Abundance, activity patterns and microhabitat of *Rhinella macrorhina*, an endemic toad from the cloud forests of the Colombian Central Andes

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The high proportion of declining amphibian populations and species without ecological information makes it imperative to conduct research on their population biology, natural history and habitat requirements. We describe the abundance, age structure, activity patterns and microhabitat of four populations of *Rhinella macrorhina*, a threatened toad from the cloud forests of the Andes of Colombia. We found similar abundances in all populations, despite differences in the level of alteration of the forest patches studied. Abundance of the species was higher during the rainy season and was related to leaf litter depth and leaf litter humidity. We found similar proportions of adults and juveniles among seasons in all populations, and we suggest that daily activity of *R. macrorhina* varies by age class, exhibiting a temporal and spatial segregation. We present insight into habitat alteration which may threaten the species, principally in the Amalfi locality where we report the largest documented population of the species.

Key words: age structure, anurans, habitat alteration, threatened species

INTRODUCTION

A mphibian populations have experienced catastrophic declines for several decades around the world (Houlahan et al., 2000), and have continued to decline faster than other taxa, such as mammals or birds (Stuart et al., 2004). Additionally, about 22.5% of the amphibian species worldwide are classified as data deficient, compared to only 0.8% of birds and 5.3% of mammals (Stuart et al., 2004). The high proportion of threatened and datadeficient amphibians makes it imperative to conduct research on their population biology, natural history and habitat requirements.

Understanding the association of amphibian species and their habitat is important because population parameters such as abundance and density are partially determined by environmental variables and habitat characteristics, which influence amphibian physiology, occurrence and behaviour (Allmon, 1991; Crump and Scott Jr, 1994; Marsh & Pearman, 1997). It is especially urgent to understand this association for endemic species of anurans that inhabit threatened areas such as the Andean mountains, which are predicted to be one of the most vulnerable zones of forest conversion in the future (Etter et al., 2006).

Rhinella macrorhina is an understudied toad, endemic to the cloud forests of the northern Central Andean mountains of Colombia. This species is known to occur in only four localities in the country (Trueb, 1971; Galeano & Urbina, 2004; Herrera & Castro, 2004). The habitat of this relatively large species (females: 50.20–65.45 mm, males: 34.77–44 mm) has been greatly diminished due to logging and land conversion for coffee plantations and livestock production (Etter & van Wyngaarden, 2000).

Published data for *R. macrorhina* are limited to accidental observations that describe it as a diurnal and nocturnal toad, living in steep terrain in unaltered or slightly altered forests. It has terrestrial reproduction and probably direct development (Herrera & Castro, 2004). The species was listed as Endangered by the IUCN (Bolívar & Lynch, 2004), and as Vulnerable by the Libro Rojo de los Anfibios de Colombia (Rueda-Almonacid et al., 2004), because of limited range (less than 5000 km²), limited area of occupancy (less than 500 km²) and continued decline in the quality of its habitat.

We describe the abundance, age structure and activity patterns of four populations of *Rhinella macrorhina* over two dry and two rainy seasons in one year, and in two of the four known localities of the species' distribution. We include microhabitat information for the species, and present insight into habitat alteration which may threaten the species in different localities.

MATERIALS AND METHODS

Study area

We conducted this study between May 2005 and July 2006 in four forest patches from the Amalfi and Guatapé localities, Department of Antioquia, northern Colombia (Table 1). The patches were mature and secondary forests, located at elevations between 1700 and 1975 m, and in regions with a bimodal precipitation regime (two rainy seasons and two dry seasons alternating in one year). The patches are classified in the humid premontane life

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Table 1. Description of four forest patches surveyed for *Rhinella macrorhina* in northern Colombia. The geographic coordinates, elevation, annual rainfall averages and time and type of human disturbances are summarized for each patch.

Locality	Forest patch	Coordinates	Elevation (m a.s.l.)	Annual rainfall (mm)	Time and type of human disturbance
Amalfi	Guayabito Los Canales	6°52'8.80"N, 75°6'12.5"W 6°49'46.1"N, 75°5'18.8"W	1700–1875 1750–1950	4000	15 years. Continuous forest clearance, logging, wood poaching
Guatapé	Rosita Don Alpidio	6°17'50"N, 75°7'26.5"W 6°18'10"N, 75°7'31.8"W	1850–1975 1850–1975	6500	≥10 years. Sporadic forest clearance

zone (Holdridge, 1967), and differed in size, ranging from 12 ha (Los Canales) to 1600 ha (Guayabito).

