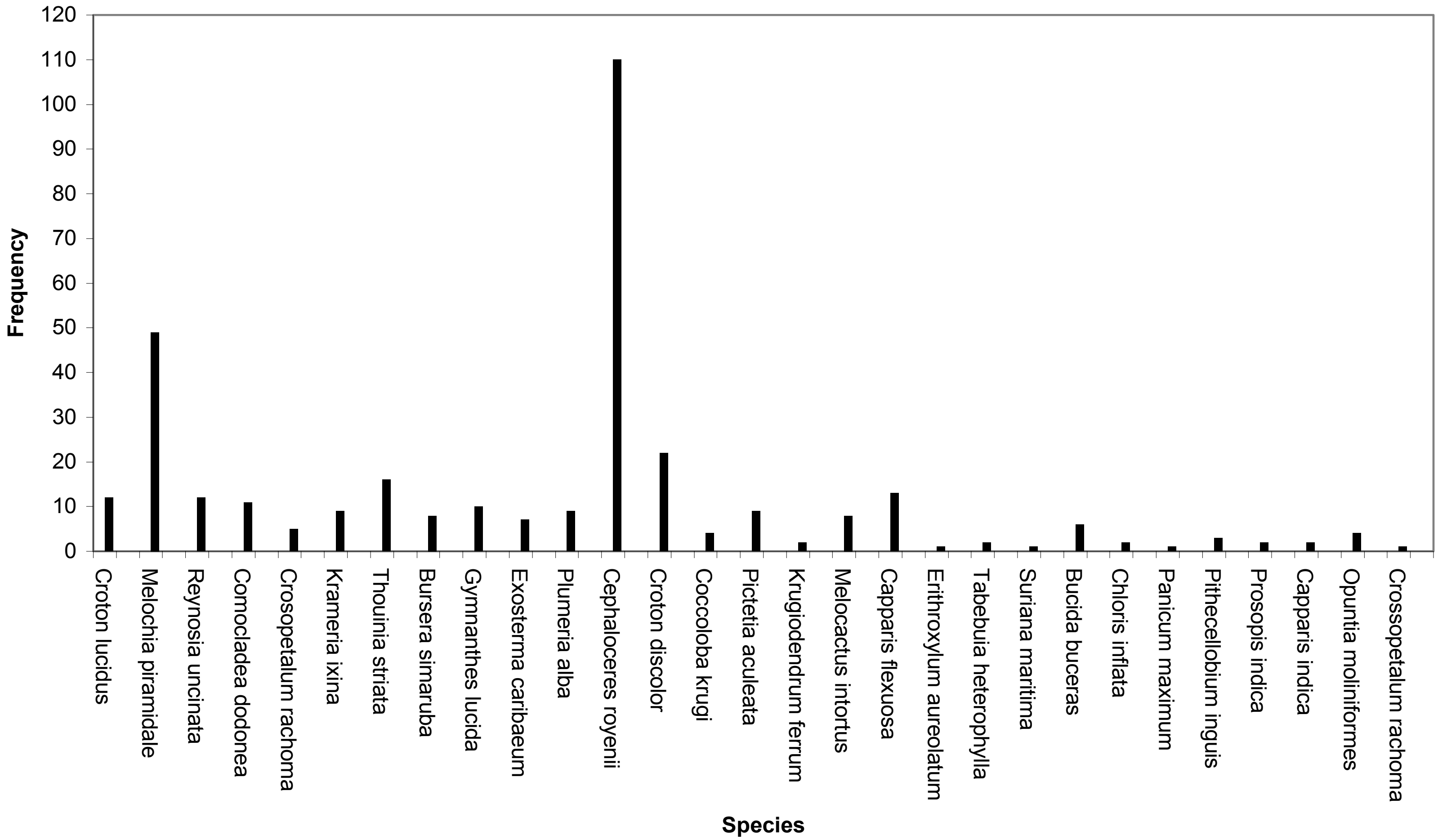

Figure 4 Vegetation Species Frequency for the Rocks & Cacti Association

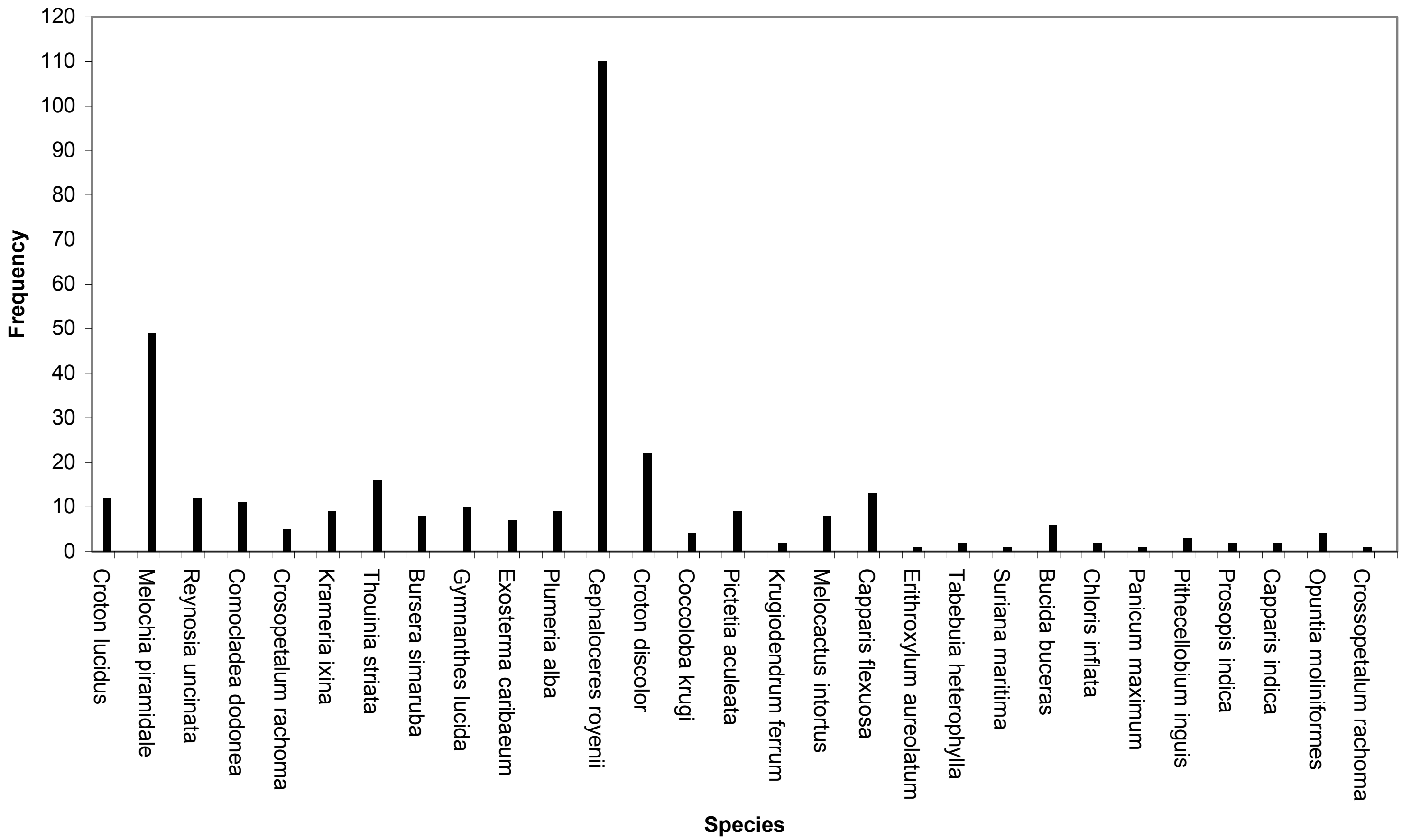

Figure 5 Canopy Cover Vegetation Composition Species for Rocks & Cacti

Table 1 Mean DBH and Composition Percentage for Canopy Cover for Rocks & Cacti Association

Species	Mean DBH (cm)	Percent (%)
Thouinia striata	2.56	10.80
Exosterma caribaeum	3.22	5.63
Bursera simaruba	9.26	4.69
Plumeria alba	4.45	5.16
Pictetia aculeata	4.10	7.04
Comocladea dodonea	2.41	2.35
Gymnanthes lucida	2.61	2.82
Erythroxylum aureolatum	4.10	0.47
Cephaloceres royenii	11.48	35.68
Bucida buceras	9.88	7.04
Coccoloba krugii	3.25	0.94
Crosopetalum rachoma	1.28	0.94
Prosopis indica	8.30	1.88
Pithecellobium inguis	3.50	3.76
Pisonia albida	12.00	0.94
Leucaena leucocephala	4.45	8.45
Capparis indica	8.56	0.47
Opuntia rubescens	6.65	0.94

The scrubland vegetation association included 29% of the studied sites. At the scrubland 4 toads were observed making 12.5% of the observations. All toads observed in the scrubland vegetation were males. The arboreus type was the predominant vegetation (58%, Figure 6) and contained trees and bushes like: *Leucaena leucocephala, Gymnanthes lucida, Capparis flexuosa, Pithecelobium inguis, Plumeria alba, Guaiacum officinale, Erythroxilum aureolatum, Adelia ricinella, Bucida buceras, Reynosia uncinata, Thounia striata, Pisonia albida, Capparis indica, Prosopis indica, Bourreria virgata, Guaiacum sanctum, Capparis bastata, Eugenia rhembea* and *Guaiacum officinale* (Figure 8). The second most predominant vegetation type was the xerophytic (12%) which contained three species: *Opuntia rubescens, Melocactus intortus* and *Cephaloceres royenii*. The weedy vegetation type scored 24%. In the scrubland there are succulent plants that compose 6% of the vegetation with the genus *Sanseviera sp.* being the dominant followed by *Batis maritima*. Canopy cover for the scrubland association was estimated in 38.23% and composed of the species described in Table 2. Canopy composition frequency of species is shown in Figure 9. Horizontal obscurity for the scrubland association was estimated as 55.10%.

The predominant surface characteristic of scrubland association was leaf litter (67%, Figure 7). The green vegetation at surface level composed 13%. Dirt composed 11% with logs and rocks composing 5% and 4% respectively. The average temperature for the scrubland was 25.93°C with an average relative humidity of 81.17%.

