A peer-reviewed version of this preprint was published in PeerJ on 24 September 2015.

View the peer-reviewed version (peerj.com/articles/1274), which is the preferred citable publication unless you specifically need to cite this preprint.

https://doi.org/10.7717/peerj.1274
Temporal stability of an endemic Mexican treefrog

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The demographic characteristics of an amphibian population fluctuate independently over time, mainly in response to the temporal variation of environmental factors, especially precipitation and temperature. These temporal fluctuations may contribute to the effective size of an amphibian population and could be used to inform the current conservation status of a species. During a five year (2004-2008) period, we studied the relative abundance, sex ratio, and size-age structure of a population of metamorphosed individuals of the endemic treefrog *Hyla eximia* in Central Mexico. We also studied the species’ relationship with climatic variables such as temperature and precipitation. We found an interannual constant abundance during the study period. However, interannual differences were observed in the population structure by age-sex category (males, females, or juveniles), with increased abundance of juveniles during the rainy months (August-November). The annual abundance of *H. eximia* was positively correlated with rainfall, but negatively with monthly temperature. We found the sex ratio was male-biased (2:1) except for year 2008. Also, differences in snout-vent length (SVL) were found between years, suggesting changes in recruitment of new individuals. We conclude that variations in abundance, and frequencies by age-sex category, of *H. eximia* are related to seasonal variations in temperature and precipitation characteristics of temperate zones. However, this temporal stability may suggest that anurans have an unusual capacity to persist even in the face of human-induced habitat change.
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Introduction

The size of a population depends on various parameters such as birth and death rates, migration and immigration, and the population’s interaction with environmental factors (Krebs, 2009). Population size is a key parameter to assessing the conservation status of a species (IUCN, 2013) and changes in population size or structure may give us clues about its current status. It is also recognized that parameters such as sex and age of individuals contribute differentially to the effective size of amphibian populations (Lee, Saether & Engen, 2011).

In amphibians, population variations are determined primarily by patterns of precipitation and temperature (Blaustein et al. 2010; Rohr & Palmer, 2013). Their physiological characteristics make them highly dependent on temperature and humidity for maintenance of fitness functions (Duellman & Trueb, 1994; Pough et al., 2003). This dependence on abiotic environmental conditions is reflected in both the start and duration of reproductive activity and survival (Blaustein et al., 2010).

Amphibian population sizes naturally fluctuate within wide margins (Joseph, Pechmann & Wilbur, 1994; Houlahan et al., 2000) and it can be difficult to determine the cause of a population decrease because disturbances such as habitat loss, disease, and climate variations can play a large role. It is necessary to have long-term data to reliably determine the temporal stability of an amphibian population (Kiesecker, Blaustein & Belden, 2001).

Moreover, the sex ratio (number of males:number of females) determines patterns of competition, mate choice, and reproductive potential of a population (Krebs, 2009). The bias in the sex ratio leads to variations in reproductive events which may cause the loss of genetic variability, the fixation of mutations, and reduced population size (Cotton & Wedekind, 2009),
which may in turn increase the risk of extinction of small, isolated populations (Stelkens & Wedekind, 2010).

The age or size of the individuals also affect the dynamics of populations. While young individuals usually have lower survival rates, the recruitment of youth is key to maintaining a population and is an important measure of its conservation status. High recruitment rates may also act as a means to offset lower survival rates caused by adverse environmental conditions (Muths, Scherer & Pilliod, 2011).

Interpreting the effect of environmental changes on population dynamics is necessary to identify temporal and spatial variations of population parameters (Coulson et al., 2001).

Obtaining this information is important in planning and evaluating population studies (Hiert & Moura, 2010; Gillespie, 2011), and for the maintenance and conservation of amphibian populations. For example, knowing the temporal pattern of activity of a species is useful for determining optimal times to conduct studies and population inventories. Determining the ecology of amphibian populations is critical to the interpretation of their temporal and spatial variations, as well as for maintenance and conservation.

