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Ecology of the Plateau Tiger Salamander (*Ambystoma velasci*) in the Biosphere Reserve Sierra Gorda-Guanajuato, Mexico

Ecología del ajolote del Altiplano (*Ambystoma velasci*) en la Reserva de la Biosfera Sierra Gorda-Guanajuato, México

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***Abstract.—Ambystoma velasci*, an endemic axolotl species of Mexico, faces significant conservation challenges. Classified under Special Protection by Mexican regulations, this species is among the 18 endemic axolotl taxa at risk. Unfortunately, there are only a few ecological studies for this species, and aspects of their populations are generally unknown hindering effective conservation strategies. The present study addresses this gap by providing demographic insights on a population of *A. velasci* within a temperate environment of the Sierra Gorda Biosphere Reserve, Guanajuato (SGBR-G). The population exhibits facultative paedomorphosis and it comprises four age classes: hatchlings, juveniles, subadults, and metamorphosed adults, subadults were the most abundant class. Significant differences were observed in the occupation of the microhabitat and activity patterns across these age classes. Key threats to this population include the extraction of specimens, water contamination, and siltation of the primary pool, which is a critical refuge, breeding site, and foraging area. Given its symbolic importance to the SGBR-G and its ecological role, *A. velasci* warrants priority status in regional conservation programs. Long-term monitoring initiatives are essential to track population dynamics and inform effective management strategies for this species.**

Keywords. Ambystomatidae, ecology, PNA, conservation, Xichú.

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***Resumen.—Ambystoma velasci*, una especie de ajolote endémico de México, enfrenta serios retos de conservación. Clasificada bajo la categoría de Protección Especial, conforme a la normatividad mexicana, esta es una de 18 especies de ajolotes endémicas en riego. Desafortunadamente, existen pocos estudios sobre la ecología de esta especie, y sus aspectos poblacionales son en general desconocidos, afectando una estrategia de conservación efectiva. El presente estudio aborda esta deficiencia proporcionando información demográfica sobre una población de *A. velasci* en un ambiente templado de la Reserva de la Biosfera Sierra Gorda, Guanajuato (RBSG-G). La población es paedomórfica facultativa y se compone de cuatro clases de edad: crías, juveniles, subadultos, y adultos con metamorfosis, siendo los subadultos, la clase más abundante. Se observaron diferencias significativas en la ocupación del microhábitat y patrones de actividad**

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en las clases de edad. Las principales amenazas para la población incluyen la extracción de ejemplares, la contaminación y asolvamiento de la poza principal, la cual es un sitio de refugio, crianza y alimentación de la especie. Dada su importancia simbólica para RBSG-G y su función ecológica, *A. velasci* deber ser una prioridad dentro de los programas de conservación. Iniciativas de monitoreo a largo plazo son esenciales para el registro de las dinámicas de esta población y así poder desarrollar estrategias de conservación efectivas para esta especie.

Palabras Claves: Ambystomatidae, ecología, ANP, conservación, Xichú.

Introduction

One of the main causes of loss of biological diversity is the significant modification of the primary elements of the landscape (water, soil, and vegetation), which with time lands on the decrease of populations of species that are particularly sensitive to changes (Badii et al. 2015; Parra-Olea et al. 2014), such as amphibians, which in Mexico are represented by near 423 species (Balderas-Valdivia and González-Hernández 2023; Ramírez-Bautista et al. 2023), and of those, 43% are in critical risk (Biaggini et al. 2018; García-Padilla et al. 2021; Parra-Olea et al. 2014; SEMARNAT 2018), reflected in negative effects in their reproduction, ecology, distribution, and the expression of bacterial and fungal infections associated to unhealthy habitats (Ballesteros-Barrera et al. 2022; Cruz-Elizalde et al. 2022; Leyte-Manrique et al. 2022). An example of species highly susceptible to habitat changes are the ambystomatids (Ambystomatidae), locally known as ajolotes, and comprised by 30 to 33 species (Frost 2025) with 18 distributed in Mexico (Everson et al. 2021; Herpetología Mexicana 2025; SEMARNAT 2018). They occur from Chihuahua, Sonora, and Coahuila (Gatica-Colima et al. 2024; Hossack et al. 2022; Lemos-Espinal and Smith 2007) to the Trans-Mexican Volcanic Belt in Puebla and Guerrero (SEMARNAT 2018). Within the ambystomatids, *Ambystoma mexicanum* has been a study model for a variety of research lines in the last three decades, such as physiology, embryonic development, genetics, reproduction, and diet (Ávila-Akerberg et al. 2021); however, there are a few studies regarding its ecology (Ávila-Akerberg et al. 2021; SEMARNAT 2018; Zambrano-González et al. 2003).

