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Population Characteristics, Habitat, and Diet of the Large-crested Toad (*Incilius cristatus*; Anura: Bufonidae): A Critically Endangered Species Endemic to Mexico

***Marco Tulio Oropeza-Sánchez*[1,2], *Eduardo Pineda*[2,3], *and Ricardo Luría-Manzano*[2]**

[1]*Facultad de Ciencias Biológicas, Benemérita Universidad Autónoma de Puebla 72570, Puebla, México*
[2]*Red de Biología y Conservación de Vertebrados, Instituto de Ecología, A.C. Carretera Antigua a Coatepec 351, Col. El Haya, Xalapa 91070, Veracruz, México*

[3]*Corresponding author, email: eduardo.pineda@inecol.mx*

***Abstract*.—Understanding the status and distribution, natural history, and threats to amphibian species is urgent, particularly for those that are threatened. *Incilius cristatus*, the Large-crested Toad, is a highly threatened species that inhabits the cloud forests of eastern Mexico and is rarely detected in the field. In this study, we evaluate the status and distribution of *I. cristatus* in localities where it has been recently detected. Specifically, we examine its relative abundance, population structure, spatial distribution, habitat, and diet in four forest fragments. With a sampling effort of 1,000 person-hours (250 h per locality) between May and November 2013, we recorded a total of 172 toads. We found differences in spatial distribution between juveniles and adults, and females and males, with respect to distance to rivers and edge of forest fragments. We found 16 categories of prey, which varied in importance between ages and sex. The total number of toads we recorded in this study is greater than the largest number of post-metamorphic toads reported to date. Our documentation of four populations of *I. cristatus*, three in Veracruz and one in Puebla, and a population recently recorded in Veracruz, indicates that there are more populations than just the two indicated by the International Union for Conservation of Nature (IUCN), and that the species can persist in habitat fragments. These findings represent an opportunity to conserve the species in each of the cloud forest fragments where it persists and highlights the need to explore other forest fragments that could harbor populations in need of protection.**

Key Words.—amphibians; cloud forest fragments; endangered species; microhabitat use; population structure; sexual dimorphism

Introduction

Estimates indicate that more than one third of amphibian species worldwide are threatened (i.e., in a high risk of extinction category: Vulnerable, Endangered, or Critically Endangered). In fact, 33 species have been formally declared extinct and 550 more are listed as Critically Endangered by the International Union for Conservation of Nature (IUCN 2017). Collecting current data about the status and distribution of populations, natural history, and threats that these species face is an urgent task that, when undertaken, not only allows us to better understand species, but also informs conservation programs by helping to prioritize strategies and strengthening conservation efforts (Marsh et al. 2007; Mace et al. 2008).

The family Bufonidae comprises 602 species, making it the fourth most numerous amphibian family worldwide (Frost, D. 2017. Amphibian Species of the World: an Online Reference. Version 6.0. Available from research.amnh.org/vz/herpetology/amphibia/ [Accessed 17 November 2017]). However, with respect to the number of high-risk species, Bufonidae has the second most threatened species (233 species), behind Craugastoridae (282 species), but with more species listed as Critically Endangered (107 species) than Craugastoridae (59 species; IUCN 2017). In Mexico, there are 35 bufonid species (Frost. 2017. op. cit.), of which 10 are biologically threatened (Frías-Alvarez et al. 2010) and only the Large-crested Toad (*Incilius cristatus*; Wiegmann 1833) is listed as Critically Endangered (Santos-Barrera et al. 2010). Mexican legislation has this species listed as at risk of extinction and it is in the Under Special Protection (Bajo Protección Especial) category (Secretaría de Medio Ambiente y Recursos Naturales [SEMARNAT] 2010). *Incilius cristatus* is endemic to Mexico, its geographic distribution is restricted and severely fragmented; it inhabits cloud forest in the mountains at intermediate elevations in the states of Veracruz and Puebla in the eastern part of the country (Mendelson 1998). The disappearance and disturbance of the forests it inhabits, as well as the pollution and desiccation of streams and rivers, are considered the greatest threats to this species (Santos-Barrera et al. 2010).

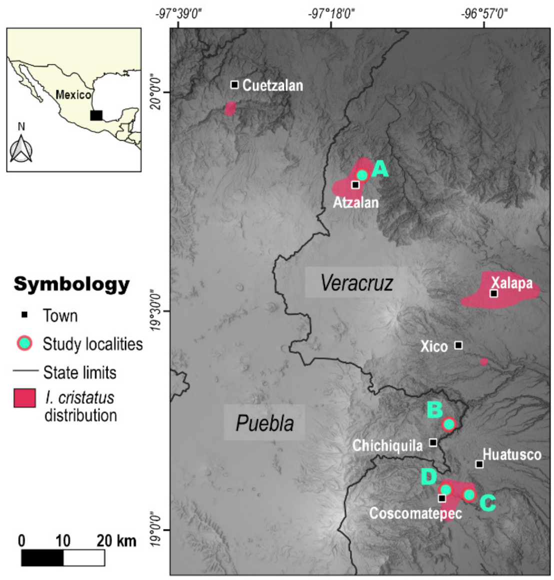

Figure 1. Four localities (A-D) in Mexico where the study of the Large-crested Toad (*Incilius cristatus*) was conducted. The red polygons are areas with populations of the toad.

