Importance of cacti and agaves in the diet of different feeding guilds of birds in Neotropical arid zones: Contrasting agricultural *vs*. wild areas. Venezuela
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1. Introduction

Arid zones in the Caribbean have a poor legal protection and are exposed to high pressure of urban, industrial and agricultural development (Dinerstein *et al*. 1995). The preservation of the ecosystem integrity of this habitat type is essential to maintain the high variety of ecosystems represented in the region. In order to reach this goal, it is important to establish conservation priorities based on the relative importance of the different species in the maintenance of biodiversity in those environments.

Cacti (Cactaceae) and agaves (Agavaceae) are two plant families representative and abundant in the neotropical arid zones. These plants offer a variety of food resources, nesting places, and refuge to a great deal of species that live in these habitats. Nevertheless, we don't know the relative importance of cacti and agaves in the maintenance of species diversity in the arid zones in comparison with other plant species.

In this project we use a bird assemblage as a model of study to evaluate the relative importance of cacti and agaves as food sources to the vertebrates in one Venezuelan arid ecosystem under both presence and absence of agricultural activity. Birds are appropriate study models to address this problem, because they are the most diverse group of terrestrial vertebrates in the neotropics, and moreover, bird assemblages are structured by many and contrasting feeding guilds associated with different trophic levels.

We will use stable isotope analysis of carbon and nitrogen in blood and feathers samples to make inferences on the relative importance of these plants in the maintenance of the bird community. Two types of environments will be considered: (1) lands transformed by agricultural activities, and (2) pristine arid zones, with no disturbance. With this study we expect to answer the following questions: What is the relative importance of cacti and agaves in the maintenance of the different bird feeding guilds that occur in a neotropical arid ecosystem?, how does cactus and agave use changes along the year?, and what is the effect of agriculture on the food habits of the different feeding guilds of birds?

Proving that cacti and agaves are key groups for the maintenance of vertebrate biodiversity in Venezuelan arid zones, will allow us to generate solid arguments in favor of their conservation. In addition, it is important to elucidate if agriculture in this type of environment damages or benefits the wild species associated to it. This study will also contribute to the knowledge of the feeding ecology of Venezuela birds. This will be the first time that stable isotopes analysis is use to evaluate the diet of a bird community in Venezuela. At the present, the available knowledge on bird's diet in Venezuela comes from studies with fecal samples, stomach contents, and field observations.

General objective

To evaluate the relative importance of cacti and agaves as food sources for the bird assemblage associated with wild *vs*. agricultural areas in an arid ecosystem in Venezuela.

Specific objectives

- Identification of the feeding guilds of birds presents in the study areas.

- To determinate changes in the species richness in the two habitat types.

- To examine the trophic structure of the bird assemblage in both pristine and agricultural areas.

- To estimate the relative importance of cacti and agaves as food sources for the birds of the different guilds in both pristine and agricultural areas.

- To determinate changes in the use of these plants as food sources by the birds along the year in each habitat type (dry *vs*. wet season).

2. Study Area

The study has been conducted in the east margin of "Cerro Saroche" National Park, at 20 km W from Barquisimeto City, Lara State, Venezuela. This area has a medium annual temperature at 27 °C and 300-600 mm annual rainfall that occurs from July to September (ParksWatch, 2003).

The vegetation of this area is dominated by the Fabaceae *Acacia farnesiana* and *Prosopis juliflora*, and the Cactaceae *Cereus repandus*, *Stenocereus griseus*, *Pilosocereus lanuginosus*, and *Opuntia caracasana* (Smith & Rivero 1991). More than 110 plant species live there, including 69 woody species that represent almost the 50 % of all species of trees and shrubs of arid zones of Venezuela.

Several endemic birds species live in the study area including the red siskin (*Carduelis cucullata*) and locally endangered species like the bare eyed pigeon (*Columba corensis*), tocuyo sparrow (*Arremonops tocuyensis*), pale-brested spinetail (*Synallaxis albescens*), slender-billed tyrannulet (*Inezia tenuirostris*), orinocan saltator (*Saltator orenocensis*), or the blue-hooded euphonia (*Euphonia musica*).