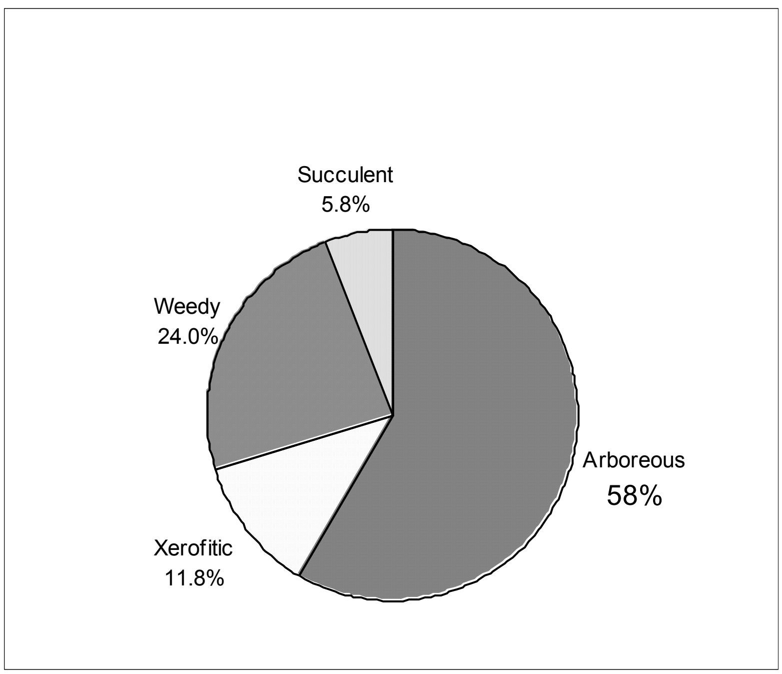

Figure 6 Vegetation characteristics for Scrubland Association

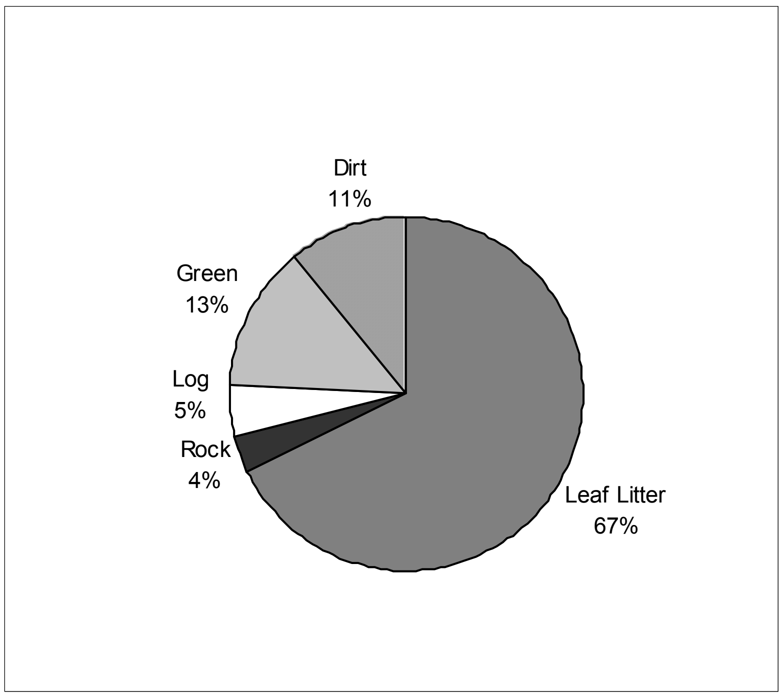

Figure 7 Surface characteristics for Scrubland Association

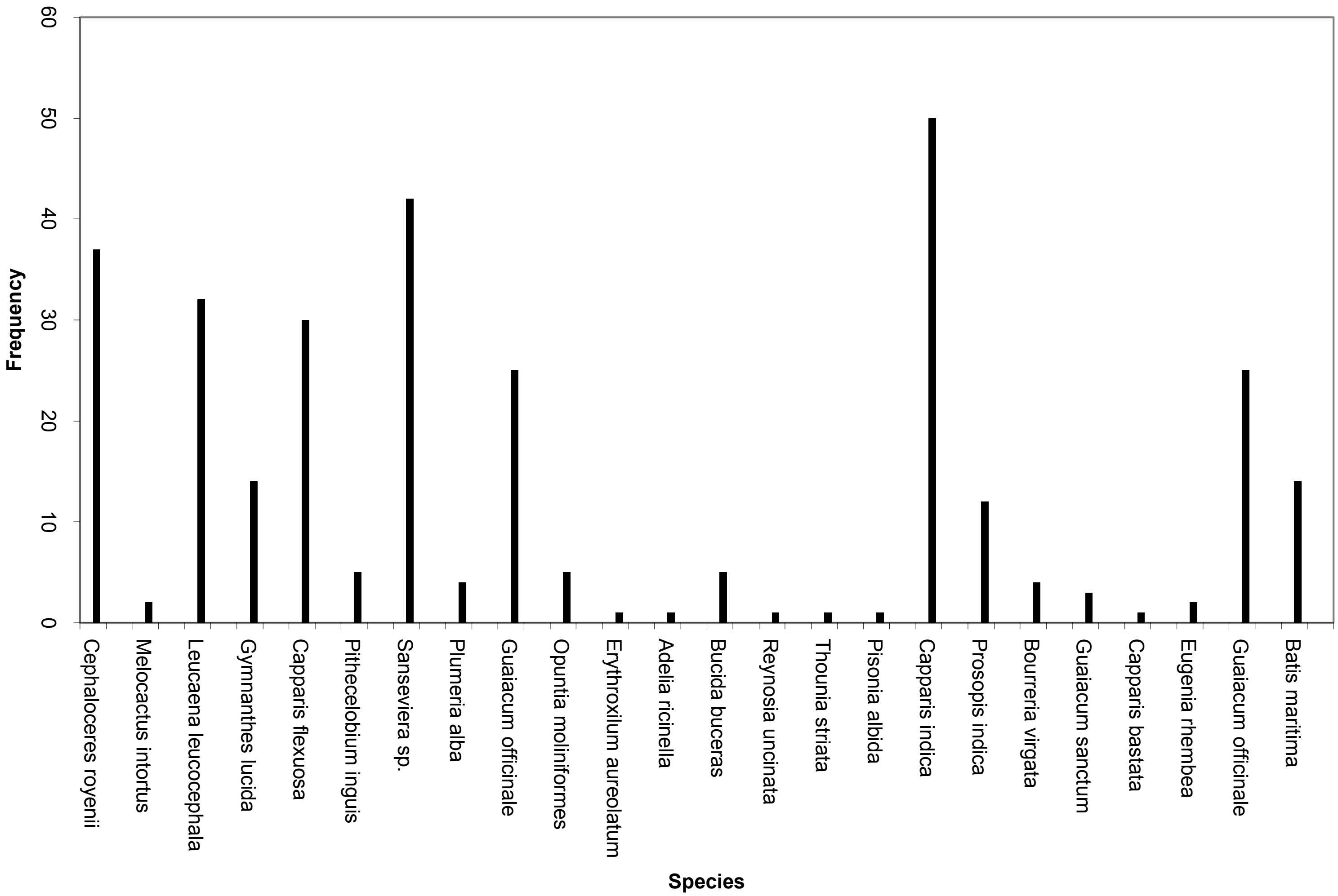

Figure 8 Vegetation Species Frequency for Scrubland Association

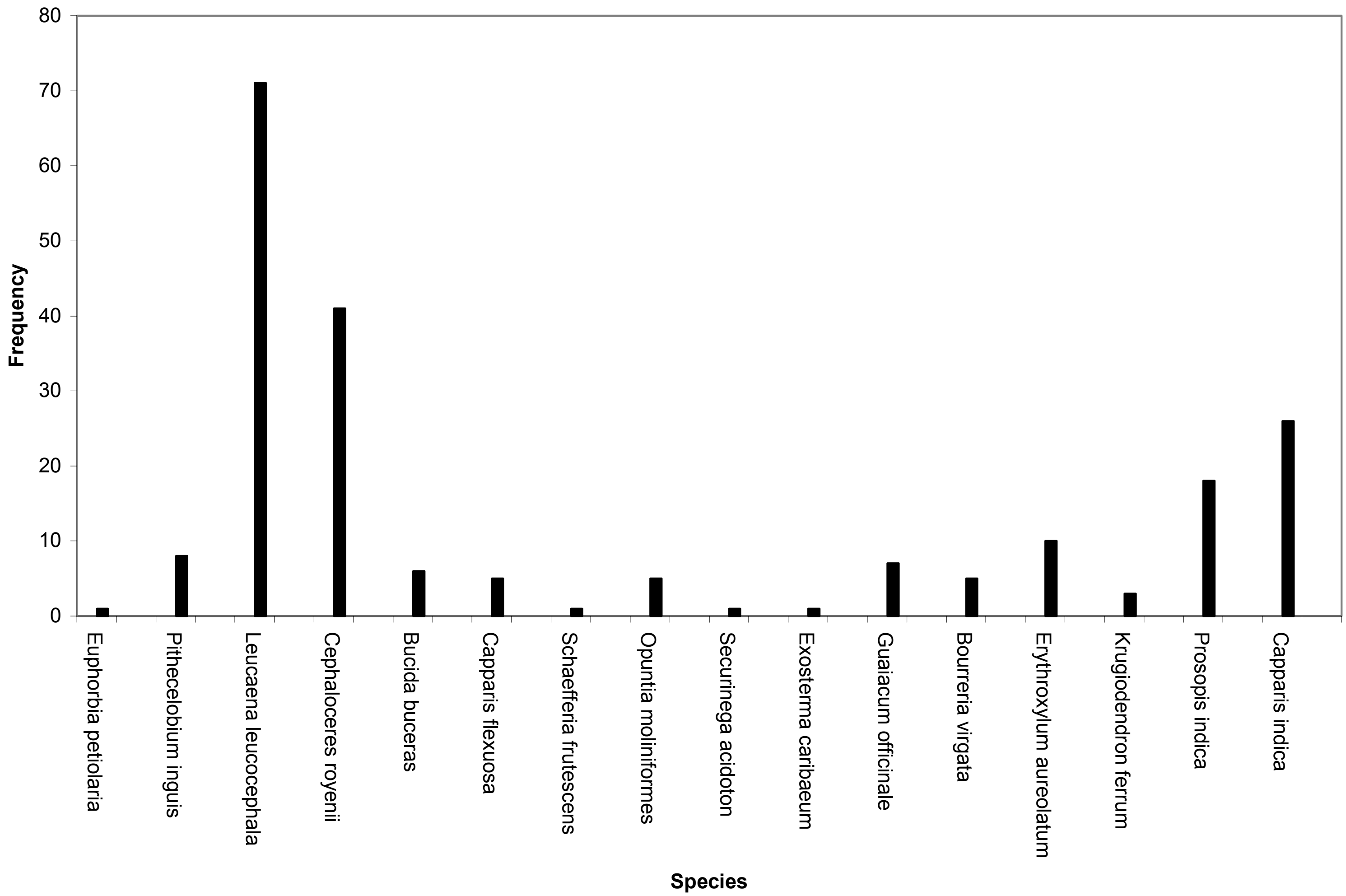

Figure 9 Canopy Cover Species Composition Frequency for Scrubland Association

Table 2 Mean DBH and Composition Percentage for Canopy Cover at Scrubland

Species	Mean DBH (cm)	Percent (%)
Euphorbia petiolaria	7.90	0.48
Pithecelobium inguis	2.33	3.83
Leucaena leucocephala	3.755	33.97
Cephaloceres royenii	8.76	19.62
Bucida buceras	9.93	2.87
Capparis flexuosa	7.30	2.39
Schaefferia frutescens	1.00	0.48
Opuntia rubescens	8.8	2.39
Securinega acidoton	1.00	0.48
Exosterma caribaeum	2.00	0.48
Guaiacum officinale	10.29	3.35
Bourreria virgata	1.00	2.39
Erythroxylum aureolatum	3.27	4.78
Krugiodendron ferrum	6.2	1.44
Prosopis indica	7.57	8.61
Capparis indica	3.08	12.44