Another species is *Ambystoma velasci*, which has the largest distribution area in the country, inhabiting a variety of environments and latitudinal gradients in the states of Chihuahua, Nuevo León, Jalisco, Michoacán, Colima, Querétaro, Guanajuato, San Luis Potosí, Morelos, Puebla, Estado de México, Tlaxcala, and Hidalgo (Ávila-Akerberg et al. 2021; Gatica-Colima et al. 2024; Frost 2025; SEMARNAT 2018). Within this ample distribution range, the species is more specifically found in lakes and reservoirs with cool temperatures at elevations from 1800 to 2400 m, primarily in the Trans-Mexican Volcanic Belt region (Ávila- Akerberg et al. 2021). Populations in Guanajuato are found in streams and natural pools in pine and oak forests with typically low ambient temperatures in northeastern region, towards the Sierra Madre Occidental in the limits with the states of Querétaro and San Luis Potosí at temperatures between 10 and 20 °C (Báez-Montes 2018; De la Cruz-Beltrán et al. 2017; Leyte-Manrique and Domínguez-Laso 2014; Leyte-Manrique et al. 2015), and also in the southern portion of the state (Báez-Montes 2018; Hernández-Arciga et al. 2018). Adults tend to be active during the night and day, and the juveniles and hatchlings at night (Leyte-Manrique and Domínguez-Laso 2014). Information regarding their reproduction in the wild is rare; however, researchers such as Juárez-López and Garza-Castro (2008) stated that females of *A. velasci* from Tejomulco lagoon in Hidalgo have oocytes in summer. Also in Hidalgo, Ramírez-Bautista et al. (2014), recorded clutch sizes of up to 100 eggs. For Sierra Gorda in Guanajuato, De la Cruz-Beltrán et al. (2017), reported the presence of 50 eggs in January. In the case of other members in the genus *Ambystoma*, information on clutch size has been reported as well. For example, Aguilar-Miguel et al. (2009) recorded clutch sizes for *A. granulosum* in laboratory conditions that spanned from 17 to 1,187 eggs and for *A. lermaense* from 110 to 1,691 eggs.

With regards to *A. velasci*, even though it is a widely distributed species, most of its life history is practically unknown, including information regarding ecology, demography, and any other data pertinent to its conservation (Molina-Vázquez 2010; Morales-García et al. 2021; SEMARNAT 2018). The state of Guanajuato has considerable industrial, farming, and mining activities with increasing negative impacts on the native flora and fauna (Leyte-Manrique and Alejo-Iturvide 2024; Leyte-Manrique et al. 2022). Furthermore, these activities also affect ajolote populations, since they are highly sensitive to changes in their immediate surrounding environments (Guerrero de la Paz 2018; Guerrero de la Paz et al. 2020), and *A. velasci* particularly could be extirpated before its natural history and its importance in the ecosystems are fully known (Leyte-Manrique and Alejo-Iturvide 2024). Importantly, the presence of *A. velasci* is reported in Reserva de la Biosfera Sierra-Gorda-Guanajuato (RBSG-G; Báez-Montes 2018; Cruz-Elizalde et al. 2022; Hernández-Arciga et al. 2018; Leyte-Manrique et al. 2022); however, few works have addressed aspects of its life history, been mostly anecdotical (Leyte-Manrique 2022; Leyte-Manrique and Alejo-Iturvide 2024). Given the current panorama for this species in the state of Guanajuato, the generation of information