3. Methods

Six collections of bird samples were carried out every two months during 2006. Once finished the collections, the samples were sent to the Stable Isotopes Laboratory at the University of Miami, Coral Gables to make the isotope analyses.

Species richness in agricultural *vs*. wild areas were compared using rarefaction method. The analyses were realized using the software EcoSIM 7 (Gotelli & Entsminger 2004).

We collect tissue samples of birds and food resources (plants and insects present) in two habitat categories, agricultural and wild areas. The birds were captured using mist nets. We use among 8 to 10 mist-nets of about 6 to12 meters long. Sampling times beginning at sunrise, near 05:30 a.m., finishing at midday, since in the morning the birds are more actives, increasing the capture probabilities. Once captured, the birds were identified using the available guides of the birds of Venezuela. After collected and identified, the birds were put inside paper bags for about one hour to obtain fecal samples. The feces were kept in the same bag even following analysis.

About 100-200 µL (only in birds above 20 g) of blood samples were be obtained following international recommendations for bird safety (The Ornithological Council, 1999). The blood was collected by piercing in the tarsal vein using syringes and heparinized capillary tubes. Blood were being place in microtubes with 70 % ethanol to preserve it. In addition, we obtain feather samples from the pectoral areas. The feathers were cut using surgical scissors. The feathers samples were placed in cardboard envelopes until following analysis. After sample collection the birds were liberated in the same places where they were captured.

Subsequently, we collect insects samples using light traps (night) and entomological nets (day), and plant samples (flowers, leaves, and fruits) of the most abundant species in the study area, including cacti, agaves and non-succulent species. These were packed in paper bags until following processing.

At the laboratory, the feces samples were analyzed to determine the presence of insect fragments, seeds (fragmented or entire) or any other particle. Based on these analyses, bird species were classified in different feeding guilds like insectivores, frugivores, granivores, omnivores, etc. (Soriano *et al*. 1999). We support these analyses with previous researches of feeding ecology and field watching of the birds with 10x50 binoculars.

Blood, insect and plant samples were dried in a convection oven at 40 °C and stored at –20 °C in a freezer until time of analysis. About 1-2 µg of the animal samples (blood, feather, and insects) and 5 µg of plant samples were processed by 800 °C combustion during 3 hours in Vycor ampoules with 1 g of cupric oxide, 1 g of copper, and 50 mg of silver foil. The CO_2 and N_2 released were separated and purified cryogenically and stored in glass ampoules until analysis.

The isotopic ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) were estimated using a mass spectrometer. These ratios were expressed in parts per thousand ($^{0}/_{00}$) using delta notation as the following equation (Mizutani *et al*. 1992, Fleming 1995, Wolf *et al*. 2002, Herrera *et al*. 2002).

$$d_X = \left(\frac{X_{sample} - X_{standard}}{X_{standard}} \right) x1000$$

X_{sample} and $X_{standard}$ are the proportions $^{13}C/^{12}C$ or $^{15}N/^{14}N$ of the samples and the standards used respectively.

For the carbon the standard used is the fossil carbonate of *Belemnitella americana* from the Peedee formation from North Carolina (Smith & Epstein 1971, Craig 1953, 1957 in Tiezsen *et al.* 1983). For the nitrogen, the standard used is the atmospheric nitrogen (Minagawa & Wada 1984, Peterson & Fry 1987).

The relative contribution from cacti and agaves to the bird diet was estimated with the following equation (Fleming 1995, Wolf *et al*., 2002; Herrera *et al*., 2002):

$$d^{13}C_{bird} = a\left(d^{13}C_{CAM}\right) + \left(1 - a\right)\left(d^{13}C_{C^3}\right) + \Delta_C$$

$\delta^{13}C_{bird}$ is the proportion of ^{13}C in the tissues (blood, feathers, C^3 or CAM plants), α is the proportion of cacti and agaves in the birds diet, and Δ_C is the discrimination factor for the carbon between diet and tissue of birds.

The trophic level of the bird species were calculated using the following equation (Herrera *et al*. 2003):

$$NT_C = 1 + \left[\left(d^{15}N_C - d^{15}N_P\right)/3.1\right]$$

NT_C is the trophic lever of the consumer; $\delta^{15}N_C$ and $\delta^{15}N_p$ are the means of $\delta^{15}N$ in the consumers and plants respectively; and 3.1 is the average value of ^{15}N fractionation between diet and the tissues of birds (Hobson & Clark 1992 in Herrera *et al*. 2003).

