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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.

Temporal stability of an endemic Mexican treefrog

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5 amphibian population and could be used to inform the current conservation status of a species.
6 During a five year (2004-2008) period, we studied the relative abundance, sex ratio, and size-age
7 structure of a population of metamorphosed individuals of the endemic treefrog *Hyla eximia* in
8 Central Mexico. We also studied the species' relationship with climatic variables such as
9 temperature and precipitation. We found an interannual constant abundance during the study
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19 habitat change.

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36 **Introduction**

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

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

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

54 Moreover, the sex ratio (number of males:number of females) determines patterns of
55 competition, mate choice, and reproductive potential of a population (Krebs, 2009). The bias in
56 the sex ratio leads to variations in reproductive events which may cause the loss of genetic
57 variability, the fixation of mutations, and reduced population size (Cotton & Wedekind, 2009),

58 which may in turn increase the risk of extinction of small, isolated populations (Stelkens &
59 Wedekind, 2010).

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

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

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

73 Hylidae species are experiencing rapid population declines (Stuart et al., 2004). In
74 Mexico, 61 anuran species are listed as endangered, and of these, 43 (70.5%) belong to the
75 family Hylidae (NOM-059-ECOL-2010). The endemic mountain treefrog, *H. eximia*, is partially
76 sympatric with *H. plicata* (Smith et al., 2007) in parts of the Mexican Plateau. Both species
77 occupy the same mating ponds in a 500-m altitudinal band (2400–2900 masl) where their
78 distribution ranges overlap, but only *H. plicata* is considered threatened (NOM-059-ECOL-
79 2010). *H. eximia* is not included in any red list of threatened species, however the lack of studies,
80 and little knowledge of their ecology and distribution in the Central Mexican Plateau, where

81 some of the highest human-populated zones of México exist, signals a potentially dangerous
82 scenario for this species (Hammerson & Canseco-Márquez, 2010).

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

91 **Methods**

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

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

102 We visited this temporary pond every two weeks for five consecutive years (2004-2008)
103 to visually record the number of individuals around the margin of the pond. Each visit was

104 conducted by one person between the hours of 1000 and 1400. At the end of each visit some
105 frogs were captured to record their snout-vent length (SVL in mm), length of tibia (Lt in mm)
106 and body weight (g). Where possible, the sex of each individual was determined by observation
107 of external sexual characteristics as in Duellman (2001). Males present a dark sub-gular sac as an
108 external secondary sexual characteristic, while females lack this pigmentation and have a light
109 colored belly with homogeneous pigmentation. The reliability of this visual sex determination
110 was verified by dissection (presence of ovaries or testes) of 47 frogs collected in a previous study
111 of the same population. These 47 frogs were also measured for SVL, Lt, and reproductive or non-
112 reproductive status.

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

116 **Statistical Analysis**

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

124 We performed ANOVA analysis to identify interannual variations in body size for each
125 sexual category. The reduction in mean could indicate recruitment of recently transformed

126 individuals in each sexual category (Donnelly, 1999). The annual sex ratio and possible
127 interannual differences were explored with a chi-square test assuming a 1:1 sex ratio.

128 The monthly abundance of frogs was obtained by adding up the number of individuals
129 viewed per month in the pond. These data were adjusted by a quadratic transformation according
130 to that described by Hiert & Moura (2010) and Zar (1984).

131 Temporal changes in the population structure were estimated by correspondence analysis,
132 which is a multivariate statistical test that creates fewer dimensions of the association between
133 two categorical variables (Gotelli & Ellison 2004). In this study, the associated variables were
134 the sex-body size category and the month when individuals were observed. The number of
135 dimensions that were used in interpreting the results contained at least 75% of the data variation.
136 The categories found nearby in the scatterplot showed a greater association (capture rate) than
137 those pairs of categories that were found distant. The null hypothesis to be tested was the
138 intermonthly equity of frogs recorded by sexual category. This hypothesis was contrasted with an
139 alpha value of 0.05

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

143 Body sizes (SVL) are reported as mean \pm 1SD.

144 **Results**

145 **Population structure**

146 Drawing on morphological and sexual variables we grouped the captured frogs into three
147 age-sex categories: juveniles (SVL < 20 mm), adult males (SVL \geq 20 mm and dark sub-gular),
148 and adult females (\geq 20 mm SVL, light sub-gular; Fig. 1). Adults showed sexual dimorphism,

149 with females larger (28.14 ± 3.11 mm) than males (25.3 ± 2.02 ; Kruskal-Wallis $H_2 = 661.86$, $p =$
150 0.001 ; Fig. 2). Of all dissected *H. eximia*, all females were gravid with abdominal free oocytes,
151 the testicles of males showed developed fatty bodies, and juveniles had immature sexual organs.
152 These results suggest that sexual maturity and morphological differentiation is achieved when
153 individuals of both sexes exceed 20 mm SVL.