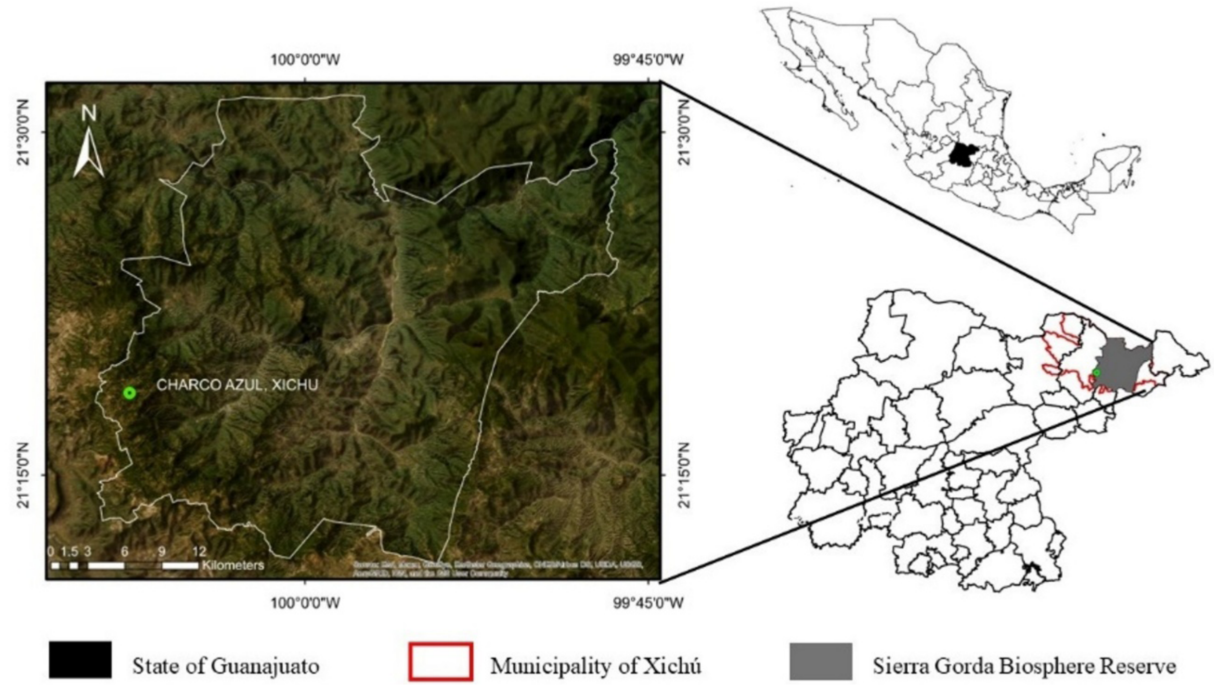

Fig. 1. Study site and location of the *A. velasci* population in Reserva de la Biosfera Sierra Gorda-Guanajuato (RBSG-G).

would be vital for its effective conservation and its use as bioindicator, and a flag species, particularly for RBSG-G. The main goals of this study were: 1) to determine the seasonal cycles of the population structure of *A. velasci*, 2) to characterize the species microhabitats selected according to their developing stage, and 3) to determine the relationship between activity, body temperature, and microhabitat selection by sex and age class.

Materials and Methods

Study site

This study took place at El Ocotero "Charco Azul" (21.309972° N; 100.127611° W; datum WGS84; Fig. 1), in the municipality of Xichú, Guanajuato, embedded in the nucleus of Reserva de la Biosfera Sierra Gorda-