The caducifoleous association makes 17% of the sites and contained 12.5% of the toad's observations. One of the three females found in the study was observed in the caducifoleous association. The arboreus type composed 44% (Figure 10) of the vegetation and included trees and bushes species like: *Thouinia portorricensis, Bucida buceras, Reynosia uncinata, Croton lucidus, Pisonia albida, Bursera simaruba, Leucaena leucocephala, Exosterma caribaeum, Pictetia aculeate, Clusia rosacea, Comocladea dodonea, Eugenia sp., Sideroxylon fruticosum, Capparis cynophallopora, Adelia ricinella, Capparis flexuosa, Lasiacis divaricata, Pithecellobium inguis, Erythroxylum aureolatum, Capparis indica, Prosopis indica* (Figure 12). Succulent plants form 26% of the vegetation with the genus *Sanseviera* being the predominant. Weedy vegetation makes 23% of the proportion and xerophytic vegetation makes 6% with species like *Cephaloceres royenii* and *Opuntia rubescens*. Species frequencies composing the canopy are show in Figure 13. Canopy coverage was estimated in 46.11% and predominantly composed of the species described in Table 3. Horizontal obscurity for the caducifoleous forest was estimated as 52.37%.

The predominant surface characteristic (Figure 11) at the caducifoleous association was the leaf litter (51%). Other important surface characteristics at the caducifoleous association were: green vegetation at surface level (28%), rocks (16%), logs (35%) and dirt (2%). The average temperature found at the caducifoleous association was 27.87°C with an average relative humidity of 76.32%.