Forest patches in the Amalfi locality have been altered during the last 15 years by human activities such as forest clearance for wood commercialization and supply of local needs (i.e. fuel for cooking and material for construction). Guatapé forest patches have experienced less alteration relative to the forests in Amalfi for ≥ 10 years, mostly due to low human population density. Currently, forest patches in the Amalfi locality suffer continuous logging and wood poaching for livestock and use of wood as fuel for wood stoves. In addition, the extraction of macana palms (*Wettinia kalbreyeri*), a species used as support in tree tomato cultivation (*Cyphomandra betacea*) and as construction material (Henderson et al., 1995), represents the loss of a significant component of the forest (Borchsenius & Moraes, 2006).

Anuran survey

We sampled each forest patch for two rainy and two dry seasons in one year. We visited each patch once every season, following the same order of visits every time. During each sampling period, we randomly selected 33 transects of 25×2 m within each forest fragment for anuran surveys. Seven transects were surveyed at night between 2030 and 2300, while the other 26 were surveyed during the day between 0900 and 1700, with a sample effort of 87 person-hours at each forest patch. This sampling effort did not include the time spent setting up the data of the transect and species information. Sampling effort was greater during the day than during the night due to concerns for personal safety in some localities. Each transect was surveyed by two people walking at a constant speed while turning over leaf litter, logs and rocks, and scanning vegetation below a height of 1.8 m looking for toads.

We captured individuals by hand and placed them in plastic bags until each transect was completed. We measured snout-vent length (SVL) with digital calipers (± 0.02 mm) and recorded the date, hour, activity, substrate and height above ground for each toad captured.

We compared the abundance of R. macrorhina among forest patches and seasons by bootstrap resampling, using the observed frequencies of each transect (R Development Core Team, 2007). Bootstrap analysis was employed because the extremely skewed distribution of individuals impedes the use of parametric or nonparametric tests (Manly, 1991). We conducted R × C tests of independence to evaluate if the frequency of adults and juveniles was evenly distributed across seasons in each fragment. To differentiate adults from juveniles we used the minimum length at which males and females of R. macrorhina reach sexual maturity. The minimum length at sexual maturity for the species was identified in a previous study, using histological examination of 25 individuals representing a wide range of snoutvent lengths (Galeano & Urbina, 2003). Active spermatogenesis was used to identify sexually mature males, while the presence of yolked follicles and wide convoluted oviducts sensu Saidapur (1983) was used to identify sexually mature females. We used the measurements obtained by Galeano & Urbina (2003) to distinguish R. macrorhina adults and juveniles. All individuals with SVL between 34.7 mm and 44.0 mm (mature males), or above 50.2 mm (mature females), were categorized as adults. Individuals with SVL smaller than 34.7 mm were categorized as juveniles and those with sizes between 44.0 mm and 50.2 mm were eliminated from the analyses, as it was not possible to determine if they were immature females or mature males. We tested differences in activity hours between adults and juveniles using chi-square tests. Analyses were carried out in SAS version 9.1 (SAS Institute, Cary, N.C.).

Microhabitat

We established a 1 m² plot at each location where we found an individual of *R. macrorhina*. We measured ambient temperature (\pm 1.0 °C) and relative humidity (\pm 1%) by putting a digital thermohygrometer in the centre of each plot. We measured leaf-litter depth (\pm 1 cm) in the centre of each plot, and estimated litter humidity by collecting litter in the centre of the plot, and thereafter calculating the weight difference between dried and non-dried material. We also quantified the number of trees with a diameter at breast height of more than 10 cm, logs of more than 10 cm diameter, and terrestrial bromeliads at each plot. Additionally, we estimated canopy cover in the centre of each plot using a densiometer, and terrain slope by visual inspection establishing two categories: low (0–30°) and

			R1		 D1		R2		D2	
Locality	Errogmont	Adulta		Adulta		Adults		Adults	Juveniles	Total
Locality	Fragment	Adults	Juvennes	Adults	Juvennes	Adults	Juvennes	Adults	Juvennes	Total
Amalfi	G	12	15	3	10	5	29	4	10	88
Amalfi	LC	4	6	2	2	0	1	1	3	19
Guatapé	DA	2	11	2	9	0	9	2	14	49
Guatapé	DR	3	10	7	12	2	14	3	7	58
Total		21	42	14	33	7	53	10	34	214

Table 2. Number of individuals of *Rhinella macrorhina* captured in four forest patches located in the Central Andes (Colombia) in two rainy and two dry seasons over a year (G = Guayabito site, DA = Don Alpidio, DR = Dona Rosa, LC = Los Canales, R = rainy seasons, D = dry seasons).

high (30–45°). We identified the family of plants used as perch sites by individuals of *R. macrorhina*. These variables were selected because of their documented influence on anuran physiology, occurrence and behaviour (Scott, 1976; Fauth et al., 1989; Allmon, 1991; Crump & Scott Jr, 1994; Vonesh, 2001).