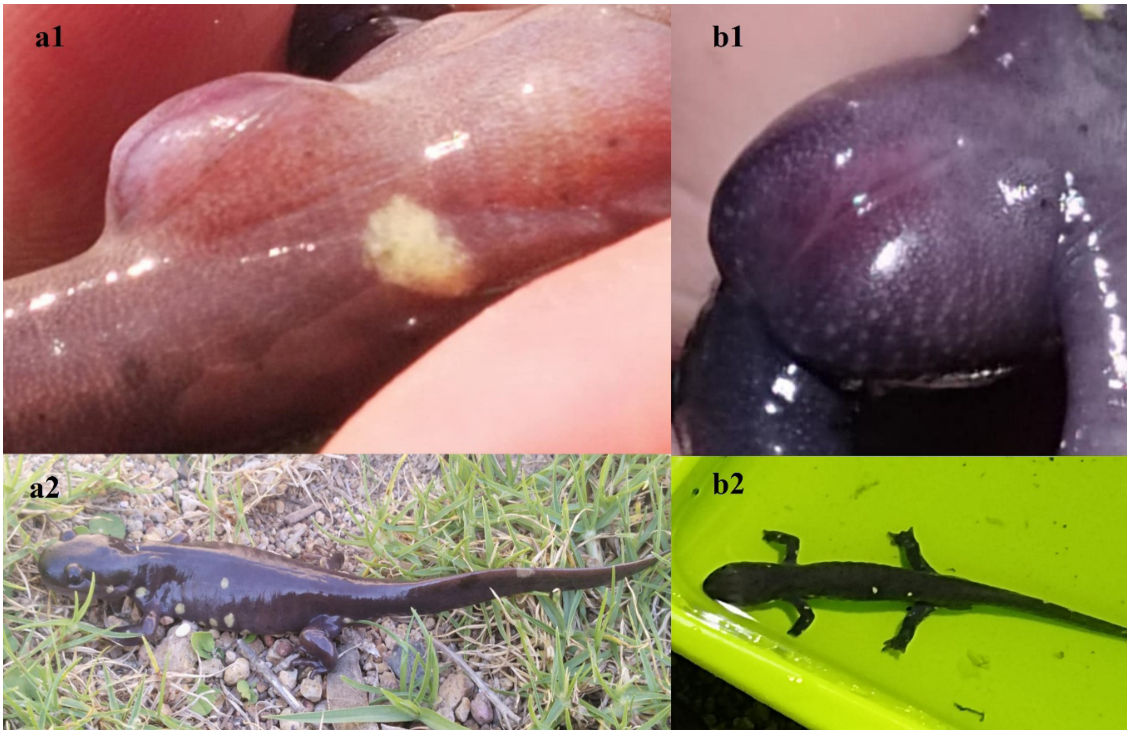

Fig. 2. Cloacal swelling present in reproductive males and females of *A. velasci*; **a1** – swelling in female, **a2** – adult female; **b1**- swelling in male (notice the dark coloration when compared to the female); and **b2** – reproductive male. *Photos by Adrian Leyte Manrique*

Guanajuato (RBSG-G). The elevation at the area ranges from 2237 to 2383 m, and the climate is temperate subhumid with rains in summer C(w2) and C(w2)(w), according to Köppen's climate classification and modified by García (2004). Annual mean temperature is 16 °C, with a minimum of 5° and a maximum of 28 °C, while annual precipitation is 670 mm (García 2004). The vegetation is represented by pine forest, oak forest, cedar forest, xerophytic scrub and submontane scrub, with areas containing crops associated to ponds and reedbed (Leyte-Manrique and Domínguez-Laso 2014; Rzedowski 2006). The ponds in Charco Azul are fed by streams that run from the Mezquital and Manzanares rivers that connect to the Santa María river in the Xichú basin (INEGI 2009; Walter and Brooks 2009).

Field Work. Data from *A. velasci* were gathered in January, and from May to December 2013 by direct observation (Casas-Andreu et al. 1991; Scrocchi and Krezschmar 1996). Animal search was carried out in streams and ponds, and surrounding vegetation from 0900 to1200 h, and from 1700 to 2400 to cover the ample activity spectrum of the salamanders (see Aréchaga-Ocampo, 2025; Guerrero de la Paz 2018; Guerrero de la Paz 2020). Organisms were handled briefly with a fishing net or directly by hand and released at the collecting sites immediately after body measurement (snout-vent length [SVL]) and body temperature were recorded and age class determined (Leyte-Manrique et al. 2022). Body temperature was obtained by placing the tip of a thermometer on the external area of the cloaca. Reading was taken withing seconds immediately after capturing the specimens. Additionally, information such as microhabitat type, ambient temperature, and water temperature were also recorded. The scientific collecting permit SGPA/DGVS/09749/2012 was granted to ALM by the General Directorate of Wildlife of SEMARNAT.

Sex and sexual maturity determination. *Ambystoma velasci* is a species that present facultative paedomorphic stages (Barriga-Vallejo 2021; Pough et al. 2016; Vitt and Cadwell 2014) and some difficulty might exist when identifying females that are sexually mature. More information on these aspects would prove beneficial for telling the sexes apart and obtaining a better identification of the class age. To address this complication, we followed Krebs and Brandon (1984) and Aguilar-Miguel et al. (2009) by observing reproductive morphological characteristics, such as cloacal swelling or physiological hyperplasia (Krebs and Brandon 1984; Ramírez-Macal et al. 2022), which is more pronounced in males than females (Figure 2). The reproductive stage in males was classified in three categories: 1) non-reproductive (hatchlings, juveniles, and aquatic subadults), 2) reproductive aquatic paedomorphs, and 3) reproductive paedomorphs (aquatic-terrestrial). These categories were based on Barriga-Vallejo (2021) and adapted to this study incorporating SVL (mm) the reproductive status for males and the presence of cloacal swelling metamorphosis of individuals. Furthermore, individuals with non-apparent cloacal swelling but with similar size to well identified males, were classified as adult females (Fig. 2.).