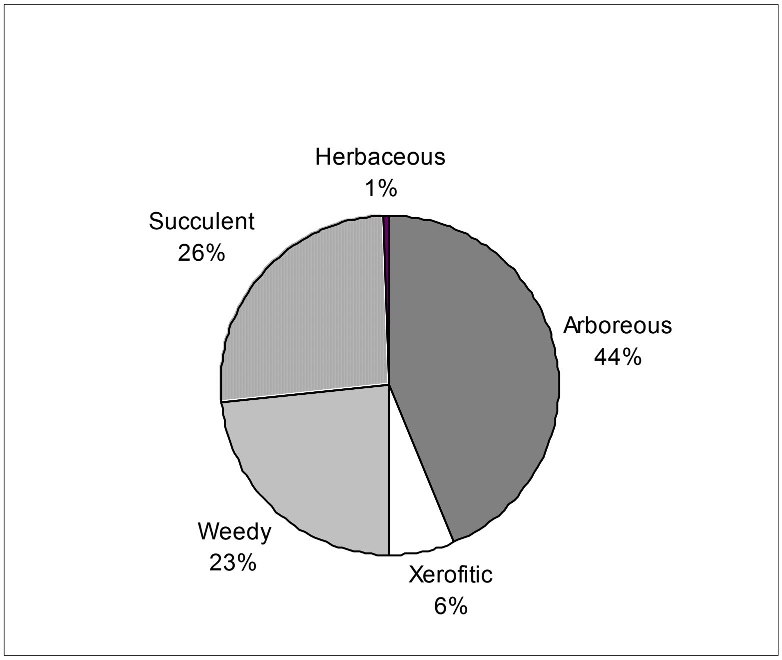

Figure 10 Vegetation Characteristics for Caducifoleous Association

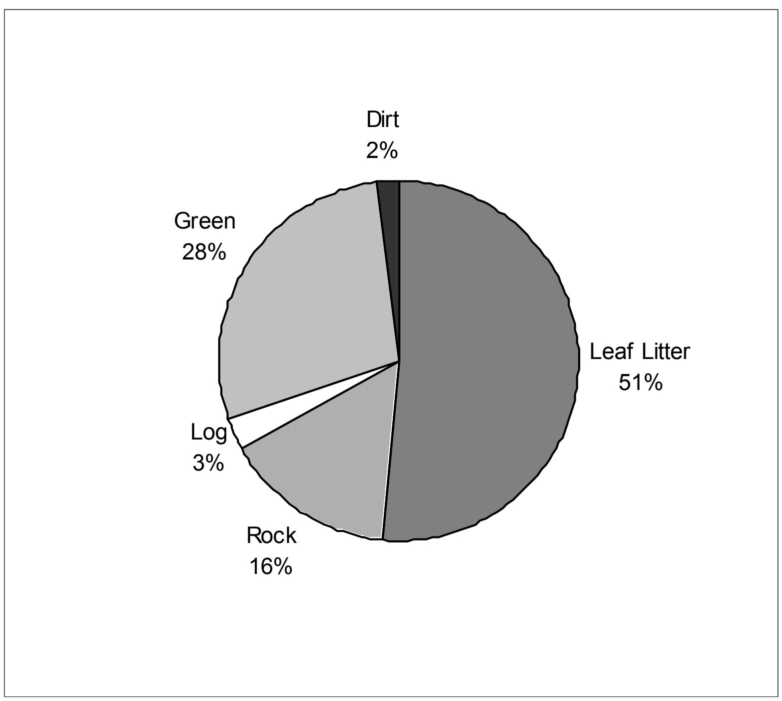

Figure 11 Surface Characteristics for Caducifoleous Association

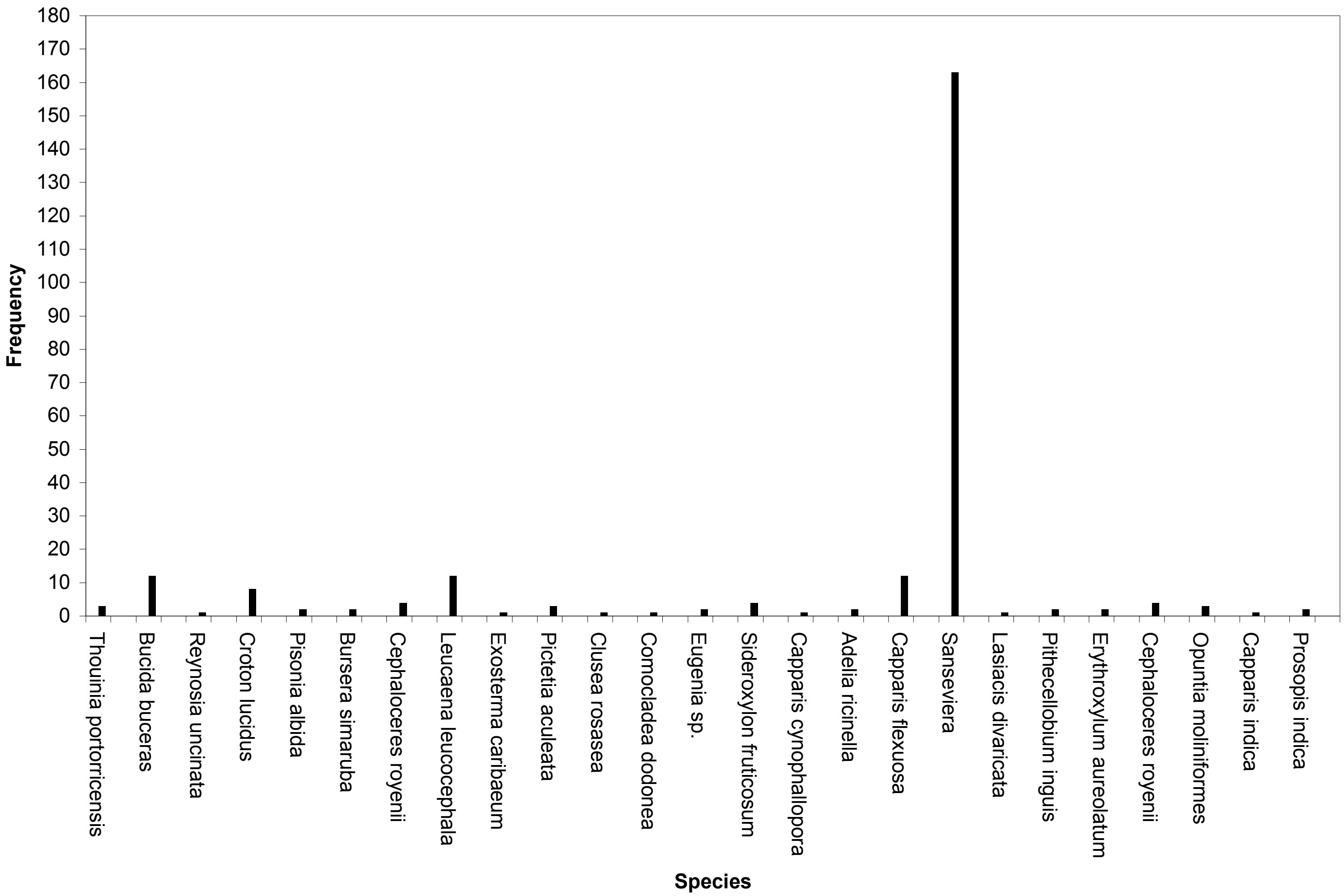

Figure 12 Vegetation Species Frequency for Caducifoleous Forest Association

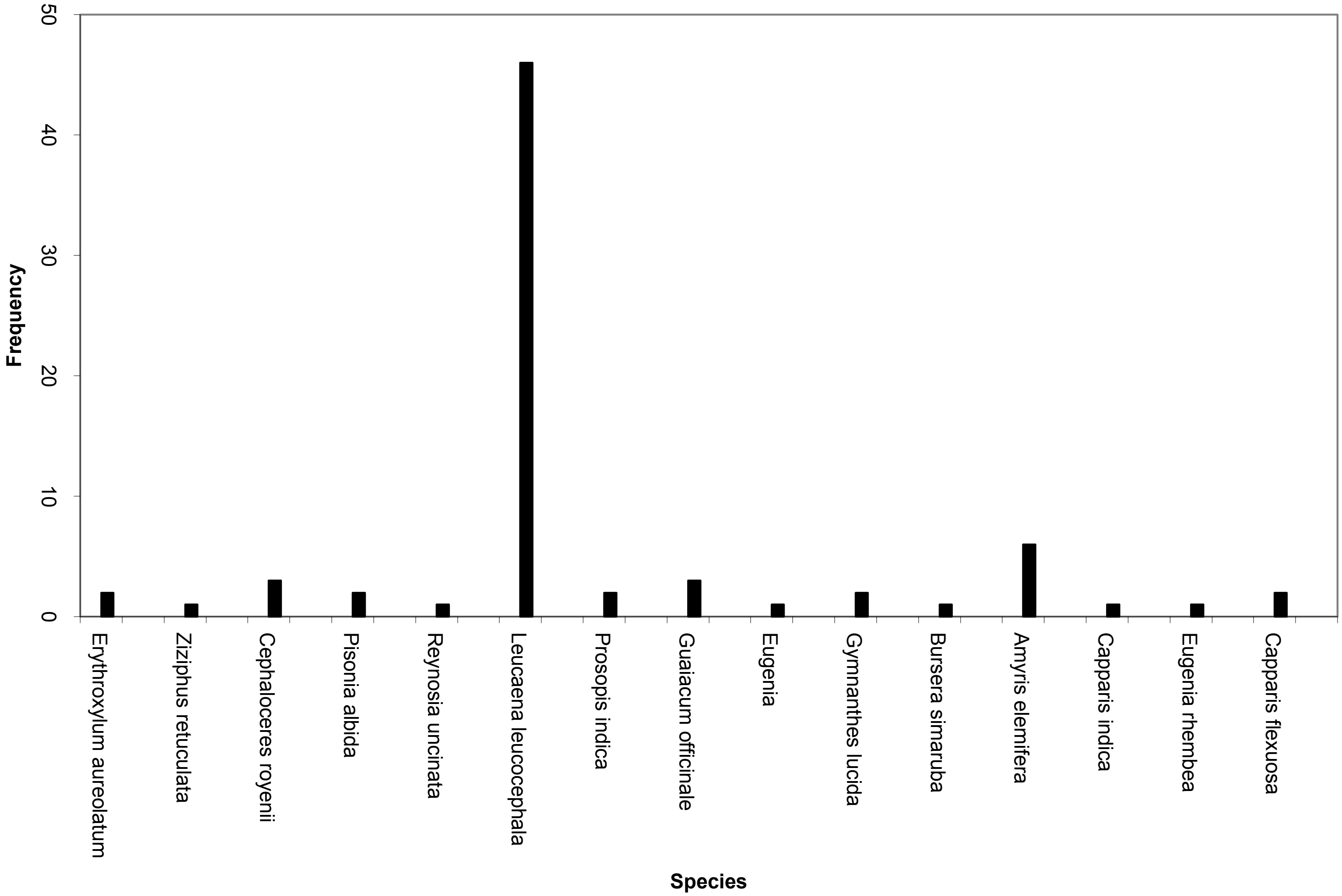

Figure 13 Canopy Cover Vegetation Species Frequency for Caducifoleous Association

Table 3 Mean DBH and Composition Percentage for Canopy Cover for Caducifoleous Association

Species	Mean DBH (cm)	Percent (%)
Erythroxylum aureolatum	4.50	2.70
Ziziphus reticulata	20.50	1.35
Cephaloceres royenii	8.70	4.05
Pisonia albida	13.25	2.70
Reynosia uncinata	2.25	1.35
Leucaena leucocephala	4.84	62.16
Prosopis indica	6.70	2.70
Guaiacum officinale	7.50	4.05
Eugenia sp.	1.50	1.35
Gymnanthes lucida	4.50	2.70
Bursera simaruba	26.30	1.35
Amyris elemifera	3.01	8.11
Capparis indica	3.75	1.35
Eugenia rhembea	4.85	1.35
Capparis flexuosa	1.60	2.70

The evergreen association makes 8% of the studied sites. Twenty two percent (22%) of the toads were observed at the evergreen association including two other females. The vegetation types composing the evergreen association were: arboreus (75%), weeds (22%), xerophytes (2%) and succulents (1%), Figure 14. The predominant tree and bush species for the evergreen association were: *Prosopis indica*, *Eugenia rhembea*, *Capparis indica, Capparis flexuosa, Eugenia rhembea, Capparis flexuosa, Erythroxylum aureolatum, Pithecellobium inguis, Leucaena leucocephala, Bucida buceras, Pictetia aculeate, Krugiodendron ferrum* and *Myrciantes fragans* (Figure 16). Canopy cover for the evergreen forest was estimated as 79.84 %. Canopy composition included the species shown in Table 4. Figure 17 shows the frequencies of the canopy composing species. Horizontal obscurity for the evergreen forest was estimated as 65.50%. The surface characteristics of the evergreen association consisted of only three components: leaf litter (82%), rocks (12%) and green vegetation (6%). The average temperature found at the evergreen association was 27.09°C with an average relative humidity of 83.11%.

