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RESEARCH ARTICLE

The underground sex life of the Guatemalan Spike-thumb Frog (*Plectrohyla guatemalensis*)

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Abstract

Two populations of *Plectrohyla guatemalensis* from two different sites in Sacatepéquez, Guatemala, were monitored for three consecutive rainy seasons, and noteworthy reproductive behavior was observed. The objective of the surveys was to assess the impact of nearby high-tension power lines, but additional information could be obtained. Our observations that male vocalizations to attract females occurred in the crevices of rocks in streams, inside which females lay eggs, suggest that the species' reproduction is closely linked to the presence of such crevices. Any clutches were then guarded by the male until hatching. We inferred the existence of male-male competition for gaining control of these reproduction sites building upon previous observations, remarks about the genus and other species with similar patterns, and evidence of such aggression, although we did not directly observe any. Also, with the help of local wardens, we recorded vocalizations in mid-January, extending the previously reported reproductive period for this species. We therefore consider the conservation and restoration of rocky streams with adequate forest coverage to be an important conservation action for this species.

Keywords

Conservation, microhabitat, reproductive behavior, territoriality

Introduction

The Guatemalan Spike-thumb frog (*Plectrohyla guatemalensis* (Brocchi, 1877)) is a tree-frog belonging to the Hylidae family. It occurs in El Salvador, Guatemala, Eastern Honduras and Chiapas in the Mexican Sierra Madre (Campbell and Vanini 1989; Santos-Barrera and Canseco-Márquez 2010), and inhabits montane and cloud forests. It is listed as Critically Endangered by the IUCN Red List, mainly as a result of habitat loss and degradation (Duellman and Campbell 1992; Santos-Barrera and Canseco-Márquez 2010). *Plectrohyla* species have also been severely affected by *Batrachochytrium dendrobatidis*, a fungal pathogen involved in the decline of several frog groups worldwide (Mendelson et al. 2004; Zamora 2014).

Males of *P. guatemalensis* can grow up to a snout-vent length (SVL) of 52 mm while females can grow up to an SVL of 54 mm. Size is not a differentiating factor of sexual dimorphism for *P. guatemalensis* (Duellman and Campbell 1992), which is in line with previous reports that male and female territorial frogs have a similar size (instead of the males being substantially smaller) as an adaptation by males to aggressive behavior against other males (Wells 1978; Shine 1979). Its coloration is variable through its geographic range, spanning from a green-brown to a matte green with brown and green marks on the dorsum (Duellman and Campbell 1992). The dorsum, head and extremities have tubercules. It has a bifid prepollex, but no rostral keel (Duellman and Campbell 1992).

Records on the ecology and behavior of *P. guatemalensis* are limited. Duellman and Campbell (1992) stated that observations of the reproductive behavior of the genus *Plectrohyla* were scarce and that their behavior had to be inferred by the available observations. The few observations of amplexus were axillary. The swelling of the upper lip glands appear to be characteristic of the genus (Duellman and Campbell 1992). Scars and male size in relation to females suggests aggressive male-male interactions (Shine 1979), possibly by fighting with their prepollex. However, combat behavior has never been observed in *Plectrohyla* (Duellman and Campbell 1992).

A better understanding of the reproductive behavior and yearly patterns, competition, and habitat use of *P. guatemalensis* would both help conservation efforts that are focused on protecting crucial habitats and improve monitoring of endangered populations. To do so, *P. guatemalensis* was monitored and observed for three years in the Guatemalan highlands.