We conducted a principal component analysis (PCA) for data reduction of the microhabitat variables measured, and only considered the variables included in the first two components for further analyses. We tested the relationships among those variables and *R. macrorhina* abundance using Spearman rank correlations.

We used mixed models to analyse repeated measures of ambient temperature and relative humidity throughout the year in all forest patches. We considered plots as a random effect, and fragment and season as fixed effects. We used different correlation structures and Akaike's information criterion (AIC) or the Bayesian information criterion (BIC) to choose among the models (Littell et al., 1996). Finally we tested for differences in leaf-litter depth, leaf-litter humidity, trees and logs among forest patches using Kruskall–Wallis tests. All analyses were carried out in SAS version 9.1 (SAS Institute, Cary, N.C.).

RESULTS

Abundance patterns

We found 216 individuals of *R. macrorhina* overall, the highest number in the Guayabito fragment, Amalfi locality (Table 2). The abundance of the species was similar

Table 3. Loadings of microhabitat variables thatexplain the maximum variability for principalcomponents 1 and 2 of the PCA.

Variables	Axis 1	Axis 2
Relative humidity	0.4994	-0.007
Temperature	0.5925	0.1100
Leaf litter humidity	-0.5928	-0.1096
Leaf litter depth	0.0628	0.4000
Fallen trunks	-0.1956	0.4772
Trees DBH >10 cm	-0.0637	0.6619
Bromeliads	0.042	-0.3871

across all patches (G=5.996, P=0.1118, n=214), and was correlated with seasonality, with more individuals observed during the rainy season (G=9.746, P=0.02, n=214; Table 2). We found a similar proportion of adults and juveniles across seasons in all the populations (Guayabito χ^2 =7.69, P=0.08; Don Alpidio χ^2 =2.78, P=0.62; Dona Rosa χ^2 = 2.98, P=0.43; Los Canales χ^2 =1.41, P=1.00), with a higher number of juveniles than adults in all the fragments (Table 2). Only 52 individuals (24%) were adults, while all the others were juveniles.

Microhabitat

The two first components of the PCA explained 53% of the variability. The first component explained 37% of the variation and was a humidity–temperature component, represented by relative humidity, ambient temperature and leaf-litter humidity (Table 3). The second component explained 16% of the variance and was a vegetal component, represented by leaf-litter depth, trees and logs (Table 3).

R. macrorhina abundance was positively associated with leaf-litter humidity (R^2 =0.76, P=0.017) and negatively associated with leaf-litter depth (R^2 =-0.5221, P=0.007). These two variables and the number of trees were similar among forest patches (KW>6.73, P>0.06). We did not find an association between abundance and ambient temperature, relative humidity, trees or logs (R<0.47, P>0.1). Ambient temperature and relative humidity differed among forest patches over time (F_{Temp} =5.98, P<0.0001; F_{Hum} =7.50, P<0.0001), and similarly, the number of logs (KW=9.265, P=0.026) differed among forest patches.

Activity patterns

We observed juveniles active during both day and night, while adults were only active at night (λ^2 =4.55, *P*=0.02). Adults were usually resting in a flattened posture below the leaf-litter during the day. *R. macrorhina* individuals were principally found in leaf litter 2–6 cm deep (82%, 172 individuals), while all other individuals were found in places with depth varying from 6.5 to 14 cm. Both adults and juveniles were found at night, walking on leaf litter, or perched atop vegetation 3–70 cm above the ground. Perching vegetation included Araceae, Arecaceae, Asteraceae, Bromeliaceae and Lauraceae families, which were found at all study sites. Additional perching substrates included tree and palm roots, bark pieces,

fallen palm leaves, fallen palm bracts, and trees with exposed cavities with leaf litter and mosses.

We found similar numbers of *R. macrorhina* individuals on low (40%, 88 individuals) and high slopes (42%, 92 individuals). We found two females carrying eggs during the rainy season, but we did not find egg clutches of the species in any season. Hatchlings were observed on the ground in all the forest patches during both rainy seasons and during the first dry season of the year.

DISCUSSION

The abundance of R. macrorhina was similar across all fragments, even though forest patches differed in levels of alteration and some of the microhabitat variables considered here. R. macrorhina abundance was related to leaf-litter depth and leaf-litter humidity, supporting the hypothesis that leaf litter is important to the abundance of the species. These two variables did not differ among forest patches. Leaf-litter characteristics such as depth and humidity have been reported to be related to the abundance and density of other anuran species (Scott, 1976; Lieberman, 1986, Fauth et al., 1989; Stewart, 1995; Van Sluys et al., 2007; Whitfield et al., 2007). Leaf litter provides refuge from predation, prevents excessive drying of the frogs (Stewart, 1995) and harbours a great number of arthropods (Lieberman, 1986). Additionally, leaf-litter humidity is important for species that have terrestrial reproduction such as R. macrorhina (Herrera & Castro, 2004), which differs from other toads of the same genus with aquatic reproduction (Duellman & Trueb, 1986).