The variation in the isotopic ratios of carbon and nitrogen were examined simultaneously for the following factors:

Guilds: the different feeding guilds identified.

Time: 6 sampling periods during one year.

Habitat : agricultural *vs*. wild zones.

A general linear model (SAS Institute, 2001) were used to determine the significance of the 3 fixed effects (guild, time and habitat) and their interactions (guild x time, guild x habitat, and time x habitat, guild x time x habitat).

4. Results

Feeding guilds

Six feeding guilds w ere identified in the two zones: insectivores (30 spp), granivores (14 spp), omnivores (13 spp), frugivores (10 spp), nectarivores (4 spp) and carnivores (3 spp).

Species richness and composition

A total of 2347 specimens and 74 species were captured. From these, 10 species were exclusive of pristine zone (PZ) and 22 of agricultural zone (AZ). Comparatively more species were found in AZ (64) than in PZ (52). 10 orders were identified. Passeriformes and Columbiformes were the best represented in both sites. *Columbina passerina* (Columbidae) and *Tiaris bicolor* (Emberizidae) were the most common species in both sites.

Bird species richness was significantly higher in AZ than in PZ. The rarefaction method reduced the sample size of birds captured in AZ (1208) to the sample size obtained in PZ (1139). Based on this reduction, the new reduced sample of AZ should contain between 61 and 64 species. Because this interval is higher than the number of species captured in the PZ (52), this result is indicative of a real difference in species richness between the two zones.

In the case of carnivores and nectarivores, abundance and richness levels were too low to use the rarefaction method. Only three carnivores and four nectarivores were captured in mist nets. Carnivores were rarely trapped in the mist nets. Of the nectarivores, *Leuccipus fallax* (Trochilidae) and *Coereba flaveola* (Coerebidae) were the only abundant nectarivores. The latter was more abundant in PZ (16 specimens) than in AZ (5 specimens).

For granivores, the sample from AZ included 766 individuals and 14 species, and the sample from PZ 607 individuals and 9 species. The rarefaction method predicted between 11 and 14 species in the AZ sample after reduction to the size of the PZ sample. Since this species interval is higher than the 9 species captured in AZ, the result indicates that bird species richness was significantly higher in AZ than in PZ.

Almost all granivore species were more abundant in the AZ than the PZ. Several rare species were present exclusively in the AZ and not in the PZ, e.g. *Sporophila minuta, Volantinia jacarina* (Emberizidae), and *Zenaida auriculata* (Columbidae).

The samples of frugivores included, 134 individuals and 7 species for PZ, and 75 individuals and 8 species for AZ. Since the PZ sample was higher in size but lower in richness, rarefaction was not useful. The most abundant species were *Forpus passerinus* (Psittacidae), *Thraupis glaucocolpa* and *Euphonia trinitatis*

(Thraupidae). These species were captured more frequently in PZ than in AZ. In addition, *T. glaucocolpa* and three rare species were found in PZ exclusively.

For the insectivores, the sample from AZ included 154 individuals and 27 species and the sample from PZ 211 individuals and 20 species. It is not necessary to use rarefaction to conclude that AZ has higher species richness than PZ. Although common species were more abundant in PZ, 10 rare species were only captured in AZ.

Omnivores were the only feeding guild that showed higher species richness in PZ. In this zone 115 birds of 11 species were captured. The sample from AZ included 152 individuals and 10 species. The most abundant species were *Icterus nigrogularis* (Icteridae) in AZ and *Sublegatus arenarum* (Tyrannidae) in PZ.

Stable isotope analysis: Agricultural *vs*. Pristine zones

All analyzed species evidence feeding use of insects and C^3 and CAM plants, but *L. fallax*, *T. bicolor,* and *Icterus icterus* (Icteridae) show higher dependence from the CAM plants as food resources.