Incilius cristatus is rarely observed in the field and has, in fact, been referred to as an enigmatic species (Mendelson 1998). In 1995, after not having been observed for 25 y, *I. cristatus* was collected near Apulco, Puebla (Mendelson and Canseco-Márquez 1998). Then, in 1998 the toad was recorded near Xalapa, Veracruz (Pineda and Halffter 2004), the type locality, but was not subsequently observed, even though multiple surveys occurred at the same localities. In recent years, the toad was found in Barranca de Xocoyolo, Puebla (Vázquez-Corzas 2012). In 2010, individuals from this population were collected and used to start a captive colony (Hernández-Díaz 2013). In the summer of 2010, we observed *I. cristatus* near Huatusco and Coscomatepec, Veracruz (unpubl. data) and between 2012 and 2014 Clause et al. (2015) reported several toads in four sites near Atzalan, Veracruz. In 2012, adults of the species were again seen near Huatusco and Coscomatepec (Meza-Parral and Pineda 2015), and that year the species was also observed near Chichiquila, Puebla (Nochebuena-Alcázar, pers. comm.), a locality where *I. cristatus* was not known to be present. In all these instances, only a few adults were detected.

The recent observations of *Incilius cristatus* at several localities present an opportunity to carry out comprehensive studies of the ecology and natural history of this enigmatic species. Specifically, we examined the relative abundance and population structure; analyzed the spatial distribution of individuals; and described the habitat and other aspects of natural history of this species at four localities. Data gathered in this study will provide a better understanding of this highly endangered species and support and promote its conservation.

Materials and Methods

Study area and localities.—The study area (19°00' and 19°50'N, 96°55' and 97°18'W) is located in the Sierra Madre Oriental and includes the states of Veracruz and Puebla, Mexico. To select the study sites, we initially included localities with recent records of *I. cristatus*, gathered from: (1) our research; (2) the Global Biodiversity Information Facility (Global Biodiversity Information Facility [GBIF]. 2013. The Global Biodiversity Information Facility. Available from www.gbif.org [Accessed 12 February 2013]), and (3) HerpNet (HerpNet. 2013. HerpNet. Available from www.herpnet.org [Accessed 12 February 2013]). We confirmed that these sites still had arboreal cover by examining aerial images using Google Earth in 2012 (Google LLC. 2012. Google Earth. Available from www.google.com/earth/index.html [Accessed 12 February 2013). Between April and May 2013, we visited preselected localities to verify the presence of *I. cristatus* and request permission to access these sites from local authorities and landowners. We selected four localities (Fig. 1): one in the municipality of Atzalan, Veracruz (Locality A), one in the surroundings of the Chichiquila site in Puebla (Locality B), one to the south of Huatusco, Veracruz (Locality C), and one north of Coscomatepec, Veracruz (Locality D). All four sites are between 1,300–1,700 m elevation where livestock pastures and shade coffee plantations primarily surround the fragments of Cloud Forest. The area of Cloud Forest in localities A, B, C, and D is 64 ha, 75 ha, 124 ha, and 84 ha, respectively.

Field work.—We sampled each locality five times between May and November 2013, with intervals of 30 to 45 d between each sample. We detected toads using Visual Encounter Surveys (Crump and Scott 1994), searching leaf litter, fallen tree trunks, under rocks, in water, and other microhabitats where toads might be found. We conducted searches mainly in and near forest fragments, bodies of running water, and coffee plantations. The sampling effort consisted of 50 person-hours of searching, for a total of 250 person-hours per site and 1,000 person-hours for the entire study. We conducted searches (by three to five people) in the evening (1600–2000) and night (2100–0100) hours.

We captured toads, georeferenced their location using a Garmin eTrex 30 GPS, and determined their sex and transported them to camp, where they were measured and marked. To avoid recording the same individual more than once, we marked toads by injecting a Visible Implant Elastomer tag (VIE; Northwest Marine Technology Inc., Shaw Island, Washington, USA) following the methodology of Hoffman et al. (2008)

and using the numbering code of Donnelly (1989). To determine sex, we used secondary sex characteristics such as the presence of preorbital crests in females and the presence of nuptial excrescences in males (Mendelson 1997). We measured snout-vent length (SVL) with a digital caliper (± 0.1 mm). We released all individuals at the site of capture.