154 The body size in the age-sex groups showed differences among the five years of records
155 (ANOVA: females $F_4 = 4.39$, $p = 0.0023$; males $F_4 = 3.99$, $p = 0.0038$; juveniles $F_3 = 19.35$, $p =$
156 0.0001 ; Fig. 3). In 2007, the females had a lower body size (26.87 ± 3.39 mm) than other years;
157 46% of females ranged from 20.00 to 25.41 mm SVL. The predominant body size in the other
158 years ranged from 25.41 to 36.0 mm SVL. The males had the lowest body size in 2004 ($24.87 \pm$
159 1.87 mm SVL) when 94% of males were between 24-28 mm SVL. For juveniles, the lowest
160 body size occurred in 2005 ($13.51 \pm$ mm SVL) with 93% of juveniles between 10.3 - 16.65 mm
161 SVL.

162 The sex ratio (2:1) was skewed towards males during four years of records (Fig.4), but in
163 2008 the sex ratio was close to 1:1 ($X^2_1 = 0.033$, $p > 0.05$; Fig. 4).

164 **Temporal abundance**

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

170 Correspondence analysis showed seasonal variations in frequency of age-sex groups
171 observed in the pond ($X^2_{42} = 3575.90$, $p = 0.0001$). Adults of both sexes were more frequent from

172 July to September, while the juveniles were more abundant in the later months, from August to
173 November. Although apparently this seasonal pattern shows some variation between the years of
174 study, the Kruskal-Wallis test showed no significant differences in abundance by age-sex groups
175 between years (juveniles $H_3 = 1.45$, $p = 0.693$; females $H_4 = 1.05$, $p = 0.83$; males $H_4 = 1.83$, $p =$
176 0.76).

177 Considering the months that we observed frogs, the annual relative abundance of *H.*
178 *eximia* was positively correlated with rainfall (Spearman $r_{58} = 0.50$, $p = 0.0001$), but negatively
179 correlated with monthly maximum temperature (Spearman $r_{58} = -0.07$, $p = 0.0001$). The
180 variations observed in the rainfall and monthly maximum temperature are seasonal in nature. The
181 most notable differences were observed in periods marked by low rainfall from December to
182 April. While rainy months were August and September (309.72 and 267 mm, respectively). The
183 monthly maximum temperature ranged from 13.72°C to 20.76°C. The highest temperatures
184 occurred in March and April (18.77° C and 19.95° C, respectively). Comparisons between years
185 for both variables show no difference (temperature: $H_{59} = 0.69$, $p = 0.951$; rain: $H_{59} = 1.24$, $p =$
186 0.87).

187 **Discussion**

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

193 In our five-year study we did not observe significant interannual variations in the
194 abundance of frogs. Stability in the abundance of a population must also take into consideration

195 survival and longevity of adult individuals (Wells, 2007) that act as buffer to the negative effects
196 of larval mortality and immigration rate during dry periods (Price et al., 2012). The greater
197 number of males of *H. eximia*, compared to females (sex ratio 2:1) is consistent with that of most
198 species of frogs (Duellman & Trueb, 1994). There are numerous possible causes for this bias,
199 some of which include, late sexual maturation of females, the postponement of reproduction in
200 females, and a possible differential survival between the sexes, such as a high survival or high
201 mortality of males or females (Wells, 2007).

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

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

213 This seasonality marks the beginning of reproduction (late May - early June) as well as
214 differences in the abundance by age-sex categories. During June-July when the pond begins to
215 form, the frequency of adults in the pond is low. During August-November, the number of
216 juveniles is higher, coinciding with the highest rainfall. No unusual environmental conditions
217 (prolonged drought or frost) were observed during the five years of study.

218 In this study, no differences were found in the yearly abundance of frogs *H. eximia*, but
219 the analysis of SVL gives an indication of possible changes in the age-size structure. It would be
220 necessary to complement the study with other techniques to establish the age and size of
221 individuals entering the adult population.