Definition and characterization of age classes. Age classes were defined and characterized based on anatomical features associated to SVL to minimize the number of confusing classifications and inconsistencies in the values that estimate total body length used in other studies (e.g., Valencia-Vargas and Escalera-Vázquez 2021; Zambrano et al. 2015). Consequently, age classes were assigned as follows: I) hatchlings (n = 18), with SVL of 50 mm or less (generally with anterior and posterior limbs absent); II) juveniles (n = 78) from 51 to 70 mm (with all limbs fully developed); III) subadults (n = 210), from 71 to 90 mm (initial reduction of gills with rounded tips and reduced caudal fin [Semlitsch and Wilbur, 1988]; with cloacal swelling not visible); IV) adults (n = 368), from 91 to 120 mm (cloacal swelling is visible in males, both paedomorphic or metamorphic [Aguilar-Miguel et al 2009; Barriga-Vallejo 2021; Krebs and Brandon 1984]). In the study herein, we do not use the term "larva" which was used in previous studies (e.g., Barriga-Vallejo 2021; Guerrero de la Paz et al. 2020; Semlitsch and Wilbur 1988) because technically juveniles and subadults would also be larval stages if we strictly follow the concept of paedomorphism (see Kardong 2012; Pough et al. 2016; Pough et al. 2022; Vitt and Cadwell 2014); that is, the term "larva" in paedomorphic species refers to the larval characteristics that remain present in individuals while reaching sexual maturity, which is not the case in *A. velasci* because it is metamorphic when becoming sexually mature (Leyte-Manrique and Alejo-Iturvide 2024).

Microhabitat and activity. Air temperature (Ta), water temperature (Tw), and body temperature (Tb) data were acquired using a digital thermometer (-10 to 80 °C). The type of microhabitat and time of observation (Actv) were also recorded. Microhabitats were organized in four categories according to García et al. (2016) based on their proximity to a body of water: 1-sediments (stream edge and ponds), 2-rocks (stream and ponds), 3-reedbed, and 4-grass.

Data analysis. Considering that abundance, activity time, and microhabitat selection of *A. velasci* can be an ontogenetic differential response, we assessed differences between sexes using a U Mann-Whitney test for independent non-homogenous samples (Zar 1999). For age classes and season (wet and dry season) a nonparametric ANOVA test was used (Kruskal-Wallis) since the samples were not uniform (Balzarini et al., 2015). On the other hand, a linear regression was used for sexes and age classes with the goal of identifying relationships with body, water, and activity time.

Niche overlap. Niche overlap between sexes and age classes was estimated using Pianka's index (Pianka 1986): Ojk = Σi PijPik / Σi Pij Σi; where Ojk = Pianka's value for niche overlap between species (in this case between sexes and between age classes) j and the species (in this case sexes or age classes) k (j and k), Pij = proportion of resource i is the total resources used by species j, Pik = proportion of resource i, is the total resources used by species k, n = total number of resources. Values for this index go from 0 (= no overlap) to 1 (= complete overlap). For this analysis we used the software Ecological Methodology, version 2 (Krebs 1999).

Lastly, we analyzed the relationship of the population with respect to microhabitats used, activity, and patterns between seasons through a Principal Component Analysis (PCA) (Balzarini et al. 2015). The variables considered were the following: body temperature = Tb, water temperature = Tw, activity = Actv, and age classes: I-hachtlings, II-juveniles, III-subadults, and IV- adults. We used the software InfoStat version 2.1 for this analysis.

Results

Abundance and sex proportions

The data in this study came from 368 individuals. We found that the size distribution of the adults (sexually mature, paedomorphic and/or metamorphic) did not show significant differences in sex ratio (U = 47.2; p = 0.81; females; SVL = 98.05±1.93 [90-120]; males: SVL = 98.8±2.31 [83-117). The highest abundance of reproductive adults (10 females and 16 males) was recorded in July, while the lowest abundance was observed in January (one male) and December (one female) (Fig. 3).