To describe the habitat, we set up ten 25 × 4 m plots (1,000 m^2 in total) at each locality during the last sampling event and measured tree density (trees with a diameter at breast height [DBH]; approximately 1.2 m height ≥ 15 cm), calculated basal area, and visually estimated tree height. We also measured canopy cover and the depth of the leaf litter at three points in each plot: at one extreme (0 m), in the middle (12.5 m) and at the other extreme (25 m). To calculate percentage canopy cover, we took a digital photograph at each point on the plot (30 photographs per locality) and we analyzed them following the methodology of Korhonen et al. (2006) using ImageJ software, version 1.43 (Rasband 1997. ImageJ. Available from www.imagej.nih.gov/ij, [Accessed 15 February 2014]).

Analysis of diet.—To analyze the diet of *I. cristatus*, we collected 31 toads between September and November 2013 at locality A, the locality where they were most abundant. Within four hours of capture, we flushed their stomachs using the method proposed by Legler and Sullivan (1979) and modified by Solé et al. (2005). We identified prey to order in the laboratory of the Red de Biología y Conservación de Vertebrados in the Instituto de Ecología, A.C. (INECOL), using a stereoscopic microscope and the keys of Triplehorn and Johnson (2005), with the exception of the Hymenoptera, which we classified as Formicidae or non-Formicidae (Whitfield and Donnelly 2006). We separated holometabolan insects into adults and larvae. After identification, we measured the length and width of each prey using a digital caliper (± 0.1 mm). We counted the number of prey in each category and the frequency of occurrence of each one.

Data analyses.—We considered the abundance of *I. cristatus* as the number of individuals observed over the entire study at each locality. We examined whether the observed abundance depended on the locality by using a Chi-square goodness-of-fit test (Zar 1999), where expected abundance is total observed abundance divided by the number of localities examined, given that we made the same sampling effort (250 person-hours) in each of the localities.

To analyze population structure, we looked at both age class proportion and sex ratio. We determined age classes by evaluating the presence/absence of secondary sex characteristics and measuring SVL. We assigned individuals to the adult age class (SVL ≥ 50 mm) or juvenile age class (SVL < 50 mm; Mendelson 1997). To evaluate if the age class proportions and sex ratio depended on the locality, we conducted Chi-square tests (Zar 1999). To compare size (SVL) between sexes, we used a Wilcoxon test.

To examine the spatial distribution of juveniles, adults, males, and females, we used the georeferenced data for each toad and measured the distance to the nearest flowing water body and the distance to the edge of the nearest forest fragment using Google Earth (from 2012). We considered the latter to be negative when the toads were outside the forest fragment, and positive when they were inside. Then, to compare the locations of the juvenile, adult, male and female toads with respect to the nearest flowing water body and forest fragment edge, we used the Wilcoxon's test. To compare habitat characteristics, we used ANOVAs on forest fragment attributes when assumptions of normality and homoscedasticity were fulfilled (leaf litter depth and tree height), or when parametric assumptions were not met, we used a Kruskal-Wallis test (canopy cover and tree DBH). Later, to identify which pair-wise comparisons of forest fragments differed significantly, we used the Tukey test for parametric results and the Dunn procedure for non-parametric results.

To analyze the diet of the toads, we determined the importance of each category of prey i (i.e., the order of each arthropod prey item) in each age class and for each sex using three variables: numerical percentage, volumetric percentage, and frequency of occurrence. We calculated numerical percentage ($\%N_i$) as

$$\%N_i = \frac{N_i}{\sum_{i=1}^{n}} (100)$$

where N_i is the number of prey in category i. We calculated volumetric percent ($\%V_i$) based on the volume (size) of each prey, obtained using the formula for an ellipsoid:

$$V = 3/4\pi \times (L/2) \times (A/2)^2$$

where L is the maximum length and A is the maximum width of the prey. Using these two values, we calculated $\%V_i$ with the following formula

$$\%V_i = \frac{V_i}{\sum_{i=1}^{n}} (100)$$

where V_i is the volume of prey in category *i*. We calculated frequency of occurrence ($\%FO_i$) as the number of toad stomachs in which prey in category *i* were found. We used these three variables to estimate the Index of Relative Importance (Biavati et al. 2004):