Hylidae species are experiencing rapid population declines (Stuart et al., 2004). In Mexico, 61 anuran species are listed as endangered, and of these, 43 (70.5%) belong to the family Hylidae (NOM-059-ECOL-2010). The endemic mountain treefrog, *H. eximia*, is partially sympatric with *H. plicata* (Smith et al., 2007) in parts of the Mexican Plateau. Both species occupy the same mating ponds in a 500-m altitudinal band (2400–2900 masl) where their distribution ranges overlap, but only *H. plicata* is considered threatened (NOM-059-ECOL-2010). *H. eximia* is not included in any red list of threatened species, however the lack of studies, and little knowledge of their ecology and distribution in the Central Mexican Plateau, where
some of the highest human-populated zones of México exist, signals a potentially dangerous
scenario for this species (Hammerson & Canseco-Márquez, 2010).

The aim of this study is to describe the abundance, size structure, and sex ratio of the
mountain treefrog *H. eximia*, a species classified as "least concern" by the IUCN Red List of
Threatened Species, in view of its wide distribution and presumed large population, however, we
feel may be at risk because there are no real population studies. We associate these population
parameters with environmental factors of temperature and precipitation to determine if there are
significant annual variations in the population structure over a period of five consecutive years.
This information can help scientists better understand fluctuations in populations of *H. eximia*
and can be used to prioritize and implement conservation efforts.

**Methods**

This study received the approval of the ethics committee of the Universidad Autonoma del
Estado de Mexico (number 3589/2013SF). All subjects were treated humanely on the basis of
guidelines outlined by the Society for the Study of Amphibians & Reptiles.

The study population is located within an oak forest of “El Pedregal de Guadalupe
Hidalgo” (19° 14´N, 99° 27´W; 2650 m altitude) in the city limits of Ocoyoacac, State of
Mexico, on the Central Mexican Plateau. The region has a sub-humid temperate climate with an
average annual temperature of 18°C. Annual rainfall is between 1400-1800 mm. The area
remains dry during the months of December to May, but during the rainy season intense rainfall
occurs in the months of July-August usually forming a temporary pond approximately 2.6 km²
which remains until November.

We visited this temporary pond every two weeks for five consecutive years (2004-2008)
to visually record the number of individuals around the margin of the pond. Each visit was
conducted by one person between the hours of 1000 and 1400. At the end of each visit some
frogs were captured to record their snout-vent length (SVL in mm), length of tibia (Lt in mm)
and body weight (g). Where possible, the sex of each individual was determined by observation
of external sexual characteristics as in Duellman (2001). Males present a dark sub-gular sac as an
external secondary sexual characteristic, while females lack this pigmentation and have a light
colored belly with homogeneous pigmentation. The reliability of this visual sex determination
was verified by dissection (presence of ovaries or testes) of 47 frogs collected in a previous study
of the same population. These 47 frogs were also measured for SVL, Lt, and reproductive or non-
reproductive status.

Temperature and rainfall data were obtained from daily records of the weather station “La
Marquesa” (19° 18' N, 99° 02' W, 3060 m altitude). For statistical analysis we used monthly
accumulated precipitation and monthly maximum temperature.

**Statistical Analysis**

Because SVL is not a good indicator of the age of the metamorphosed individuals
(Duellman & Trueb, 1994), we obtained age-sex categories of sex-body size, as defined by the
generalized analysis of k-means. This analysis grouped the frogs in k-groups based on three
continuous variables (SVL, Lt, and body weight) and color of sub-gular sac (dark or light) as a
categorical variable. The number of groups was previously defined by cluster analysis using
agglomerative Euclidean distance and Ward amalgamation algorithm. For data obtained from the
dissection of individuals, a Kruskal-Wallis test was performed to compare the SVL.

We performed ANOVA analysis to identify interannual variations in body size for each
sexual category. The reduction in mean could indicate recruitment of recently transformed
individuals in each sexual category (Donnelly, 1999). The annual sex ratio and possible interannual differences were explored with a chi-square test assuming a 1:1 sex ratio. The monthly abundance of frogs was obtained by adding up the number of individuals viewed per month in the pond. These data were adjusted by a quadratic transformation according to that described by Hiert & Moura (2010) and Zar (1984).

Temporal changes in the population structure were estimated by correspondence analysis, which is a multivariate statistical test that creates fewer dimensions of the association between two categorical variables (Gotelli & Ellison 2004). In this study, the associated variables were the sex-body size category and the month when individuals were observed. The number of dimensions that were used in interpreting the results contained at least 75% of the data variation.