Although abundance did not differ among fragments, it was influenced by seasonal patterns, with higher abundances during the rainy season. This pattern has been observed in other leaf-litter species (Scott, 1976, Aichinger, 1987; Allmon, 1991; Duellman, 1995), and is expected as rainy seasons are related to an increase in litter volume and humidity (Lieberman, 1986).

The diurnal and nocturnal activity pattern of *R. macrorhina* was previously reported by Herrera & Castro (2004). However, we found an age-class pattern associated with this daily activity, where juveniles are active during both the day and night, while several adults were found resting during the day. This pattern was previously reported for other species of bufonids, whose juveniles tend to be heliothermic, in contrast to conspecific adults (Duellman & Trueb, 1986).

The number of juveniles found during the day on top of the leaf litter was higher than the number of adults, who preferred secretive places beneath the leaf litter. The high number of adults found beneath the litter and the low number found on top of it suggests a spatial segregation of substrates by age class. In contrast, both litter and vegetation were used by juveniles and adults during the night. Even though *R. macrorhina* was previously associated with steep habitats (Herrera & Castro, 2004), we found that neither juveniles nor adults were restricted to steep areas.

Although we found hatchlings in both rainy and dry seasons across all fragments, we found females carrying eggs only during the rainy seasons. Similar results were found by Galeano & Urbina (unpublished data) in a previous study conducted in the Amalfi locality, where six females were found carrying eggs during the rainy season. However, while these results would suggest a reproductive peak in the rainy season, it would be difficult to ascertain due to the low sample size of gravid females and lack of egg clutches.

Threats to the species and conservation

Habitat loss and degradation are some of the main threats faced by *R. macrorhina*. During this study the Guayabito forest fragment in the Amalfi locality suffered considerable loss of important structural elements such as the macana palm (Wettinia kalbreyeri). This extraction could affect leaf-litter depth, leaf-litter humidity and number of palm tree roots, characteristics associated with R. macrorhina abundance (this study), all of which provide important perching substrates and cover (Whitfield & Pierce, 2005). First, the extraction of vegetal material can lead to a reduction in canopy cover, resulting in a reduction of leaf-litter input. Second, this extraction could lead to a reduction in the number of tree buttresses for the species, such as roots, fallen palm leaves and fallen palm bracts. Third, logging affects the microclimatic conditions by increasing temperature and dryness, thereafter affecting the presence of amphibians (Marsh & Pearman, 1997).

The higher number of juveniles found across all the forest patches, and the similar proportion of adults and juveniles across seasons, suggest similar reproduction dynamics within the patches, despite the different anthropogenic pressures to which the patches are submitted. This similarity in the proportion of adults and juveniles and the similar abundance of the species across patches, suggest that the alteration of forest fragments in the Amalfi locality have not yet affected the population abundance of *R. macrorhina*. These population conditions can change in forest patches that are most threatened by human activities, such as Guayabito in the Amalfi locality, which has the largest documented population of *R. macrorhina*.

Conservation actions, such as the establishment of protected areas like the National Natural Reserve Park in Florencia in 2005 (Samaná locality) and the Arrierito Antioqueño Bird Reserve in 2006 (Anorí locality), could benefit *R. macrorhina*. However, there is no information about the current status of the populations of *R. macrorhina* in Samaná, while studies conducted in the Anorí locality suggest that the population is not in good condition. Less than five individuals were found after a search effort of more than 4800 person-hours in forest patches within the Anori locality over three years (Galeano & Urbina, 2003; Gutierrez, 2005; Molina, 2007).

Additional conservation actions should be taken to preserve the habitat of at least one of the four study populations described here. Regulation of clear cutting and wood extraction should be undertaken by the environmental authorities in charge of the Guayabito patch in the Amalfi locality. This patch is one of the few remnants with more than 500 ha of intact forest located in the northernmost part of the Central Andean mountains (Cavelier & Etter, 1995; Chaves & Arango, 1998). It holds at least seven more endemic species of anurans such as *Colostethus fraterdanieli*, *Ranitomeya opisthomelas*, *Pristimantis factiosus*, *Pristimantis lemur* and *Pristimantis suetus* (Galeano & Urbina, 2003), at least six endemic species of birds (Cuervo et al., 2008), and one endemic mammal (Delgado, pers. comm.). Therefore, any conservation action taken to protect the Guayabito patch will have a great impact, not only for the conservation of *R. macrorhina*, but also for other taxa of the Central Andean mountains.

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