$$IRI_i = (\%N_i + \%V_i + \%FO_i)/3$$

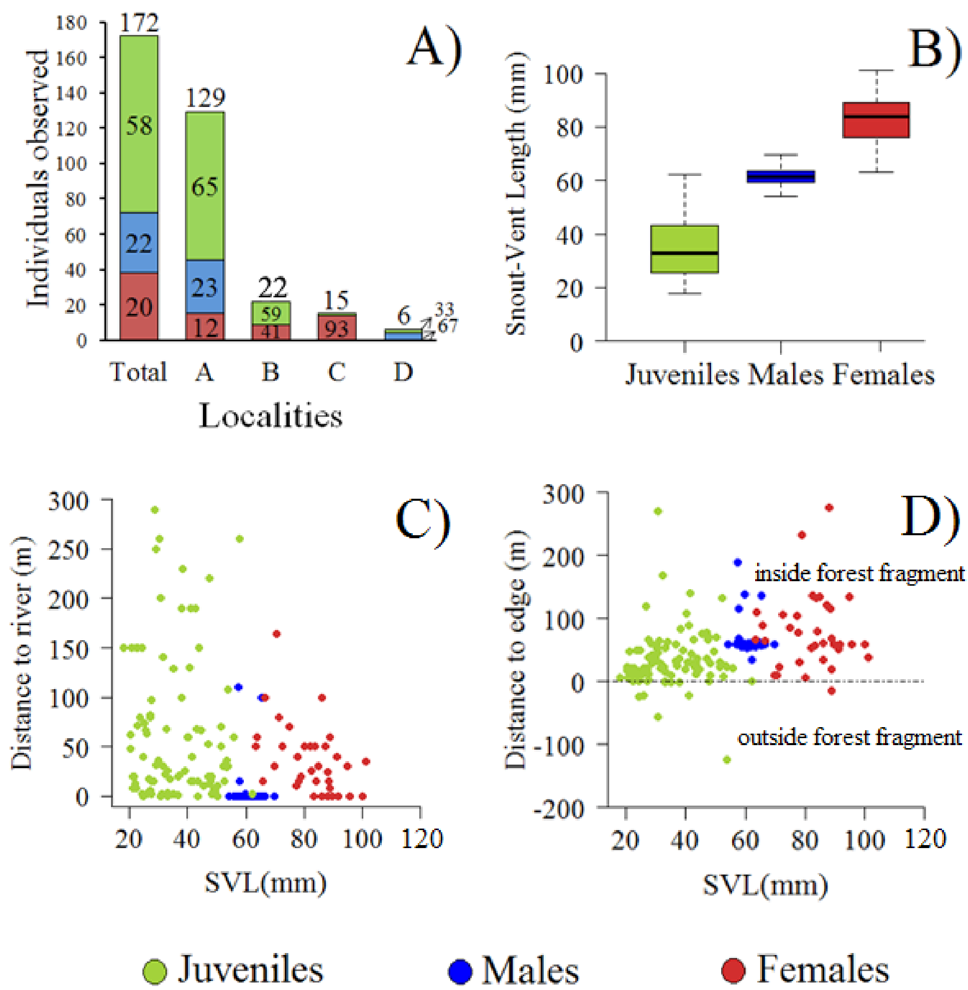

Figure 2. Population characteristics of the Large-crested Toad (*Incilius cristatus*) in four localities of Mexico. (A) The number of toads observed at each locality: the number above each column is the total number of toads observed and the numbers inside the columns are the percentages for each category. (B) The median and quartile distributions of snout-vent lengths of juveniles, males, and females. (C) The distance (m) to rivers where toads were found. (D) Location of toads with respect to the edge of the forest fragment.

To examine differences in the number of prey categories, the number of prey, or size of prey between juveniles and adults and between males and females, we used Generalized Linear Models (GLM). We developed models for analyzing the number of categories of prey using a Poisson distribution and a square root link function. For models corresponding to the number of prey and mean prey volume, we used a Gamma distribution and a log link function. For all statistical analyses, we used $\alpha = 0.05$. We used program R, version 3.1.3 (R Core Team 2015) and the extension gmodels to perform all calculations presented in this study.

Results

Number of toads recorded.—We recorded 172 *I. cristatus*. The number of toads we encountered varied from 129 at locality A to six at locality D (Fig. 2A). The number of toads observed depended on the locality sampled ($\chi^2 = 240.3$, df = 3, $P < 0.001$). We marked 160 with a VIE tag but did not mark 12 individuals because they were either found in amplexus (five pairs) or escaped (two individuals). We recaptured four marked toads at locality A and one at locality C.

Proportion of age classes and sex ratio.—We observed more juveniles (n = 100, 58%) than adults (n = 72, 42%). The proportion of juveniles to adults varied among localities ($\chi^2 = 20.4$, df = 3, $P < 0.001$), with the greatest proportion of juveniles in locality A; we did not detect any juveniles at locality C (Fig. 2A). The sex ratio for all localities was close to 1:1, with the females accounting for 52% and the males accounting for 48% of the adult toads encountered. However, sex ratios varied

Figure 3. Pair of Large-crested Toads (*Incilius cristatus*) in amplexus, north of Atzalan, Veracruz, Mexico. (Photographed by Marco Tulio Oropeza-Sánchez).

among localities (χ^2 = 31.8, df = 3, P < 0.001) and was not 1:1 at any locality. We did not observe adult males at locality B, and we did not observe adult females at locality D (Fig. 2A).