The categories found nearby in the scatterplot showed a greater association (capture rate) than those pairs of categories that were found distant. The null hypothesis to be tested was the intermonthly equity of frogs recorded by sexual category. This hypothesis was contrasted with an alpha value of 0.05.

To explore interannual variation in rain or temperature, we used the Kruskal-Wallis test with monthly maximum temperature and accumulated monthly rainfall. Finally, these two variables were correlated with the frog abundance with nonparametric Spearman correlation.

Body sizes (SVL) are reported as mean ± 1SD.

Results

Population structure

Drawing on morphological and sexual variables we grouped the captured frogs into three age-sex categories: juveniles (SVL < 20 mm), adult males (SVL ≥ 20 mm and dark sub-gular), and adult females (≥ 20 mm SVL, light sub-gular; Fig. 1). Adults showed sexual dimorphism,
with females larger (28.14 ± 3.11 mm) than males (25.3 ± 2.02; Kruskal-Wallis $H_2 = 661.86$, $p = 0.001$; Fig. 2). Of all dissected *H. eximia*, all females were gravid with abdominal free oocytes, the testicles of males showed developed fatty bodies, and juveniles had immature sexual organs. These results suggest that sexual maturity and morphological differentiation is achieved when individuals of both sexes exceed 20 mm SVL.

The body size in the age-sex groups showed differences among the five years of records (ANOVA: females $F_4 = 4.39$, $p = 0.0023$; males $F_4 = 3.99$, $p = 0.0038$; juveniles $F_3 = 19.35$, $p = 0.0001$; Fig. 3). In 2007, the females had a lower body size (26.87 ± 3.39 mm) than other years; 46% of females ranged from 20.00 to 25.41 mm SVL. The predominant body size in the other years ranged from 25.41 to 36.0 mm SVL. The males had the lowest body size in 2004 (24.87 ± 1.87 mm SVL) when 94% of males were between 24-28 mm SVL. For juveniles, the lowest body size occurred in 2005 (13.51 ± mm SVL) with 93% of juveniles between 10.3 - 16.65 mm SVL.

The sex ratio (2:1) was skewed towards males during four years of records (Fig.4), but in 2008 the sex ratio was close to 1:1 ($X^2_1 = 0.033$, $p > 0.05$; Fig. 4).

### Temporal abundance

During the five years of study, 4,314 frogs were visualized in the pond with similar abundances between years (Kruskal-Wallis $H_4 = 2.64$, $p = 0.451$). The frogs were visually present for 5-6 months each year starting in June and ending in early November (Fig. 5). In general, the highest abundance was recorded from August to November, with monthly variations for each year (Kruskal-Wallis $H_5 = 11.9$, $p = 0.036$, Fig. 5).

Correspondence analysis showed seasonal variations in frequency of age-sex groups observed in the pond ($X^2_{42} = 3575.90$, $p = 0.0001$). Adults of both sexes were more frequent from
July to September, while the juveniles were more abundant in the later months, from August to November. Although apparently this seasonal pattern shows some variation between the years of study, the Kruskal-Wallis test showed no significant differences in abundance by age-sex groups between years (juveniles $H_3 = 1.45, p = 0.693$; females $H_4 = 1.05, p = 0.83$; males $H_4 = 1.83, p = 0.76$).

Considering the months that we observed frogs, the annual relative abundance of *H. eximia* was positively correlated with rainfall (Spearman $r_{58} = 0.50, p = 0.0001$), but negatively correlated with monthly maximum temperature (Spearman $r_{58} = -0.07, p = 0.0001$). The variations observed in the rainfall and monthly maximum temperature are seasonal in nature. The most notable differences were observed in periods marked by low rainfall from December to April. While rainy months were August and September (309.72 and 267 mm, respectively). The monthly maximum temperature ranged from 13.72°C to 20.76°C. The highest temperatures occurred in March and April (18.77°C and 19.95°C, respectively). Comparisons between years for both variables show no difference (temperature: $H_{59} = 0.69, p = 0.951$; rain: $H_{59} = 1.24, p = 0.87$).