Methods

Two populations of *P. guatemalensis* were studied during the rainy season (May-November) between the years 2016–2018. One was located in Finca Carmona (WGS84 14.509861°N, 90.701077°W), Sacatepéquez and the other in a municipal park in San Bartolomé Milpas Altas (WGS84 14.603481°N, 90.695705°W), Sacatepéquez, Guatemala. Both sites were in protected areas (private and public, respectively) in

Figure 1. Rocky streams in Finca Carmona, Sacatepéquez, Guatemala, where we found a reproductive population of *Plectrohyla guatemalensis*.

montane forests between 1500 to 1800 m. a. s. l. At Finca Carmona we focused on three shorter streams and at San Bartolomé Milpas Altas we focused on one longer stream that led to a floodplain used by local farmers. The streams in Finca Carmona were very rocky (Fig. 1). The characteristics of these streams also generated turbulence in the water and maintained it oxygenated, along with several drops. In contrast, the streams in San Bartolomé Milpas Altas were smaller, not rocky and were influenced by nearby agriculture. However, they did have a similar current to those in Finca Carmona. Although the use of pesticides is prohibited, the forest cover has been reduced in a large part of the streams.

We used Visual Encounter Surveys (VES) to find individuals. Specifically, we walked along the study streams looking for adult and metamorphic individuals on leaves, branches, or rocks, while also counting tadpoles in stream pools (following Pearman et al. 1995). We also used vocalizations to attempt to locate individuals that might have been in the mating season (following Perovic et al. 2008). Additionally, we recorded behavioral observations and microhabitat preference of the individuals of *P. guatemalensis* found during the surveys. The VES started at sundown and finished around midnight. Each survey covered approximately 400 m of streams. Our surveys focused predominately on the populations of *P. guatemalensis*

and their main objective was to assess the impact of nearby high-tension power lines, but observations of behavior, microhabitat use and observations of other amphibian species were also recorded.

Results

The surveys allowed us to observe both populations of *P. guatemalensis* with local wardens for three consecutive rainy seasons, and to find all life stages of the species (Fig. 2A–D). We found *Craugastor stuarti*, *Bolitoglossa kaqchikelorum* and *P. guatemalensis* to be the only amphibian species present in Finca Carmona, while only *B. kaqchikelorum* and *P. guatemalensis* were found in San Bartolomé Milpas Altas.

During these surveys, we also made several behavioral observations. We noticed that the vocalizing frogs were usually not on the canopy, but were instead inside the crevices of rocks in the streams where we carried out the VES. In the close vicinity of these vocalizing frogs, we found egg clutches laid in the crevices submerged in the stream water (Fig. 2A). We found such individuals in nine different sites in streams in Finca Carmona. Seven of the nine sites where vocalizing males were found were located in permanent streams, while two were found in seasonal streams that disappeared during the dry season. We also found an adult male that had several scars on its dorsum and arms (Figs 2D, 3), which, based on the hypothesis proposed by Duellman and Campbell (1992), we considered to be caused by male-male competition. From one year to the next, individuals were found vocalizing in six of the nine spots where we had heard them previously. We found a male and female together only once. We also found around 200 tadpoles (Fig. 2B) each year in different stages of their larval development in the permanent streams we surveyed, as well as several metamorphic individuals (Fig. 2C). The adults and metamorphic individuals observed during each year showed an increasing trend (Fig. 4). Although no metamorphic individuals were observed during 2016, three were observed during 2017 and three during 2018. Also adult observation increased steadily, but this was attributed to the better understanding of the behavior of the species and, consequently, more efficient sampling, rather than an actual population increase.

In the case of San Bartolomé Milpas Altas, we found only four adults during the three rainy seasons in which we monitored the population: one in the second year and three in the third. We did not find any egg clutches, tadpoles or metamorphic individuals. We found a young adult, but we could not confirm that it had spent its larval stage in the stream we visited. However, two of the adults we found were only heard vocalizing from beneath the ground in the edge of the floodplain where the stream leads to.