With respect to body temperature (Tb), microhabitat temperature (Ta+Tw), microhabitat type (Tmh), and activity (Actv) no significant differences were observed between sexes (Tb, U = 46.1, p = 0.27; Tmh, U = 45.0, p= 0.4; Mh, U = 45.3, p = 0.29; Actv, U = 39.0, p = 0.5). On the other hand, a positive correlation was observed between Tb and Tmh for both sexes for the wet season (females: r = 0.90; p = 0.001; males: r = 0.69; p = 0.001) and for the dry season (females: r = 0.62; p = 0.01; males: r = 0.59; p = 0.01) (Fig. 4). Furthermore, a positive relationship was observed between Tb and Tmh for all age classes (hatchlings: r = 0.75, p = 0.05; juveniles, r = 0.63, p = 0.01; subadults, r = 0.73, p = 0.01; and adults, r = 0.72, p = 0.001. On the other hand, a negative relationship was observed between Tb and Actv in adults (r = 0.26, p = 0.01), and subadults (r = 0.42, p = 0.01), but not in hatchlings (r = 0.009, p = 0.25) and juveniles (r = 00.3, p = 0.12).

Population structure: 368 individuals were grouped into four age classes: hatchlings (18, 4.89%), juveniles (78, 21.19%), subadults (210, 57.06%), and adults (68; 18.47%). The average SVL for the population was as follows: hatchlings (SVL = 49.5±2.2 [30-69]); juveniles (SVL = 66.2±1.05 [30-69]), subadults (SVL = 81.5±0.35 [70-90]) and adult paedomorphic and/or metamorphic (females, n = 33; males, n =35; SVL = 96.8±1.07 [83-120]) The subadults were the most abundant (n = 210), while the hatchlings were the least abundant (n =18). The subadults were most abundant in July, followed by October, and September, but in August only one individual was recorded (Fig. 5).

The population of *A. velasci* showed significant differences between age classes (Kruskal-Wallis; H = 43.5; p = 0.001) with respect to activity during the wet

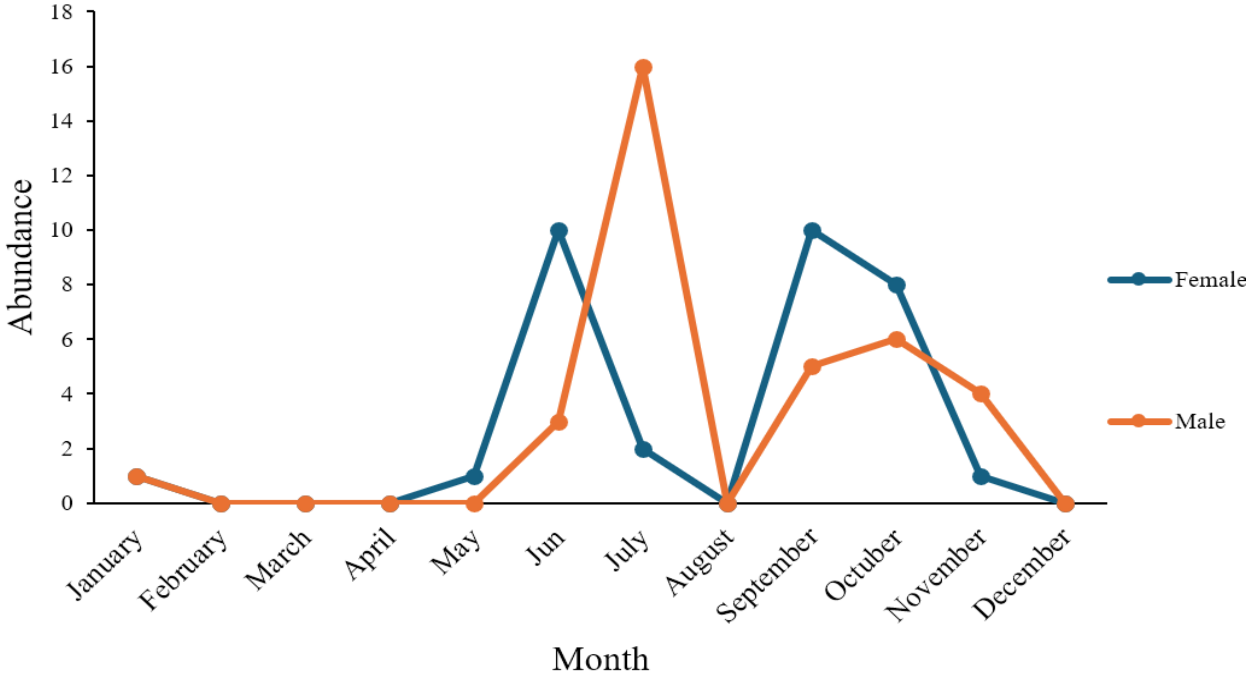

Fig. 3. Monthly abundance of reproductive male and female (both paedomorphic and metamorphic) individuals of *A. velasci* in the study site.