The mean SVL of juvenile toads was 35.5 ± 11.0 mm (mean ± SD) and ranged from 18 mm to 62 mm. The mean SVL of the males (61.5 ± 3.3 mm; range, 54–70 mm) was significantly smaller than that of the females (87.9 ± 5.0 mm; 77–101.3 mm; W = 1305, df = 70, P < 0.001; Fig. 2B). The five pairs we observed in amplexus were from locality A (Fig. 3) during the last sampling period (November). These mating females were 83, 88, 92, 96 and 100 mm SVL and the mating males were 66, 63, 61, 63 and 65 mm, respectively, for a female to male size ratio of amplectant pairs of 1.2:1 to 1.5:1. We did not we hear vocalizing males.

Spatial distribution.—The average distance from rivers for juveniles was three times greater (63.0 ± 72.4 m [mean ± SD]; range, 0–290 m) than that of adults (20.2 ± 33.3 m; 0–164 m; Fig. 2C), and average distance from rivers differed significantly between age classes (W = 1,645, df = 170, P < 0.001). Males were significantly closer to the nearest river (5.8 ± 23.5 m; 0–110 m) than females (35.7 ± 35.5 m; 0–164 m; Fig. 2C; W = 229, df = 70, P < 0.001). Juveniles were closer to the forest edge (33.6 ± 46.0 m; -124–270 m) than adults (72.5 ± 46.3 m; -15–276 m; Fig. 2D), and the difference between age classes was significant (W = 1476, df = 170, P < 0.001). We observed 14 juveniles at the edge and outside of the fragment, but only one adult outside of the forest fragment. We observed males to be slightly closer to the forest edge (67.2 ± 29.2 m; 35–189 m) than females (77.9 ± 58.9 m; -15 to 276 m; Fig. 2D). However, the difference between the sexes was not significant (W = 733, df = 70 P = 0.425).

Habitat.—Mean canopy cover ranged from 78–90%, and differences among localities were significant (H = 10.95, df = 3, P < 0.001; Fig. 4A), with locality B having the greatest of canopy cover. Mean leaf litter depth ranged from 29.0 to 42.1 mm, and differences among localities were significant ($F_{3,116}$ = 5.64, P = 0.001; Fig. 4B). Leaf litter at locality A was deepest. Mean DBH ranged from 24.1 to 52.3 cm, and differences among localities were significant (H = 11.94, df = 3, P < 0.001; Fig. 4C). DBH was greatest at locality C, and greater at locality D than at localities A and B (Fig. 4C). Mean tree height ranged from 9.2 to 12.3 m and varied significantly among localities ($F_{3,113}$ = 9.96, P < 0.001; Fig. 4D). Locality A had the shortest trees and locality D the tallest (Fig. 4D). Locality D had the greatest basal area (8.4 m^2/1,000 m^2), followed by localities C (6.8 m^2/1,000 m^2), A (2.4 m^2/1,000 m^2) and B (2.0 m^2/1,000 m^2). We recorded the greatest density of trees with a DBH > 15 cm in locality A (50/1,000 m^2), followed by C (35/1,000 m^2), B and D (30/1,000 m^2).

Diet.—Of the 31 *I. cristatus* stomachs that we flushed, 28 contained prey. We analyzed stomach contents from 13 juveniles and 15 adults (eight males and seven females). We sorted 296 prey into 16 categories total: 16 categories in juveniles, 10 in adults, nine in males, and eight in females (Table 1). Based on the Index of Relative Importance (IRI), Coleoptera were the most important prey for juveniles and males, the second most important for adults and the third most important for females. Formicidae were the most important prey for adults and females and the second most important prey for juveniles and males. Diplopoda were the second most important prey for females. The numerical percentage data (%N) indicate that Formicidae dominated the diet of all age classes, followed by Coleoptera. Diplopoda was the prey category with the largest volume for adults and females, while for males Coleoptera was the category with the largest volume (and second largest for adults and for females). Coleoptera larvae were the second largest volume for juveniles, and Dermaptera comprised the second largest volume for males. Coleoptera had the highest frequency of occurrence (%FO), while Formicidae had the second highest for both age classes and both sexes. Coleoptera occurred most frequently in all groups, as did Formicidae in juveniles and females. Formicidae were the second most frequent prey in adults and males.