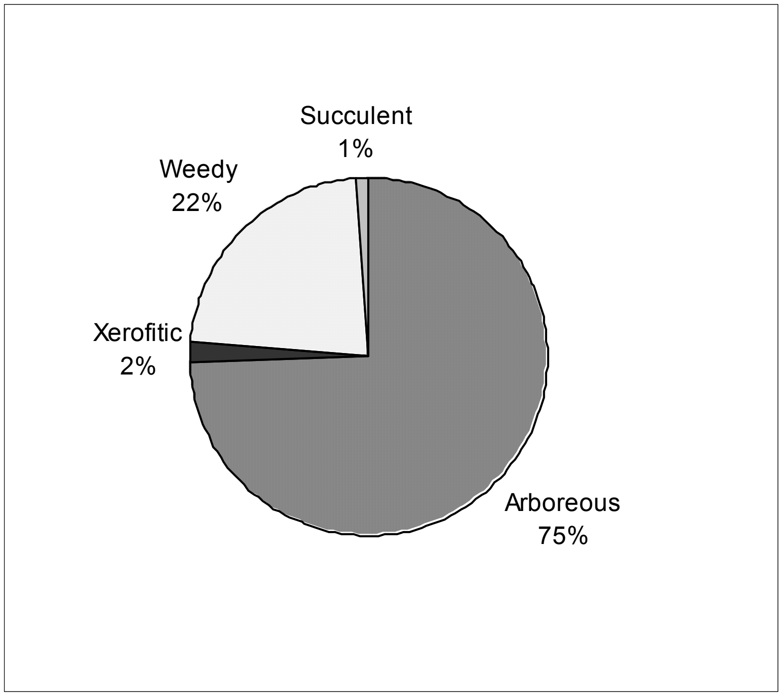

Figure 14 Vegetation Characteristics for Evergreen Association

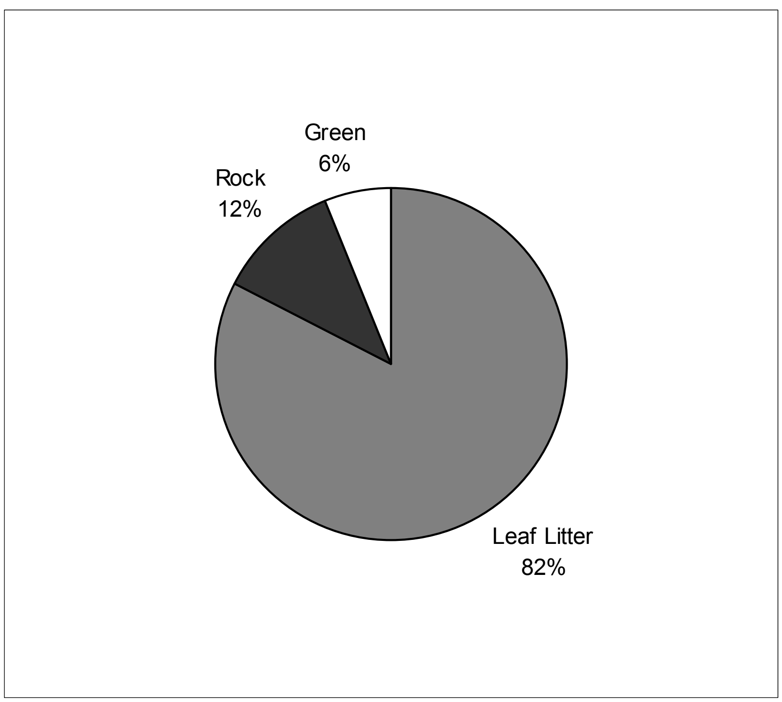

Figure 15 Surface Characteristics of Evergreen Association

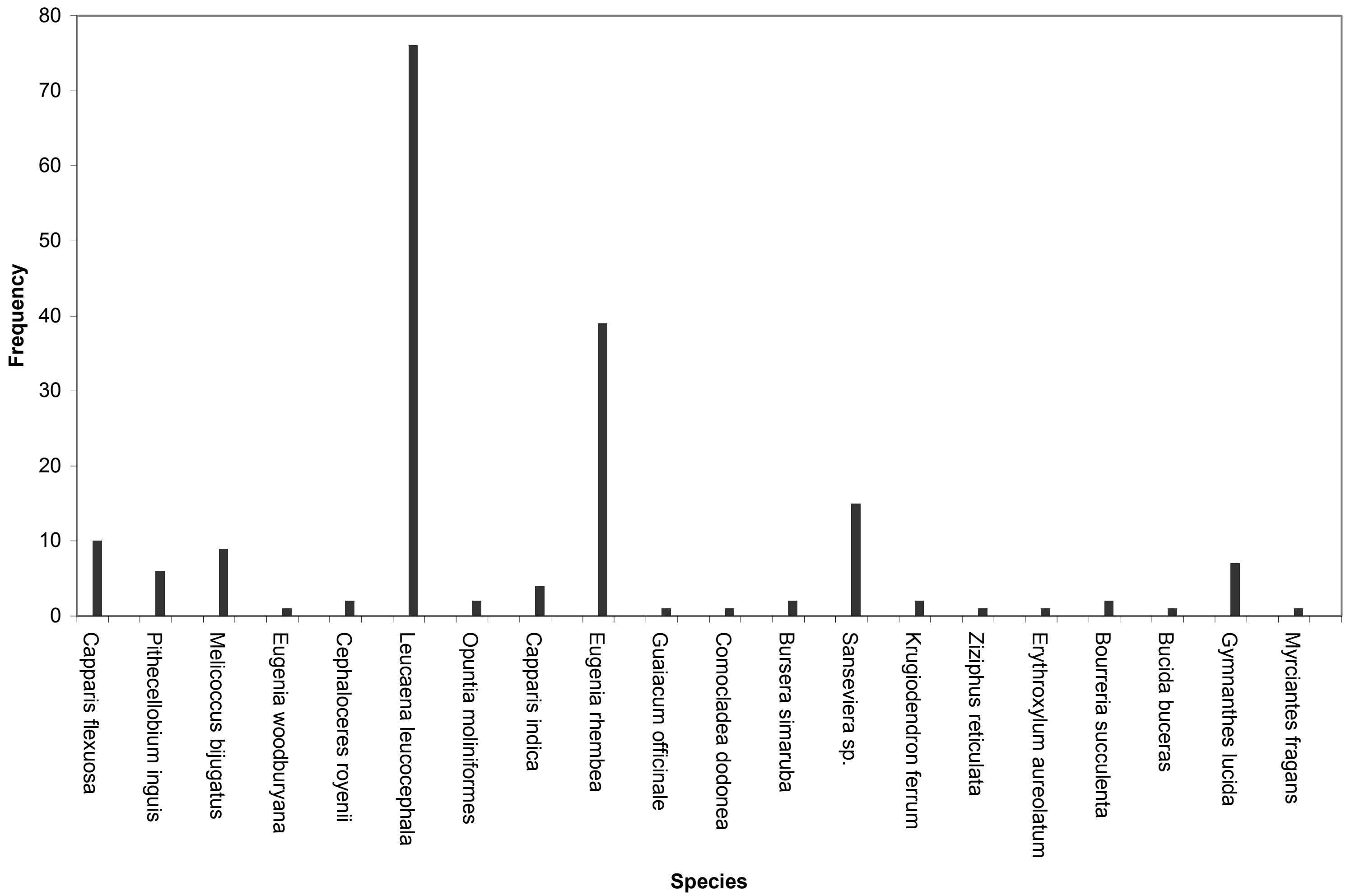

Figure 16 Vegetation Species Frequency for Evergreen Association

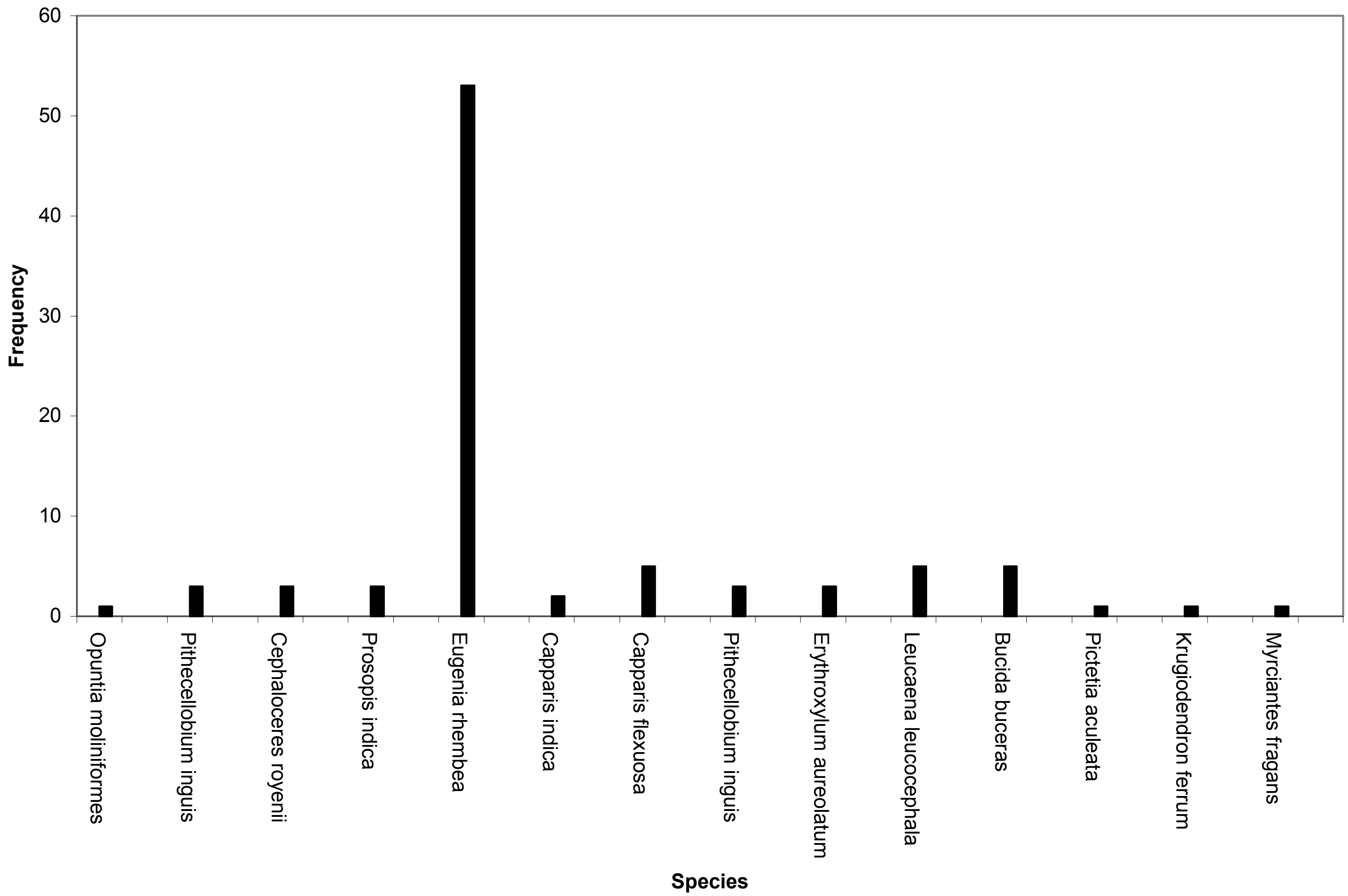

Figure 17 Canopy Cover Species Composition Frequency for Evergreen Association

Table 4 Mean DBH and Composition Percentage for Canopy Cover for Evergreen Association

Species	Mean DBH (cm)	Percent (%)
Opuntia rubescens	11.00	1.12
Pithecellobium inguis	2.85	3.37
Cephaloceres royenii	20.00	3.37
Prosopis indica	8.20	3.37
Eugenia rhembea	6.85	59.55
Capparis indica	1.90	2.25
Capparis flexuosa	8.40	5.62
Pithecellobium inguis	2.85	3.37
Erythroxylum aureolatum	3.80	3.37
Leucaena leucocephala	6.50	5.62
Bucida buceras	24.50	5.62
Pictetia aculeata	5.40	1.12
Krugiodendron ferrum	3.20	1.12
Myrciantes fragans	4.20	1.12

Table 5 Average Temperature and Relative Humidity at Vegetation Associations

Association	Temperature (°C)	Relative Humidity (%)
Rocks & Cacti	27.72	66.63
Scrubland	25.93	81.17
Caducifoleous	27.87	76.32
Evergreen	27.09	83.11

Breeding habitat

The breeding areas for *P. lemur* are the temporary ponds of Tamarindo, Atolladora and Aroma. Tamarindo pond is the most active reproductive area, located at the end of road PR 333 (17°57.260 N and 066°50.903 W). Due to the geographical characteristics of the Tamarindo pond it could be divided in three areas: Tamarindo north, Tamarindo center, and Tamarindo south. The north and center sections of Tamarindo pond are the sites where most ovipositon occurs. The average pH for the north section was 7.783. The average conductivity was 15.842 mq/cm. The average temperature was 30.53° with an average salinity of 0.97ppt. The center section of the Tamarindo pond had an average pH of 7.796, with a conductivity of 5.207 mq/cm, a temperature of 29.53°C and a salinity of 0.269ppt. In terms of ph, Tamarindo north and center sections were not significantly different ($p>0.05$) but Tamarindo north showed a significantly higher ph compared with Atolladora and Aroma ($p<0.05$) [Figure 18]. Also Tamarindo north shows a significantly higher salinity compared to Tamarindo center ($p<0.05$) but lower than Atolladora and Aroma [Figure 19]. As for temperature there was not any significant difference between Tamarindo to any other of the breeding ponds [Figure 20].

Atolladora pond is located at the coordinates 17°57.423 N and 066°51.251 W. The pH at Atolladora was of 7.59 with a conductivity was of 26.8 mq/cm, a temperature of 32.55°C and a salinity 1.57ppt. Atolladora pond has a significantly higher pH than Aroma ($p<0.05$) [Figure 18] but a significantly lower salinity ($p<0.05$) [Figure 1].

Aroma pond is located at coordinates 17°57.284 N and 066°51.048 W. The Aroma pond had an average pH of 7.306. The average conductivity was 34.1 mq/cm. The temperature averaged 33.12°C. The salinity at Aroma was 2.16ppt.