As we progressed in our understanding of the reproductive behavior of this frog species, so our surveying success increased. Once we knew that they would spend their time in the crevices between the rocks of the streams, and confirmed that the vocalizations emanating from beneath the rocks actually came from them, we managed to increase our sightings from seven in the first year to 15 in the second year,

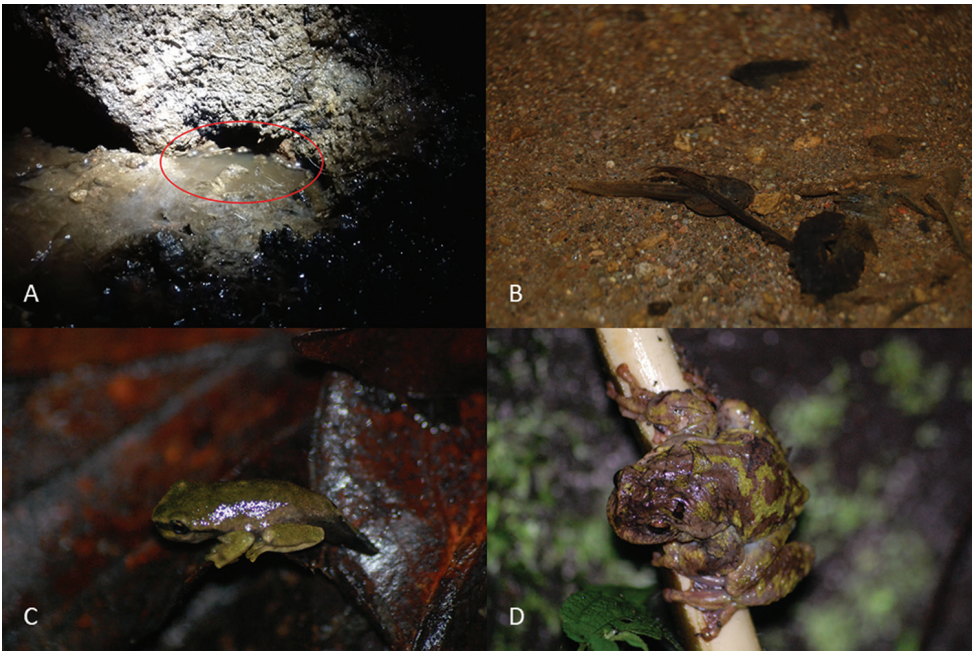

Figure 2. Life cycle of *P. guatemalensis* observed in Finca Carmona, Sacatepéquez, Guatemala A egg clutch observed submerged in a stream, attached to the bottom of a rock B tadpole of *P. guatemalensis* observed in the stream C metamorph observed in leaf litter above the stream D adult male displaying scars from male-male combat.

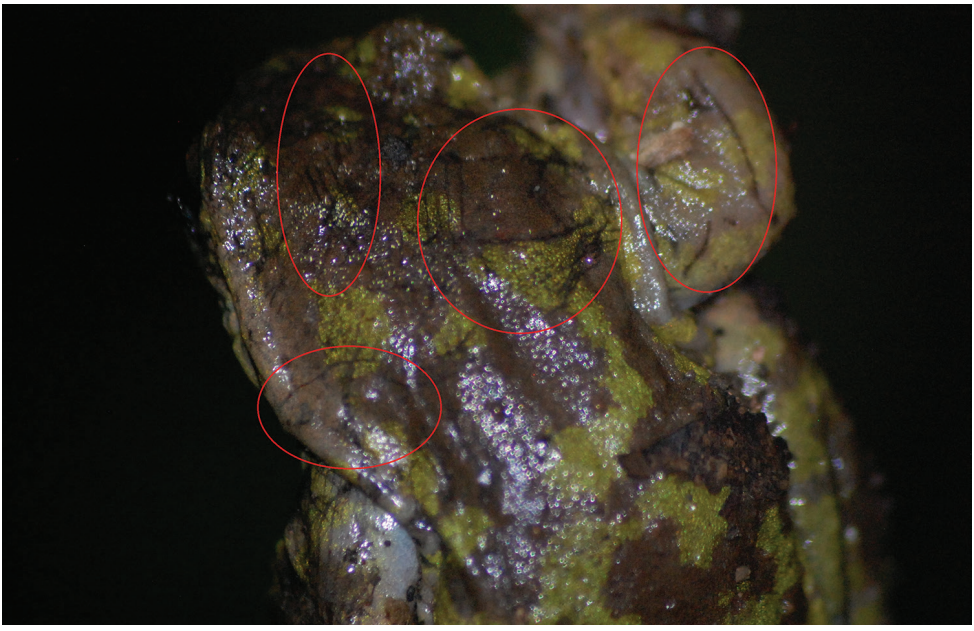

Figure 3. Adult male of *P. guatemalensis* presenting multiple scars from male-male combat with thumb spikes.