Toads consumed prey from one to eight different categories. Juveniles ate prey from a significantly larger number of prey categories (5.6 ± 1.4; range, 3–8) than adults (3.1 ± 1.8; 1–6; see Fig. 5A; χ^2 = 8.82, df = 26, P = 0.002). Males ate prey from significantly fewer categories (2.0 ± 1.4; 1–5) than females (4.4 ± 1.4; 2–6;

Table 1. Composition of the diet of the Large-crested Toad (*Incilius cristatus*) by age and sex. Values in gray highlight the categories of prey with the three highest values of IRI = Index of Relative Importance, %N = Numerical Percentage, %V = Percentage Volume and %FO = Frequency of Occurrence. Abbreviations are J = juveniles, A = adults, M = males and F = females.

	IRI				% N				% V				% FO			
Taxon	J	A	M	F	J	A	M	F	J	A	M	F	J	A	M	F
HEXAPODA																
Orthoptera	14	11		22	2	6		8	8	1		1	31	27		57
Dermaptera	7	12	15	16	1	5	8	4	3	4	25	2	15	27	13	43
Hemiptera	17	12	8	17	4	6	12	4	1	3		3	46	27	13	43
Coleoptera	53	38	43	40	29	15	27	11	29	26	40	25	100	73	63	86
Hymenoptera	28	11	5	21	8	6	4	7	6				69	27	13	57
Formicidae	52	40	33	48	40	49	35	55	15	5	16	4	100	67	50	86
Diptera	11	3	5		3	1	4						31	7	13	
Coleoptera (larva)	9	5	5	6	1	2	4	1	10	1		1	15	13	13	14
Neuroptera (larva)	3				0.4				1				8			
Lepidoptera (larva)	12				2				2				31			
Diptera (larva)	3				0.4				1				8			
CRUSTACEA																
Malacostraca	11				1				10				23			
MIRIAPODA																
Chilopoda	3	3	9		1	1	4		0.3	1	11		8	7	13	
Diplopoda	16	34	8	43	3	8	4	10	8	61	9	64	38	33	13	57
ARACHNIDA																
Acari	11				2				0.1				31			
Opilionidae	7				1				6				15			

Fig. 5A; $\chi^2 = 8.90$, df = 13, $P = 0.007$). The number of prey items found in the stomachs of toads ranged from one to 45. Juveniles ate a significantly larger number of prey (17.1 ± 9.2; 7–45) than adults (6.6 ± 6.8; 1–27; Fig. 5B; $t = 3.72$, df = 26, $P = 0.002$). Males consumed significantly fewer prey items (3.2 ± 3.2; 1–10) than females (10.4 ± 7.9; 2–27; Fig. 5B; $t = 6.81$, df = 13, $P = 0.010$). Mean volume of individual prey consumed was 0.01–260 mm^3. Juveniles consumed prey that were significantly smaller (15.3 ± 29.1; 0.3–101.8) than those eaten by the adults (42.7 ± 66.8; 0.01–260.5; Fig. 5C; $t = 24.6$, df = 26, $P < 0.001$). Males ate significantly smaller prey (13.0 ± 14.7; 0.01–43.5) than the females (58.2 ± 91.9; 2.1–260.5; Fig. 5C; $t = 10.8$, df = 13, $P = 0.025$).

Discussion

This study, based on several populations, represents the first broad examination of the ecology of *I. cristatus* and provides important information about the population structure, spatial distribution, habitat characteristics, diet, and other elements of its natural history in the Cloud Forest where it is found. Our documentation of *Incilius cristatus* of four populations, three of them in Veracruz and one in Puebla, and a population recently recorded near Atzalan, Veracruz (Clause et al. 2015), indicates that there are more populations than just the two indicated by the IUCN in municipalities in the state of Puebla (Santos-Barrera et al. 2010), and that the species is capable of persisting in habitat fragments. The presence of several populations of a critically endangered species such as *I. cristatus*, is an opportunity to conserve the species in each of the localities where it still dwells, to conserve its habitat (cloud forest fragments with rivers) and to study it from population, genetic, behavioral, and reproductive approaches.

The total number of individuals recorded in this study is the largest number of adult *I. cristatus* reported to date, although it is worth mentioning we observed large variation in the number of toads recorded among localities. This large variation may reflect actual differences in population size among our study sites, or differences in detectability among localities (or a combination of the two). Also of note, the largest female we recorded was 101.3 mm, and the largest male was 70.0 mm, both of which are larger than the largest previously reported female and male (87.3 and 54.8