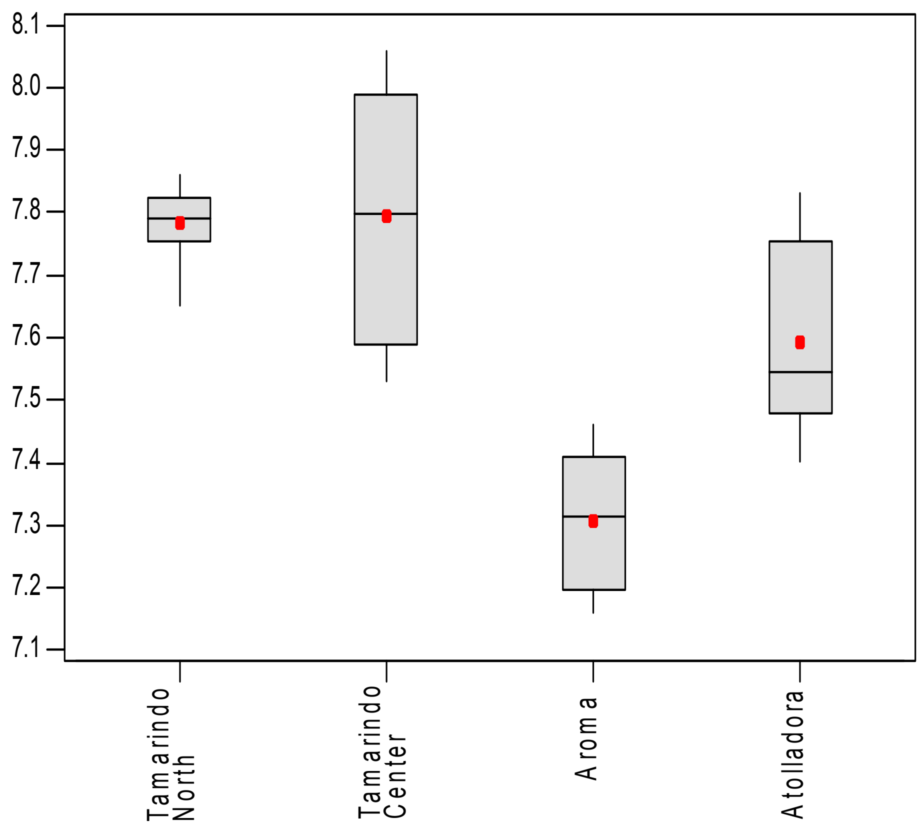

Figure 18. pH Boxplots for Breeding Ponds. (n=10)

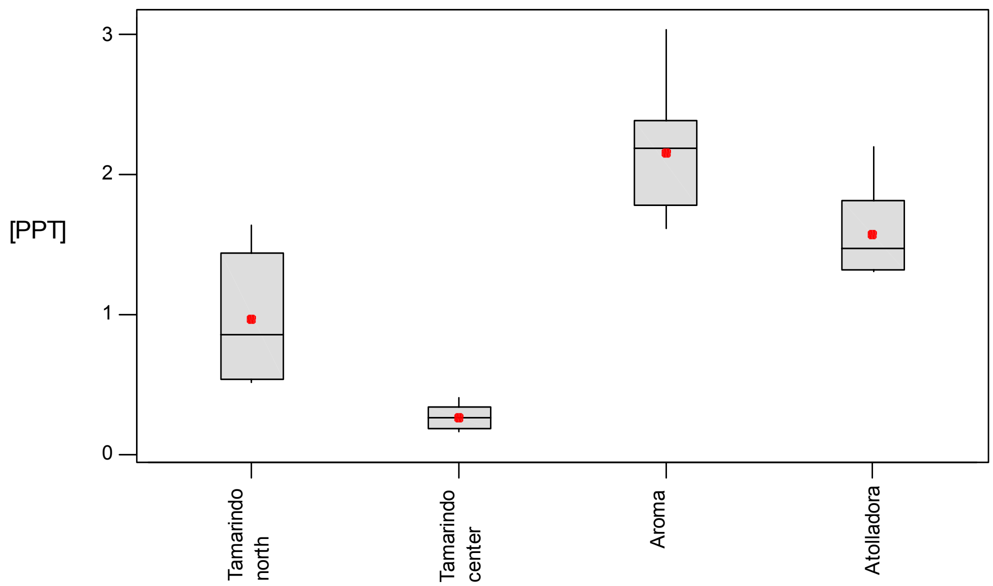

Figure 19. Salinity Boxplots for *P. lemur* Breeding Ponds. (n=10)

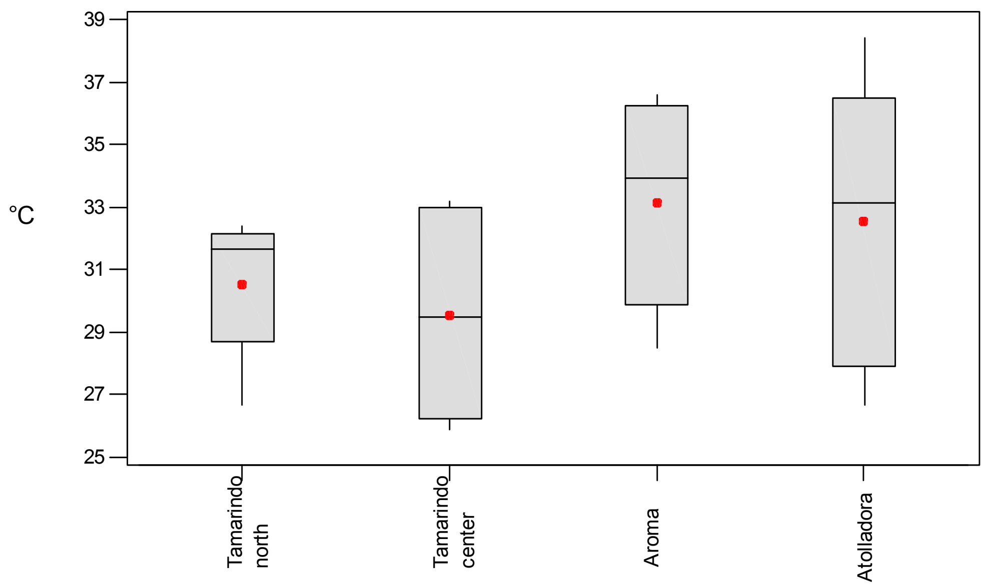

Figure 20. Temperature Boxplots for *P. lemur* Breeding Ponds. (n=10)

GIS Analysis

A prediction of *P. lemur* distribution probability through its habitat was created using GIS applications. A supervised classification of Landsat satellite images shows four vegetation types based on Near Infrared Vegetation Index (NIVI) [Figure 21]. The vegetation type with a vegetation cover over 20% was selected as a predictive variable and used in a logistic regression model obtaining a partial regression coefficient of 0.089. Using Digital Elevation Models (DEM) the distribution of rainfall runoff areas was identified through the forest (Figure 22). The runoff water image also was used in the regression model with a partial regression coefficient of 0.0056. The last variable used in the model was the distance from the breeding ponds (Figure 23), this variable has a partial coefficient of 0.004. These three variables were correlated to the sites on which toads were observed and GPS points were taken (Figure 24). The regression coefficient (R^2) obtained for the correlation was of 0.38. The probability image obtained by the GIS analysis (Figure 25) showed a higher probability (>0.10) of finding toads in the rock & cacti vegetation type areas proximal to the north of the breeding areas.