**Discussion**

Amphibian populations naturally fluctuate within wide margins (Joseph et al. 1994; Houlahan et al., 2000). For example, in a study of 15 years, the abundance of Pacific tree frog (*Pseudacris regilla*) varies from 1 to 126 individuals in northwestern Nevada (Weitzel & Panik, 1993). To observe the actual range of fluctuations in abundance long-term studies are recommended (Lips et al., 2001).

In our five-year study we did not observe significant interannual variations in the abundance of frogs. Stability in the abundance of a population must also take into consideration
survival and longevity of adult individuals (Wells, 2007) that act as buffer to the negative effects of larval mortality and immigration rate during dry periods (Price et al., 2012). The greater number of males of *H. eximia*, compared to females (sex ratio 2:1) is consistent with that of most species of frogs (Duellman & Trueb, 1994). There are numerous possible causes for this bias, some of which include, late sexual maturation of females, the postponement of reproduction in females, and a possible differential survival between the sexes, such as a high survival or high mortality of males or females (Wells, 2007).

For all age-sex categories, we observed significant variations in body size. In 2005 smaller juveniles were recorded, but this was also the year with the highest number of individuals (1430). This could indicate that in 2005 there was greater success in the survival of the larvae. Among adults, we observed sexual dimorphism, with females larger than males, similar to that observed in 90% of all species of Anura (Shine, 1979). These differences in size could be related to differences in age between the sexes, and the fertility of the females (Monnet & Cherry, 2002).

The activity of amphibian species that breed in temporary bodies of water, such as *H. eximia*, is dependent on the time of flooding and the permanence of the water body. In this study *H. eximia* abundance showed a characteristic pattern of temporary abundance of temperate zones with marked seasonality of rainfall, which was constant over the five years of study.

This seasonality marks the beginning of reproduction (late May - early June) as well as differences in the abundance by age-sex categories. During June-July when the pond begins to form, the frequency of adults in the pond is low. During August-November, the number of juveniles is higher, coinciding with the highest rainfall. No unusual environmental conditions (prolonged drought or frost) were observed during the five years of study.
In this study, no differences were found in the yearly abundance of frogs *H. eximia*, but the analysis of SVL gives an indication of possible changes in the age-size structure. It would be necessary to complement the study with other techniques to establish the age and size of individuals entering the adult population.

Conclusions

We conclude that changes in abundance and distribution of the three *H. eximia* age-sex categories, are related to seasonal variations in temperature and precipitation during the year.

The ability of *H. eximia* to persist is constrained by human-induced habitat alterations of the temperate forest habitat in the Central Mexican Plateau. The small frog species, such as *H. eximia*, may be strongly disadvantaged by habitat fragmentation. The Central Mexican Plateau contains a major metropolitan area and 44.7% of this area is used for agriculture (CONAPO, 2010). Only temperate forest habitat seems to be suitable for *H. eximia*, therefore, conservation efforts should be directed to areas where suitable ecological variables prevail and especially where human encroachment affects tree microhabitat. In consequence, most of what we know about this endemic treefrog comes from occasional captures of active animals. New studies are needed, for example a population genetic study in order to provide crucial information about this treefrog to plan effectively for their conservation.

Acknowledgements

We thank all of the students of the Evolutionary Biology Laboratory for their assistance in the field and laboratory work. We also thank Ruthe J. Smith for their comments on an earlier version of the typescript. All subjects were treated humanely on the basis of guidelines outlined by the Society for the Study of Amphibians & Reptiles. C. Zepeda, M. Manjarrez-Zepeda, and J. Manjarrez-Zepeda provided moral support to JM.
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Fig. 1. Age-sex categories of *H. eximia* defined by the generalized analysis of k-means on sex-body size. Juveniles (SVL < 20 mm), adult males and adult females (SVL ≥ 20 mm).
Fig. 2. Adult sexual dimorphism in SVL of *H. eximia* (mean ± 1SD), at Ocoyoacac, State of Mexico, with females larger than males.
Fig 3. Mean body size (SVL ± 1SD) in the age-sex categories of *H. eximia* among the five years of records at Ocoyoacac, State of Mexico.
Fig. 4. Abundance of adults and sex ratio of *H. eximia* during five years at Ocoyoacac, State of Mexico.
Fig. 5. Monthly abundance of *H. eximia* at Ocoyoacac, State of Mexico, during activity season by each year of study.