222 **Conclusions**

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

225 The ability of *H. eximia* to persist is constrained by human-induced habitat alterations of
226 the temperate forest habitat in the Central Mexican Plateau. The small frog species, such as *H.*
227 *eximia*, may be strongly disadvantaged by habitat fragmentation. The Central Mexican Plateau
228 contains a major metropolitan area and 44.7% of this area is used for agriculture (CONAPO,
229 2010). Only temperate forest habitat seems to be suitable for *H. eximia*, therefore, conservation
230 efforts should be directed to areas where suitable ecological variables prevail and especially
231 where human encroachment affects tree microhabitat. In consequence, most of what we know
232 about this endemic treefrog comes from occasional captures of active animals. New studies are
233 needed, for example a population genetic study in order to provide crucial information about this
234 treefrog to plan effectively for their conservation.

235 **Acknowledgements**

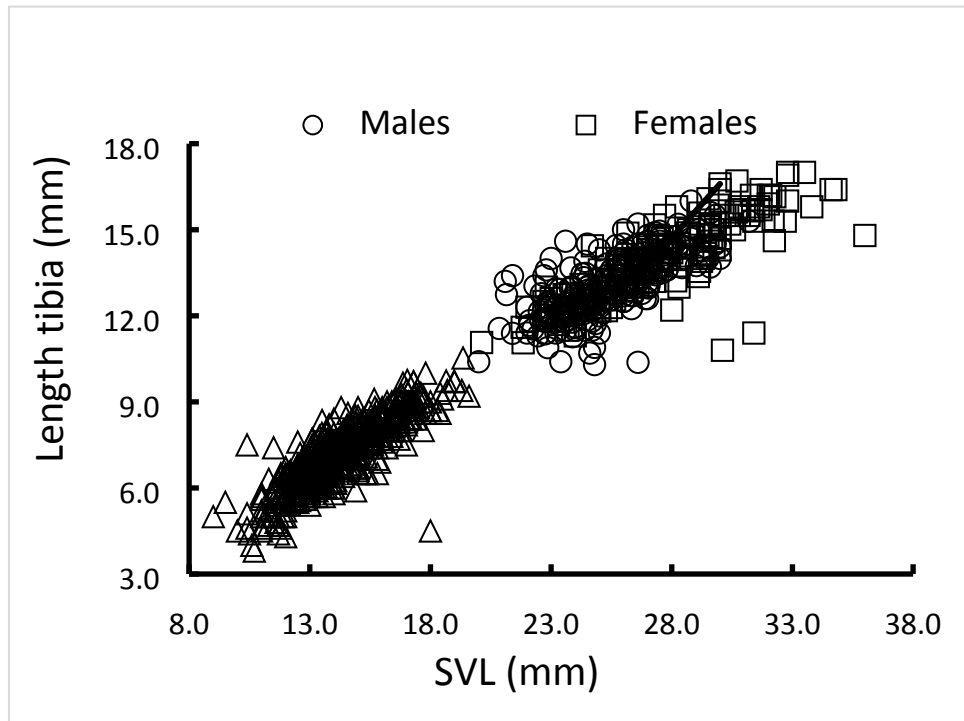
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313 Fig. 1. Age-sex categories of *H. eximia* defined by the generalized analysis of k-means on sex-

314 body size. Juveniles (SVL < 20 mm), adult males and adult females (SVL \geq 20 mm).

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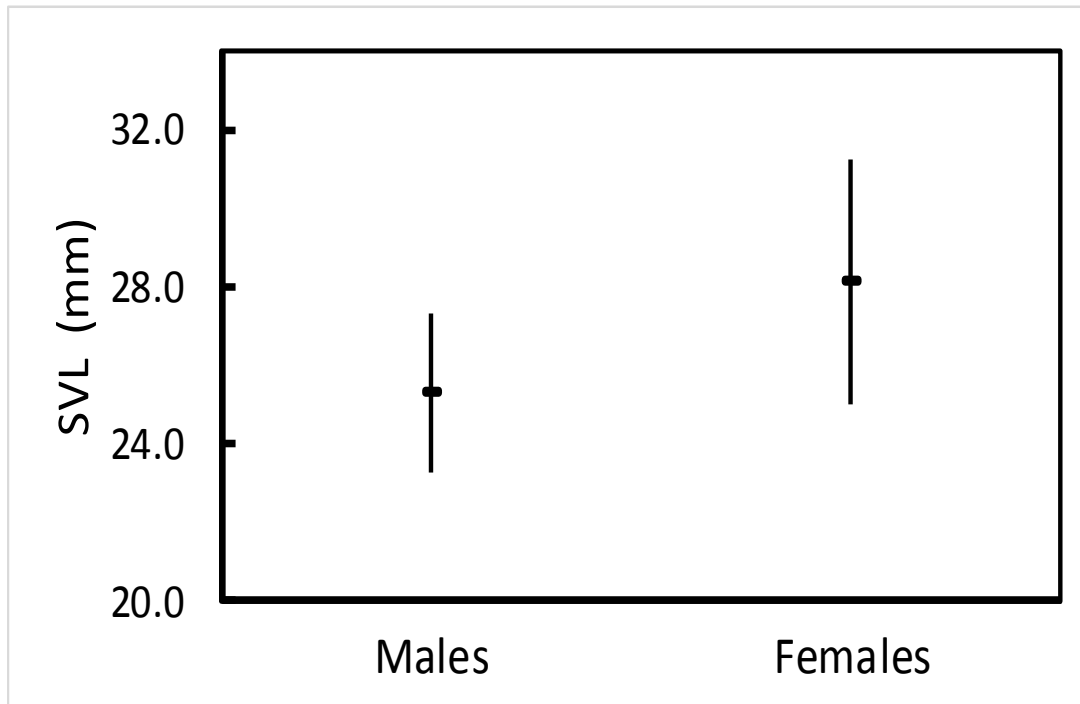
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325 Fig. 2. Adult sexual dimorphism in SVL of *H. eximia* (mean \pm 1SD), at Ocoyoacac, State of
326 Mexico, with females larger than males.