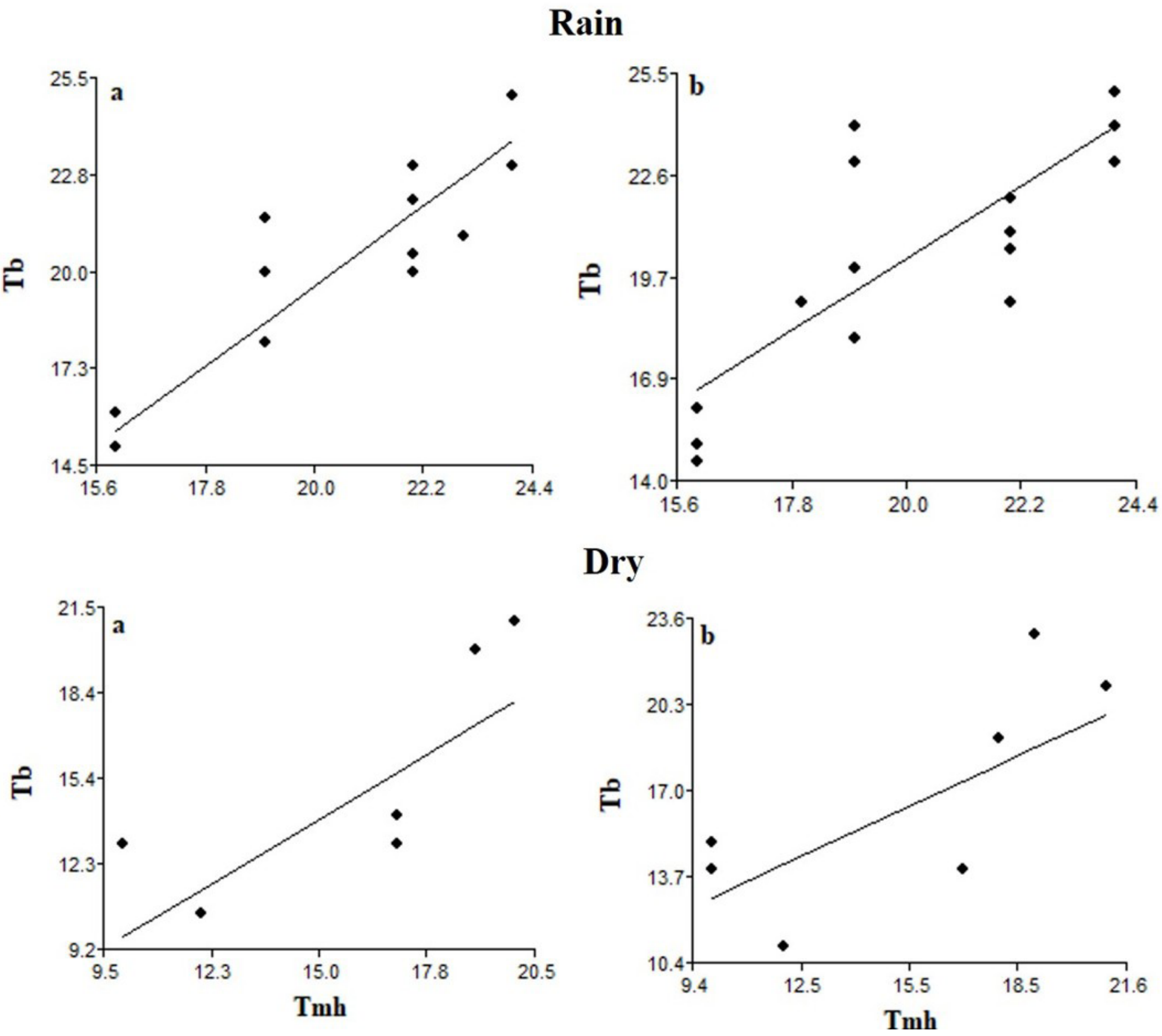

Fig. 4. Correlation between body temperature (Tb) and microhabitat temperature (Tmh) between females **(a)** and males **(b)** of *A. velasci* by season (Rainy and Dry) in the study site.