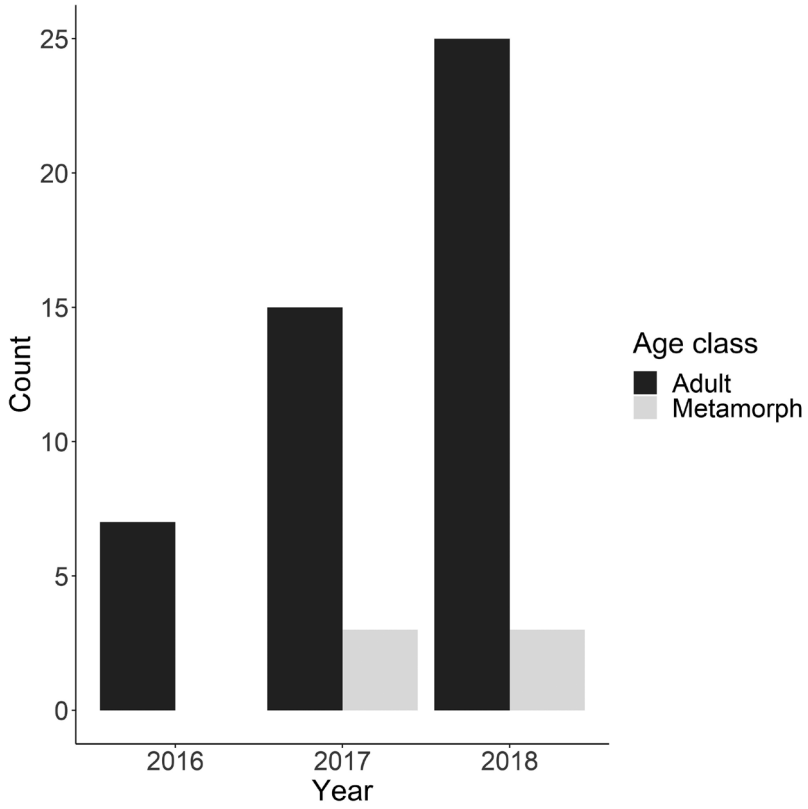

Figure 4. Adults and metamorphs found in the three years of monitoring in the population of *Plectrohyla guatemalensis* located at Finca Carmona, Sacatepéquez, Guatemala.

and then 25 in the third year of monitoring for both sites. Based on the proportion of metamorphic individuals in relation to the total number of adults observed in the population, we could determine that there was an average generational recruitment of 16.5% for the population in Finca Carmona. This showed that the population was breeding successfully in the streams at Finca Carmona.

Discussion

The observations made during our surveys give new insight into the reproductive behavior and habitat use of this species, leading us to propose that they utilize spaces such as crevices in rocks in the streams they inhabit for the purposes of attracting females, laying and fertilizing their eggs, and continued calling to attract further females. Additional to this, we also suggest that there may be competition between males to gain control of these sites, although we could not actually record observations of said competition. We elaborate below how we have reached these conclusions and how future research could further confirm them.