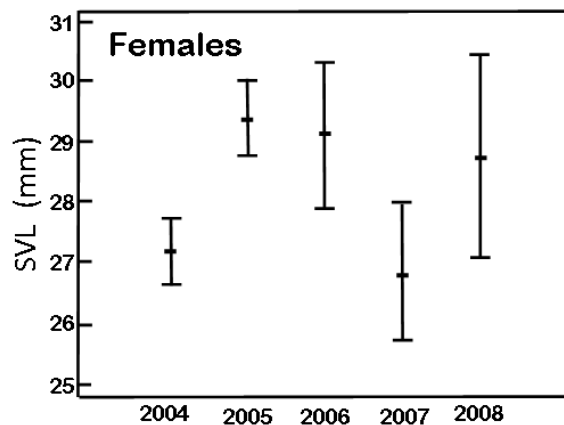
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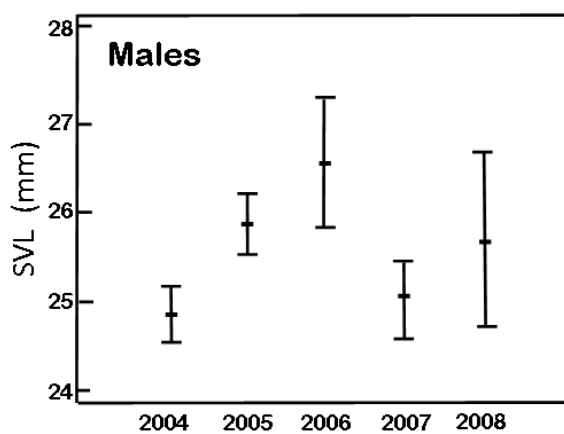
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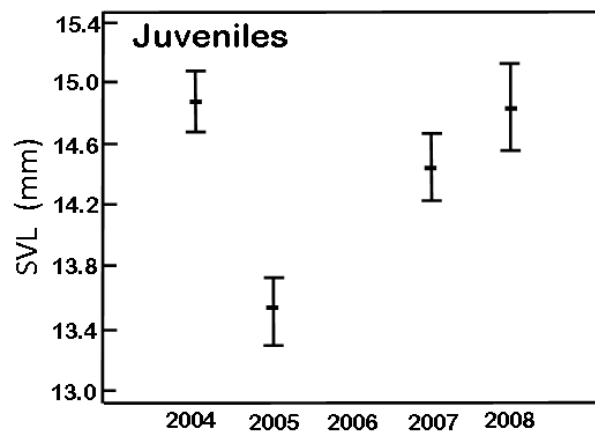
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334 Fig 3. Mean body size (SVL \pm 1SD,) in the age-sex categories of *H. eximia* among the five
335 years of records at Ocoyoacac, State of Mexico.