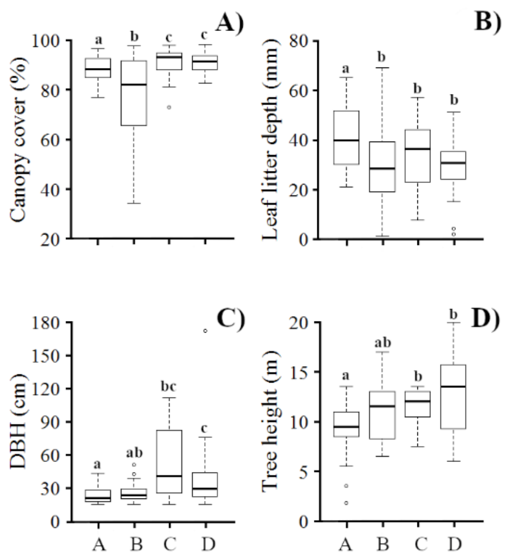

Figure 4. Habitat characteristics of study localities where the Large-crested Toad (*Incilius cristatus*) was present. (A) Percentage canopy cover; (B) Leaf litter depth; (C) Diameter at breast height or DBH (approximately 1.2 m height) of trees; (D) Tree height. Different letters (a, b, c) indicate significant differences among sites in the medians of characteristics measured.

mm, respectively; Mendelson 1997). With respect to the diet, the number and type of prey *I. cristatus* consumes suggests that the species interacts with a wide range of invertebrates. Additionally, dietary differences between age classes and sexes indicate that, depending on the age or sex, an individual may interact differently with the arthropods they consume, therefore, play a different role in the ecosystem.

The spatial distribution of the different age classes with respect to the distance to river and the forest fragment edge suggests differential behavior as a function of age and sex. For juveniles, it has been reported that higher mobility is commonly associated with migration events (toward habitats with lower population density) and with the colonization of new suitable habitats (Pittman et al. 2014). The spatial distribution of the adults in forest fragments and the distance to the river could result from the dependence of this species on running water and microhabitats suitable for reproduction, and philopatry, as reported for other amphibians (Todd et al. 2009; Liang and Stohlgren 2011). The differences between the sexes in spatial distribution have been reported for other species (Fellers and Kleeman 2007; Tozetti and Toledo 2005) and these might result from their reproductive strategies: the location of the males close to rivers and toward the interior of the forest fragments could result from their territoriality (Hunter et al. 2009) and for the females, their broader distribution could result from the need for more food, owing to their larger size (Muths 2003).

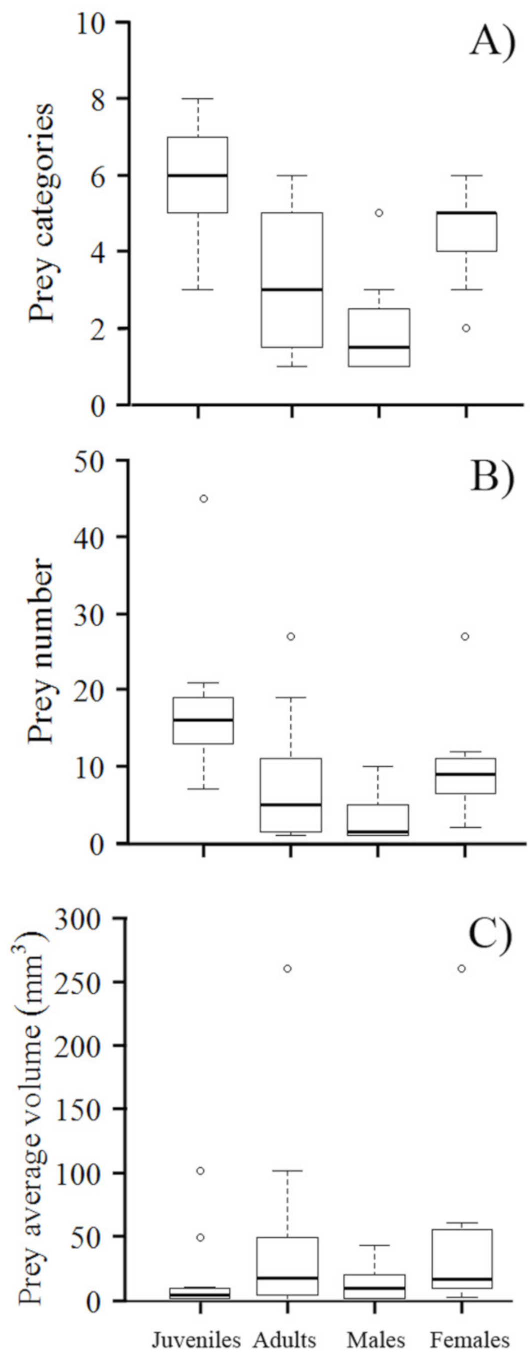

Figure 5. Dietary differences between size classes and sexes for the Large-crested Toad (*Incilius cristatus*). (A) Number of categories of prey eaten; (B) Number of prey eaten; (C) Mean volume of prey eaten.