Our observations of the reproductive behavior of *P. guatemalensis* appear to be consistent with those made by Duellman and Campbell (1992) for several *Plectrohyla* species. Considering that mating sites could be a limiting resource for the species, competition and territoriality could be expected (Wells 1978; Shine 1979). Males seem to remain in the mating sites to guard the egg clutches they fertilize, while vocalizing for more females to arrive. The presence of scars on males leads us to propose that there is fighting between males to gain control of these sites. Similarities in SVL observed between males and females of *P. guatemalensis* is also considered to be an indication of territoriality and male-male competition in other frog species (Shine 1979), which further supports our claim of male-male aggression. We found males in some of the same sites where we had heard them vocalizing in previous years and thought that they could have a degree of fidelity to the sites where they were mating. However, we could not mark and track specific individuals of the population we observed, so we are unable to determine if the males have a fidelity to the sites, or if the sites were being used by different males from year to year.

Similar vocalization behavior and submerged egg clutches were documented for *P. guatemalensis* by McCraine et al. (1987), detailing a clutch of eggs laid beneath a cluster of roots, submerged in a stream, but our observations have allowed us to build on the reproductive behavior of the species by confirming this in other populations and linking it to microhabitat preference. Also, with the help of a local warden, we learnt that in the rainy season, male frogs in Finca Carmona begin to vocalize as early as 10:00 am. Also, local wardens in San Bartolomé Milpas Altas recorded vocalizing males as early in the year as January 17th in 2019, extending the previously reported period of reproduction of this species by Duellman and Campbell (1992).

Even though the reproductive behavior we propose for *P. guatemalensis* can be supported by the studies and observations cited above, we are aware that further research effort is needed to confirm our hypotheses. Studies marking the males of the population and tracking the mating sites they use from year to year would allow an assessment of the levels of fidelity to a site or the possible rotation of individuals within each site. Characterizing different types of vocalizations could confirm if males employ aggressive calls when in the presence of other males and if the calls actively attract females to the mating sites (Hutter et al. 2013).

Our observations on the reproductive behavior of the species also suggest a strong relationship between reproductive success and specific microhabitat characteristics. For instance, neither tadpoles nor metamorphic individuals were observed in San Bartolomé Milpas Altas, while many tadpoles were observed every year in Finca Carmona. The only remarkable difference in microhabitats that we could observe between both streams was the presence of rocks in the streams at Finca Carmona, where we could observe the frogs actively reproducing. The use of specific microhabitat breeding frogs has been reported before with important implications for their ecology (Kupferberg 1996; Hodgkinson and Hero 2001; Borzée et al. 2016), and the lack of pigmentation in the eggs of *P. guatemalensis* indicates

that they might be obligated to use crevices for oviposition to protect the eggs from adverse effects due to exposure to UV radiation (Langhelle et al. 1999). Therefore, these mating sites could be important in explaining the differences in the success of both populations, as a less suitable microhabitat is present at San Bartolomé Milpas Altas for females to lay their eggs and could be the limiting factor that drives competition in males of the species.

A final consideration we must account for is that *P. guatemalensis* appears to be a species complex that needs taxonomic resolution (Campbell pers. comm.). This implies that the reproductive behavior of the species cannot be assumed continuously in its distribution, although the observations of McCraine et al. (1987) suggest similar behavior in a population in Honduras. Any further research must consider this.

In terms of conservation, our observations of greater numbers of metamorphic individuals in Finca Carmona suggest that habitats resembling that of Finca Carmona are more favorable for population stability, as they appear to better allow reproduction maintenance of a given generational recruitment. Finca Carmona provides water to several communities that need it to irrigate their coffee plantations, so a great majority of the water is captured by tubes and the water is distributed accordingly, interrupting their natural flow. The longest stream runs for about 250 m before entering the irrigation system. A proposed solution is to ensure that the streams continue flowing in their natural course while the water continues to be harvested in lower parts of the stream so as to stabilize the hydrological conditions of the stream and minimize disruption to frog breeding sites (Kupferberg 1996). When considering the habitat characteristics of these streams, however, we propose that the presence of rocks in a stream should be considered when restoration or rehabilitation is considered as a conservation action (Kupferberg 1996; Borzée et al. 2016). Other estates in the area are also concerned with conservation of biodiversity and the restoration of rocky streams with adequate forest coverage could be important actions to contribute to the conservation of this species.

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