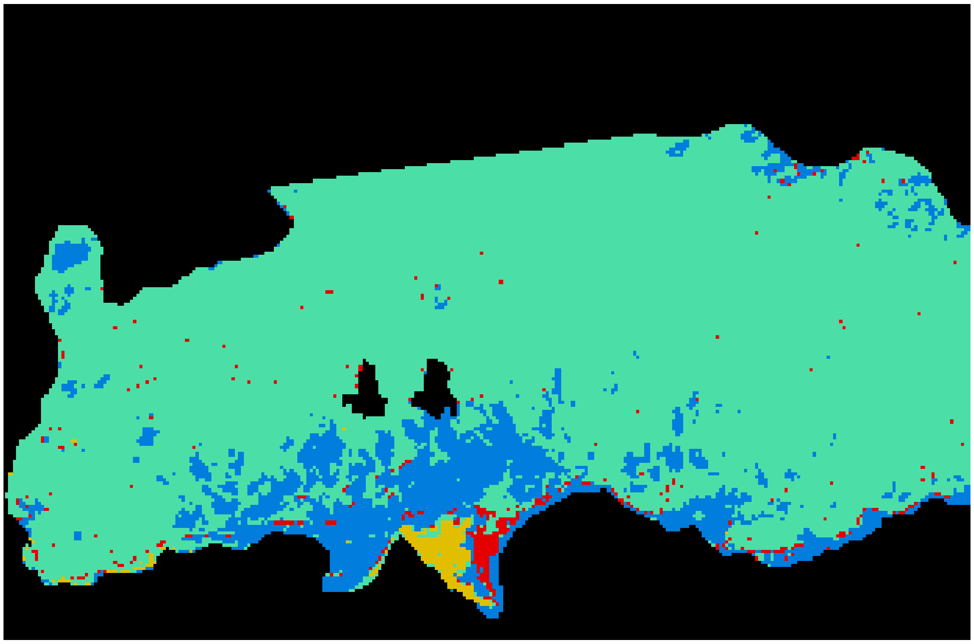

Figure 21. Supervised Classification of Vegetation Types for Guánica Forest

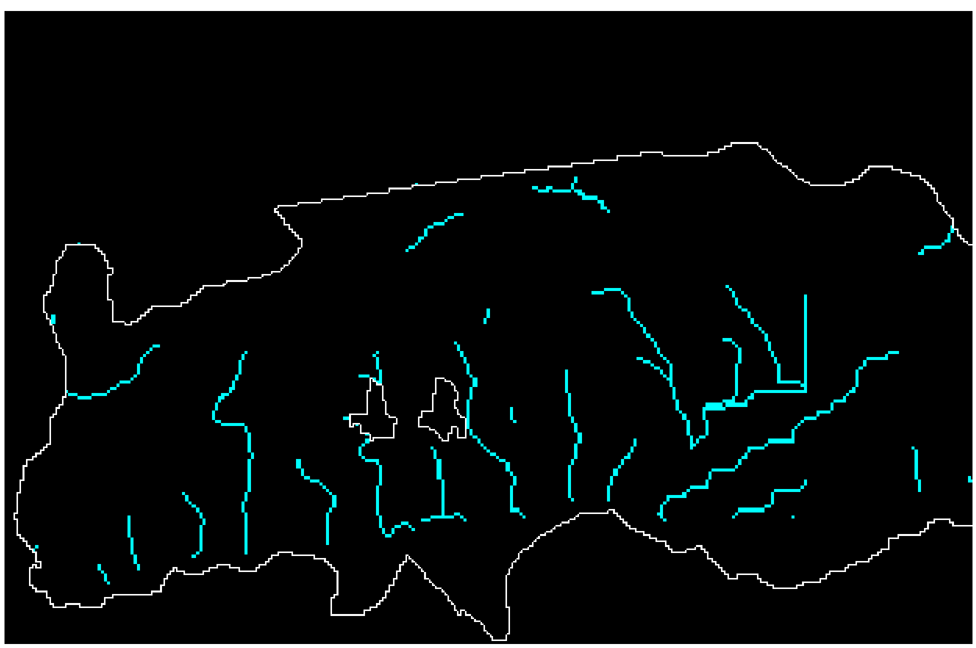

Figure 22. Rainfall Water Runoff Areas at Guánica Forest

Figure 23. Breeding ponds at Guánica Forest

Figure 24. *P. lemur* Observation Points at Guánica Forest

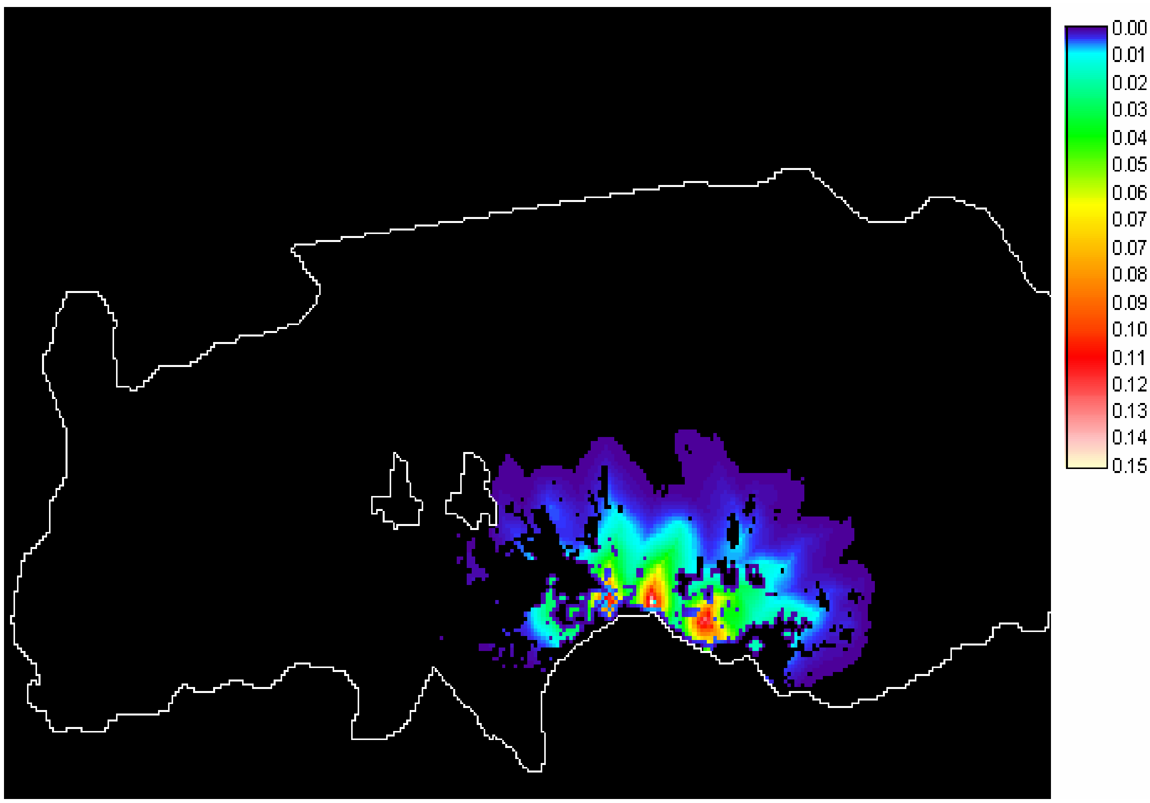

Figure 25. Distribution Probability of P. lemur at Guánica Forest

Discussion

Non breeding habitat

Study plots contained four of the nine vegetation types described by Rogers (Cited in Dugger, 1979) for the Guánica State Forest. These vegetation types or associations are defined in terms of physiognomic and soil depth variations. These variations go from the sparsely vegetated areas with very shallow soil to areas on which some mesic vegetation assemblages had developed in the most protected areas of the forest.

The rocks & cacti association was the predominant type. This vegetation type also has been described as cactus scrub by Castilleja (1991) or limestone scrub by Vilella (1989) and includes the exposed vegetation at the ridge top and southern slopes in lower altitudes (50 m) of the forest. It is associated with limestone outcroppings of shallow soils with little water retention (Lugo et al., 1978; Cintrón & Lugo, 1990.). The rocks & cacti association is characterized by a patchy distributed canopy cover (15.34 %) associated with vegetation growing in soil accumulations on the limestone. The dominant vegetation species on those patches were arboreus species such as *Thouinia striata*, *Bucida buceras*, *Pictetia aculeata*, *Exosterma caribaeum* and *Plumeria alba*; *Cephaloceres royenii* was the predominant cacti species and the introduced species *Leucaena leucocephala* was the predominant invasive species. At the surface level sclerophyllous shrubs such as *Melochia piramidale* and *Croton discolor* were predominant. With such sparse canopy cover and with a horizontal obscurity of

37.47% the rocks & cacti association is characterized by its exposed surface, which leads to the highest average temperature and lower relative humidity of the studied areas (Table 5)

Seventeen toads were observed at the rocks & cacti, which accounts for 53% of the observations. Surface characteristics could be the most important factor explaining this proportion of toads observed. Although sparse vegetation cover at surface level led to better observation of individuals, the patchy vegetation distribution does not provide homeostatic microclimatic conditions in terms of temperature and humidity to support the survival of toads for long periods of time. Other important surface characteristic is the presence of structures that serve as shelter for the toads. The predominant surface characteristic of the rocks & cacti was bare rock that contained 2% of crevices and holes. These holes and crevices are the result of limestone dissolution through ground water drainage that eventually converge in subterranean water and cave systems in which toad activity has been documented (Rivero et al., 1980; Moreno, 1985; Conde-Costas & González, 1996; Canals, pers. comm.). Active use of crevices and holes by *P. lemur* could be an important adaptative behavior for this species. For an amphibian finding a suitable shelter in a dry habitat, like Guánica, its critical for the survival of the species and could be a limiting factor to their population (Cohen and Alford, 1996). Amphibians in dry environments are subjected to high evaporative rates which limit their activities. To deal with these limitations amphibians depend on morphological, physiological and behavioral adaptations (Shoemaker, 1988; Duellman and

Trueb, 1994). Morphological adaptations include skin modifications such as changes in cutaneous vascularization; or the production of barriers to prevent water loss such as wax or multiple layers of stratum corneum (e.g. cocoon). Physiological modifications include metabolic rate reductions and modification of excretory products. Morphological and physiological adaptations are better represented in specialized arboreal frogs of the genera *Chiromantis*, *Hyperolious* and *Phyllomedusa* (Shoemaker, 1988). As for the majority of amphibians, behavioral adaptations are more readily used for exploiting dry habitats. The selection and use of suitable shelters is a key factor in the behavioral adaptations of amphibians to live in dry areas. Experimentally it has been shown that terrestrial amphibians like *Bufo marinus* selectively use moister shelters and have homing fidelity to them (Cohen and Alford, 1996). It was observed in a field study that *Bufo marinus* varied their movements and shelter type accordingly to soil moisture (Seebacher and Alford, 1999). The same study showed that rock crevices were used thoroughly by the *B. marinus* as shelter. Also it has been found that rock crevices, as a microhabitat, provided least dehydrating conditions when compared to other substrates like clumps of vegetation or open spaces (Seebacher and Alford, 2002). These observations may indicate that the use of rock crevices by *P. lemur* in the Guánica forest could be a key adaptative factor. Further studies are needed to observe the relation between the use of the cavities at the rock and cacti association and their connection to subterranean water sources.

The scrubland association included 29% of the studied sites and scored 4 toad observations (12.5%). To get an idea of the factors that could account for the difference in observations between the rocks and cacti and the scrubland associations, it is important to review the vegetation and surface characteristics of the scrubland. The areas of scrubland on which toads were observed are characterized by deeper soils that support more complex vegetation than Rocks & Cacti, with a canopy cover of 38.23% and a horizontal obscurity of 55.1%. The studied scrubland areas were characterized by a secondary growth vegetation type promoted by previous disturbances of cut and burn. This secondary growth vegetation type is exemplified by the presence of introduced species like *Leucaena leucocephala*, *Prosopis indica* and *Sanseviera* spp. The studied areas were located in the vegetation transition zone between the rocks & cacti and the caducifoleous vegetation. These areas differ from the rocks and cacti in terms of temperature, humidity and surface composition. In terms of humidity and temperature the scrubland presented higher relative humidity and a lower temperature than the rocks & cacti. Although these observations could be considered favorable factors for toad distribution, only 12.5% of the toads were observed in the scrubland.

In terms of surface composition the scrubland also differs from the rocks & cacti. The principal surface component in the scrubland was soil covered with leaf litter. Although leaf litter covering moist soil helps to create ideal microclimates for amphibians in most forests, this is not the case in Guánica. In the subtropical dry forest of Guánica mean annual leaf litter production is estimated at 241 g/m^2 with a leaf litter turnover half-life of 1.9 yr (Cintrón & Lugo, 1990). It takes a long time for leaf litter to decompose due to the low water retention capacity of the soil (4 to 33%), which exhibits a water deficit 10 months out of the year (Lugo, et. al. 1978). Dry soil conditions do not provide the humidity necessary for the development of microorganism decomposers of leaf litter. Soil humidity is important for terrestrial amphibians' movement and distribution. Studies of *Bufo marinus* movement and microhabitat use have shown that this species moved further distances as soil moisture increases (Seebacher and Alford, 1999). Also it has been shown that seasonal patterns in movement and habitat use of *Bufo marinus* were related to soil moisture more than to air temperature or relative humidity.

Another factor to consider in distribution of *P. lemur* is the availability of retreat sites. Seebacher and Alford (1999) observed that *B. marinus* rarely used leaf litter as shelter and no seasonal pattern was apparent in the use of this kind of substrate. *Peltophryne* individuals may also not be benefit from an area that is composed predominantly of dry soil with limited access to shelters.