In general, the diversity and composition of diet of *I. cristatus* are similar to that reported for other members of the family Bufonidae, which consume 13 to 28 different types of prey, predominantly Coleoptera and Formicidae (Moseley et al. 2005; Duré et al. 2009;

Hirai and Matsui 2002). These results support the idea that diet in bufonids may be highly conserved, as first suggested by Quiroga et al. (2009). A notable difference in the diet of *I. cristatus* compared to other bufonids is the predominance of Diplopoda. These prey were the fifth most important for juveniles and third most important for adults, yet it is among the least consumed prey for several species of the genus *Rhinella* (Duré et al. 2009; Quiroga et al. 2009; Maragno and Souza 2011). Only two species of the genus *Bufo* have been reported to include in their diet large quantities of Diplopoda (Hirai and Matsui 2002; Crnobrnja-Isailović et al. 2012). The importance of three types of prey (Coleoptera, Formicidae, and Diplopoda) in *I. cristatus* may result from different factors. The high proportion of Coleoptera and Formicidae may be due to their great abundance in Cloud Forests (Nadkarni and Longino 1990; Yanoviak et al. 2007). Also, the social behavior of species in Formicidae (Sampedro-Marín et al. 2011) might facilitate their availability, while Coleoptera and Diplopoda might be eaten in large quantities owing to their size and protein content (Anderson and Smith 1998; Anderson et al. 1999). These three types of prey are, in general, distasteful to many predators because of the formic acid in species of Formicidae and the quinones in species of Coleoptera and Diplopoda (Hirai and Matsui 2002). Thus, eating these types of prey could reduce the intensity of competition for trophic resources with other species of amphibians.

The differences in the number of prey and categories of prey, as well as in the mean size differences between toad age classes and the sexes could be the result of morphological limitations, different preferences in prey, or differences in spatial location. Smaller toads (juveniles) could be limited to consuming small prey that have a larger proportion of chitin owing to their morphological limitations. To obtain the same supply of energy as they would from a large prey item (Hirai 2002), juveniles would need to consume a greater number and variety of prey (i.e., a more generalist diet). The consumption of fewer, larger prey by larger anurans (adults in this case and specifically females) has been mentioned for other species (Bonansea and Vaira 2007; Sampedro-Marín, et al. 2011), and might suggest more selective feeding habits of adults as a result of the energetic requirements of reproduction, known to be greater in the females than in the males of certain species (Finkler 2013; Finkler et al. 2014). Also, a different spatial location of some prey could influence our results. In some arthropod groups, there may be a greater abundance and species richness on the edge of vegetation fragments (Dauber et al. 2005; Villada-Bedoya et al. 2017), the same area of forest fragments studied where juvenile toads were more frequent than adult toads.

Our results indicate that occurrence of *I. cristatus* is not limited to pristine and highly conserved Cloud Forest habitats as previously reported (Santos-Barrera et al. 2010). This species can inhabit small, moderately conserved fragments of Cloud Forest. This finding underscores the importance of surveying locations that have not been recently evaluated, along with fragments of cloud forest with habitat attributes where this threatened species might be present. Additionally, long-term studies of population dynamics are needed to define the current range of the species and its risk of extinction (see Rodríguez-Contreras et al. 2008; Sandoval-Comte et al. 2012).

Finally, genetic studies of *I. cristatus* are needed to inform conservation. This is especially urgent given the tremendous fragmentation and loss of Cloud Forest in the study region (Cruz-Angón et al. 2010). This understanding would inform guidelines for captive rearing and reintroductions by helping choose appropriate stock for propagation and release to regions where *I. cristatus* has been extirpated or has low genetic variability.

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Marco Tulio Oropeza-Sánchez obtained his Bachelor's degree at Benemérita Universidad Autónoma de Puebla (BUAP), his M.Sc. Degree at the Instituto de Ecología, A. C. (INECOL) and currently is a doctoral student at the Universidad Nacional Autónoma de México (UNAM). He is interested in populations, ecology, and conservation of amphibians in tropical environments. (Photographed by Andrea Iovanna Raya-Hernández).

Eduardo Pineda obtained his Bachelor's degree at the School of Sciences at the Universidad Autónoma del Estado de México (UAEM) and his Doctoral degree at the Instituto de Ecología, A.C (INECOL), in Xalapa, Mexico. His research in the INECOL is focused on understanding the relationship between the transformation of tropical forest and biodiversity at different spatial scales, recognizing the importance of conserved areas and modified habitats to maintain the diversity of amphibians, and to assess the current situation, through fieldwork, of amphibian species in imminent danger of extinction. Currently he has several graduate students addressing topics on ecology and/or conservation of amphibians in Mexico and Latin America. (Photographed by Adriana Sandoval).

Ricardo Luría-Manzano received his B.Sc. at Benemérita Universidad Autónoma de Puebla (BUAP), and his M.Sc. at Universidad Autónoma del Estado de Hidalgo (UAEH). His research focuses on ecology and natural history of amphibian and reptile populations and communities in central Mexico. (Photographed by Ricardo Luría-Manzano).