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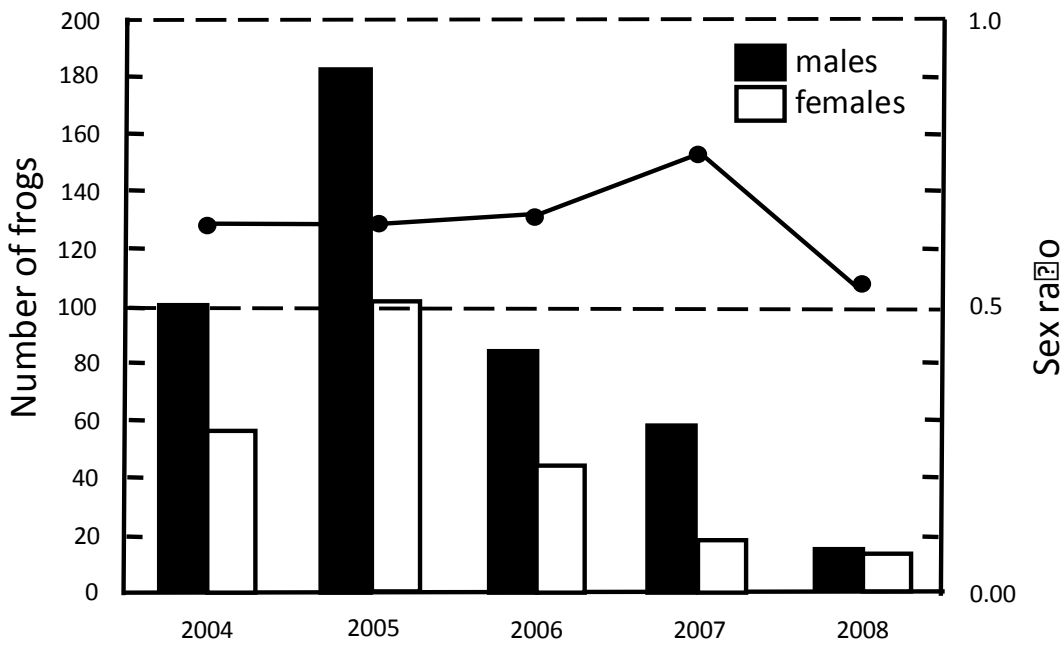
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348 Fig. 4. Abundance of adults and sex ratio of *H. eximia* during five years at Ocoyoacac, State of

349 Mexico.

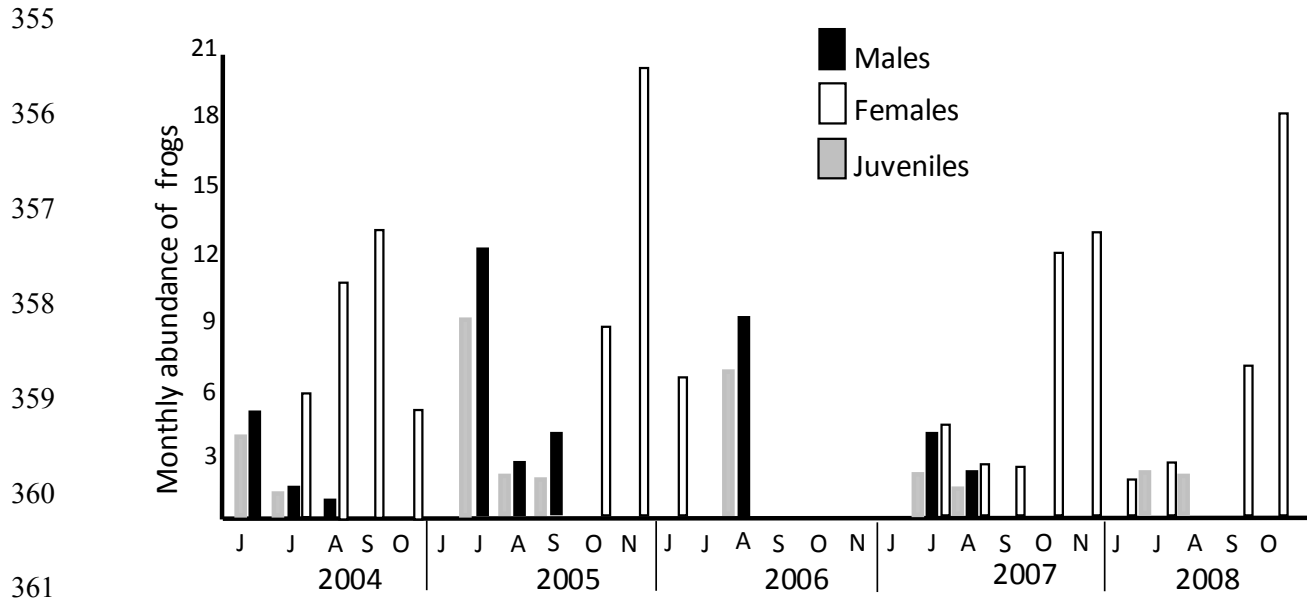
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364 Fig. 5. Monthly abundance of *H. eximia* at Ocoyoacac, State of Mexico, during activity season

365 by each year of study.

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