The caducifoleous association comprised 17% of the sites and scored 4 toad observations (12.5%). The caducifoleous, as well as the evergreen associations, share floristically distinct characteristics due to the geographical accidents, such as ravines and sinkholes, which delimit these areas. In the case of the caducifoleous association, the areas on which toad activity was observed were dry ravine beds formed by rainfall runoff. The caducifoleous forest is characterized by a stratified vegetation growth, composed of a layer of green vegetation under a developed canopy. The canopy was composed predominantly by saplings of the introduced species *L. leucocephala*, indicative of some disturbances in the area, but the presence of full grown individuals of *Ziziphus reticulata*, *Pisonia albida* and *Bursera simaruba* (DBH>13.0cm;Table 3) could be better representatives of the original forest vegetation supported by the geography of the area. Most of the green cover at the surface level is made by *Sanseviera* sp. a succulent plant that grows forming a thick layer (Figure 12). This vegetation coverage at surface level in the caducifoleous sites is supported by deeper soils with more water retention capacity. Seebacher & Alford (1999) had noted the use of dense clumps of vegetation by terrestrial toads, especially in wet seasons. This observation is particularly interesting in the case of Guánica since one of the three females was observed in such covered areas of the caducifoleous forest. More important is the fact that those areas of caducifoleous forest coincide with ravine beds that carry runoff water into the reproductive pond of Tamarindo. The studies of Seebacher & Alford (2002) had shown that due to dehydrating conditions, vegetation clumps are not suitable shelter throughout the year. In the Guánica case *P. lemur* could be using such covered areas as shelter when accessing or returning from its reproductive ponds.

The evergreen association accounted for 8% of the studied areas and 22% of toad observations. Evergreen associations developed in secluded areas formed by dry ravines beds, canyons and sinkhole formations that promoted the development of a unique assemblage of mesic plant species. The evergreen areas differ from the caducifoleous in terms of vegetation composition and microclimatic conditions. The vegetation physiognomy at the evergreen areas is a well developed canopy with an open understory. The canopy was composed predominantly by individuals of *Eugenia rhembea* and other evergreen species such as *Capparis flexuosa, Krugidendron ferrum* and *Bucida buceras* (Figure 17). Farnsworth (1993) noted that mesic vegetation assemblages at sinkholes differed from the surrounding xerophytic vegetation. This mesic assemblage includes species like *Cecropia peltata,* a plant not found anywhere else at the southern coast. The prevalence of these species over the introduced *L. leucocephala* is an indicator of fewer disturbances in those areas. The geological accidents that delimit evergreen areas protected them from human related activities and natural phenomenon such as hurricanes. Another effect of the geological conditions promoting the evergreen associations is the maintenance of homeostatic microclimatic conditions. Farnsworth (1993) found that at sinkholes, air temperatures were significantly lower with relative humidity and soil moisture higher than the surrounding areas. These special conditions at evergreen areas could be important factors determining *Peltophryne* distribution that could be attracted to these areas searching shelter during dry seasons.

The importance of soil moisture for terrestrial amphibians' movement and distribution has been shown by Seebacher and Alford (1999). At evergreen areas soil moisture could be between 3.3 and 4.1% compared to 1.0-2.3% at surrounding rocks & cacti areas (Farnsworth, 1993). Also at evergreen areas air temperature and relative humidity ranged 28.7-31.1°C and 69.3-74% respectively compared to 30.9-31.9°C and 65.8-67.3% at surroundings (Farnsworth, 1993).

These microclimatic variables at evergreen areas create suitable conditions for *P. lemur* in a dry environment like Guánica.

Surface characteristics at evergreen areas are another factor that could be influencing toad distribution. Although surface composition is predominantly of dirt covered with leaf litter (Figure 15), all of evergreen areas are delimited by rocky walls that offer access to a matrix of crevices. The importance of crevices and holes as shelter for Peltophryne especially in areas like the rocks & cacti has been stated. In the case of the evergreen areas such crevices could be having additional advantages due to the homeostatic environment provided by the geographical and vegetation conditions. Since evergreen areas of sinkholes and ravine beds are surrounded by rocks & cacti areas the matrix of crevices and holes made in the limestone could be one important link between these two vegetation associations which contained the most number of *Peltophryne* observations.

Furthermore the observation of various individuals of *Peltophryne*, including one female, in the Murcielago Cave sinkhole supports the idea that toads could be using these areas as retreats during the dry season. The Murcielago Cave is located approximately 1km from the reproductive ponds and contains a standing body of water which is part of the underground water system. Although the saline conditions of this cave pool, described as anchialine by Conde-Costas & Gonzalez (1996), may prevent its use as reproduction site for *Peltophryne*, it could serve as an important resource of humidity to endure the dry seasons. The evergreen areas encompassed by ravines, canyons and sinkholes may be acting as oases for the *Peltophryne* populations.

Breeding habitat

As for its reproductive ecology, *P. lemur* shows plasticity to differences in the breeding habitat. There are significant differences between breeding ponds in terms of abiotic factors such as pH and salinity. Such differences may be due to natural biochemical processes inherent to each pond but there also have been different events that may have contributed to them. All three ponds (Tamarindo, Aroma and Atolladora) had received salt water influx from the sea when hurricane surges have pushed water inland. Aroma has also been used in the past as an illegal dump. Between 1984 and 2003, Miguel Canals recorded 24 reproductive events at the Guánica State Forest (unpublished data). All reproductive events included Tamarindo pond while only 37% included Atolladora and 33% included Aroma. These differences in breeding habitat selection could be related to the chemical differences between the ponds that could be key factors to understanding and managing the breeding habitat for this species.

Differences in the chemical properties of breeding ponds could influence breeding habitat selection or affect larval survivorship of *P. lemur* offspring. The physical and chemical characteristics of the aquatic environment affect the development of amphibian larval stages in terms of behavior, adaptation, growing and metabolic rate (Duellman & Trueb, 1994). These physical and chemical water characteristics can also vary significantly over time along the development of the larvae affecting the ability of the tadpoles to cope with the changes. For example pH levels could vary in response to changes in dissolved gases (O_2 /CO_2) due to evaporation or biological processes in the pond. Amphibian larvae exposed to high pH levels in experiments metamorphosed significantly faster than amphibians in other environments (Gerlanc and Kauffman, 2005). The presence of contaminants such as aluminum (Al) or copper (Cu), can increase pH levels that greatly reduces larvae survival (Horne and Dunson, 1995). Other experiments have also shown that compared to neutral pH sites, low pH causes prolonged embryogenesis, higher mortality and higher proportion of deformities (Glos et al., 2003).

As for salinity, there are only a few species of amphibians capable of completing their reproductive cycle in brackish water. The tadpoles of *Bufo viridis* can survive in waters up to 15% of the sea water concentration, while the tadpoles of *Rana cancrivora* withstand concentrations up to 80% (Duellman & Trueb, 1994). Experiments with tadpoles of *Bufo calamita* have shown that under a saline treatment larvae showed a slower developmental rate completing metamorphosis 4 to 9 days later than the control (Gómez-Mestre and Tejedo, 2003). Breeding at the Tamarindo pond (salinity ≈ 0.97 ppt) occurs in conditions similar to freshwater. At Atolladora and Aroma (salinity 1.57-2.56 ppt) breeding occurs in brackish conditions that can present challenges to the survival of the tadpoles.

Although our investigation with *P. lemur* did not correlate abiotic parameters of breeding ponds and survivorship of the larvae it is important to understand the differences between the breeding areas that could affect breeding habitat selection and larvae survival for future management decisions.

GIS analysis

The use of Geographic Information System (GIS) provided tools to explain and predict the distribution of *P. lemur* throughout its habitat. The GIS model was based on the creation of a logistic regression model containing three variables that could affect the distribution and habitat selection by *P. lemur*. The use of “distance to vegetation type”, “distance to ravine beds” and “distance to breeding ponds” as predicting variables of *P. lemur* distribution were based subjectively on the capacity of these areas to provide humidity and shelter.

The “vegetation type” variable explained most of the data variability with a partial regression coefficient of 0.089. The “vegetation type” variable was used as an estimate of the vegetation associations that supported 20% or more of vegetation cover. Comparing the information obtained in the vegetation association description with the probability image (Figure 25) it shows that the rocks & cacti vegetation type is predominant in areas with more probability of finding *P. lemur* individuals.

As previously described, rocks & cacti association characteristics such as availability and access to shelters could be key factors for habitat selection by *P. lemur*. The proximity of these areas to breeding ponds makes them advantageous to access the breeding sites during reproductive events. Although radio telemetry studies showed that *P. lemur* individuals moved up to 1.5 km (0.9 miles) from breeding ponds into the forest (Lentini, A. 1990) there is no evidence indicating that most of the *P. lemur* population could invest such energy. The probability image shows that most of the *P. lemur* population might prefer proximal areas north of the breeding ponds.

The partial regression coefficient for the correlation between toad distribution and ravine beds was 0.0056. Dry ravine beds correspond with evergreen association areas that contained 22% of toad observations. It has been stated that homeostatic microclimatic conditions at evergreen areas could be important factors for *P. lemur* habitat selection and distribution. The probability image (Fig. 25) shows a higher probability trend around the breeding areas. This trend seems to follow the geographic pattern of dry ravine areas. The partial regression coefficient (0.0056) indicates that distance from ravines explain more data variability than distance from the breeding ponds. Although partial regression coefficients values were small, the regression coefficient (R^2) obtained for the logistic regression was of 0.38. Clark and Hosking (1986) showed that for a logistic regression model any R^2 above 0.20 indicated good explanatory power. The R^2 obtained for the logistic regression model shows that the selected variables are good predictors of toad distribution. The introduction of other variables such as relation of toad distribution to underground water systems, telemetry, homing fidelity and the inclusion of more data points are necessary to better understand, model and predict *P. lemur* distribution.

Conclusion

The Puerto Rican Crested Toad (*Peltophryne lemur*) has adapted to survive and exploit different habitats within the Guánica State Forest. Most individuals of *P. lemur* were observed in the "rocks & cacti" vegetation type that is characterized by a sparse canopy cover, exposed bare rock, high temperatures and low humidity. Habitat selection might be associated with surface characteristics of the limestone formations and could reflect evolutionary adaptations related to the historical distribution of this species in karstic areas. *P. lemur* has also adapted to variations in its reproductive habitat, which shows significant differences in terms of pH and salinity between breeding ponds. GIS analysis shows more probabilities to find *P. lemur* individuals in areas of "rocks and cacti" proximal to the breeding ponds and dry ravine beds were evergreen association develops. Other important considerations that could lead to better understand the relationship between *P. lemur* and its habitat includes studies of the underground water systems that could be correlated to toad distribution. Telemetry studies of the other terrestrial amphibian *Bufo marinus* are important to understand how these two species share the same habitat at Guánica. In order to develop a better mathematical model to predict habitat selection and distribution of *P. lemur* is important considerate further variables and to include more data points of observation. The understanding of the use and distribution of *P. lemur* throughout its habitat is of critical importance to the conservation efforts of this unique and fascinating species of the Puerto Rican herpetofaouna.

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