At assemblage level, we found similar values in the ^{13}C signature in AZ and PZ. This suggeststhat there are no differences in the importance of CAM and C^3 plants as food resources for the birds in both habitats. Only five species of birds show zonal differences in the dependence on CAM/C^3 plants: *Mimus gilvus* (Mimidae), *Pitangus sulphuratus* (Tyrannidae), *Melanerpes rubricapillus* (Picidae), *Saltator orenocensis* (Cardinalidae), and *Xiphorhyncus picus* (Dendrocolaptidae). All these species has more dependence on CAM plants on the AZ than in PZ.

^{15}N signatures in the PZ were higher than in the AZ, and evidence that in the PZ the insects are more important as food resources for the birds. Only three bird species: *M. gilvus*, *S. orenocensis*, and *X. picus*, show similar values for ^{15}N in both habitat zones.

Stable isotope analysis: dry *vs*. wet season

In this tropical xeric ecosystem the bird assemblage has:

1) Marked seasonality in dependence on insects as protein source: High during the wet season and low during the dry season.

2) A balanced dependence on C3 and CAM plants.

Dietary habits of frugivore and insectivore birds are the most affected by the seasonal regime: Frugivores turns secondary consumers in the wet season, and Insectivores turns omnivores in the dry season.

CAM and C3 plants are equally important food sources year round for most guilds considered.

Insects are an important protein source for the majority of bird species associated to this neotropical xeric environment. The importance of insects increases during the wet season.

Specialization on CAM or C3 plants is restricted to a small number of taxa in the bird community. Most species rely on a mixture of succulent and non-succulent plants

Compared to bird assemblages from Neotropical wet and dry forests, birds from tropical xeric habitats present:

1) Higher consumption of insects.

2) Reduced importance of C3 plants in their diet

5. Concluding remarks

Higher species richness in the agricultural zone suggests that certain level of agricultural intervention might contribute to increase species richness of bird assemblages in Neotropical arid zones, allowing foreign species to live in the area by opening new ecological niches and increasing food and water resources.

In spite of this result, the agricultural activities seem to have negative effects on some species that show lower abundance or are absent in the disturbed area, e.g. *F. passerinus*, *T. glaucocolpa*, *E. trinitatis* (frugivores), *Xiphorrynchus picus*, *Inezia caudata*, *Myiarchus tyrannulus*, *Camptostoma obsoletum* , *Synallaxis candei* (insectivores) and *Sublegatus arenarum* (omnivore).

6. Data publishing

At present, our work has been presented in the following international scientific events:

- González-Carcacía, JA & Nassar, JM. Species diversity in bird assemblages in a neotropical arid zone: natural *vs*. agricultural areas. 24th Internacional Ornithological Congress. August 2006. Hamburg, Germany.
- González-Carcacía, JA; Nassar, JM; Herrera, LG & Martínez, HM. Use of cacti and agaves by bird assemblages in an arid ecosystem with different agricultural disturbance levels in Venezuela. VIII Neotropical Ornithological Congress. May 2007. Maturin, Venezuela.
- González-Carcacía, JA; Nassar, JM; Herrera, LG & Martínez, HM. Seasonality and origin of dietary protein in neotropical arid zone birds inferred from stable isotope analysis. Annual Meeting of the Association for Tropical Biology and Conservation. July 2007. Mexico.

Three manuscripts will be published with the data obtained:

- González-Carcacía, JA; Nassar, JM & Martínez, HM. Species richness of bird assemblages in a neotropical arid zone: natural *vs*. agricultural areas (in. prep.).
- González-Carcacía, JA; Nassar, JM; Herrera, LG & Martínez, HM. Origin of dietary protein in a neotropical arid zone bird assemblage (in. prep.).
- González-Carcacía, JA; Nassar, JM & Herrera, LG. Origin of dietary protein in bird assemblages in a latitudinal gradient of american arid zones (in. prep.).

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7. Budget.

Item	FUNDING AGENCIES			
	IVIC	UNAM	Rufford	INPARQUES
1. Travel and living expenses				
Accommodation				2520
Meals	1550		1320	
Fuel	80		65	
Vehicle maintenance and services	200		120	
Field assistantship for one assistant	660		660	
2. Field equipment				
8 Nylon mist nets + shipping expenses	100		860	
3. Laboratory Analyses				
C and N Stable Isotope Analysis (£8,9/sample x 960 samples)		6569	1975	
4. Other expenses				
Budget per Funding Agency	2590	6569	5000	2520
Total budget: £ 16679.00				

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