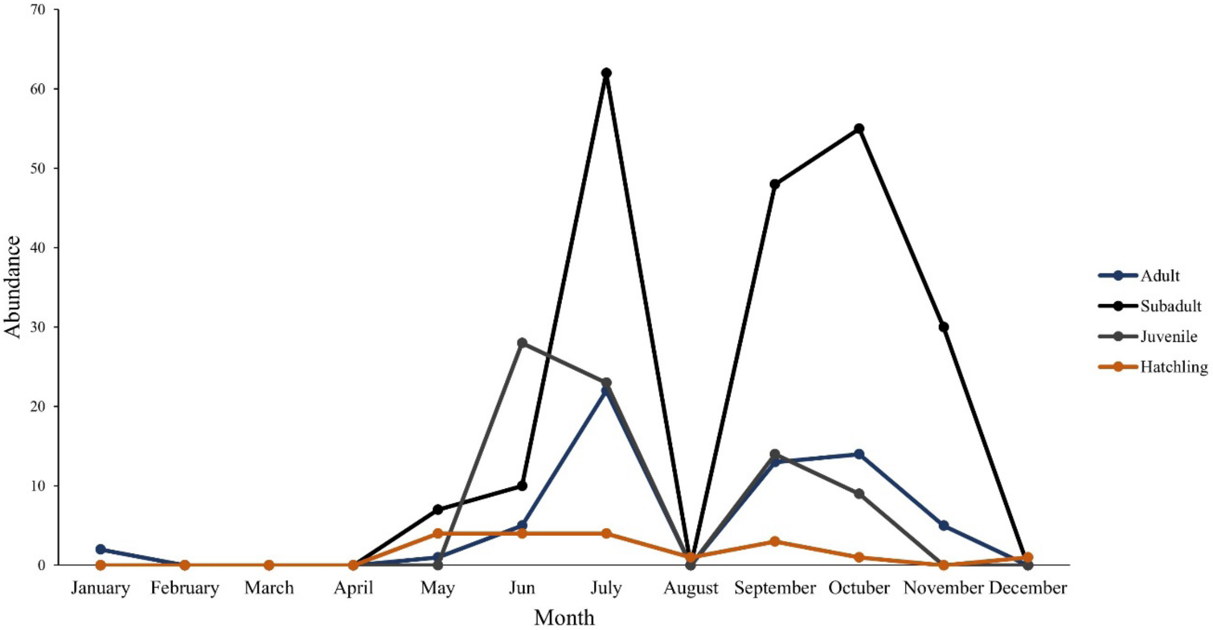

Fig. 5. Monthly abundance of *A. velasci* by age class in the study site.

season but not during the dry season ($H = 5.74$; p = 0.12). However, there were no observed differences in Tb, Tmh, and Mh between size classes by season (Table 1).

Microhabitat preference

Males and females preferred sediments in streams and ponds with no significant differences (U-Mann-Whitney, $U = 42$, $p = 0.9$, x = 1.1±0.1; 1-4), being able to use rocks, reedbed, and grass (in the metamorphic state). Significant differences were observed by age class for the wet season (Kruskal-Wallis, $H = 11.8$, $p = 0.01$; adults, x = 1.3±0.10 [1-3]; subadults, x = 1.1±0.03 [1-3]; juveniles, x = 1.5±0.10 [1-3]; hatchlings, x =1.6±0.23[1-3]), but that was not the case for the dry season ($H = 0.44$, $p = 0.66$). The microhabitat mostly used in both seasons was sediments in stream edge and ponds, and reedbed and grass were used only during the wet season (Fig. 6).

Niche overlap. According to the overlap index values for the wet season, hatchlings, juveniles, subadults, and adults showed an overlap for sediments from 0.22 to 1.0, while juveniles and adults showed an overlap for rocks of 1.0, while the least overlap was observed for reedbed and grass. With respect to the dry season, the highest overlap value was shown for hatchlings and juveniles (1.0), while the least overlap was for subadults and adults (0.20).

The PCA showed the correlation between environmental variables, body temperature, and activity between age classes by season (Table 2). For the wet season CP1 explained 0.57% of the relationship, while CP2 explained 0.24%, and the total relationship was explained by 0.81%. For CP1 the variables that showed high correlation between age class were Actv, Tb, Ta, and Tmh; and for CP2 was Mh (Figure 7); with a total correlation of 0.96. With respect to the dry season, CP1 explained 0.63% of the relationship, and CP2 only 0.18%, with a total relationship for both components of 0.81%. The dry season showed a high correlation for Tb, Actv, Ta, and Tmh in CP1, and Mh in CP2, with a total correlation of 0.96% (Table 1; Fig. 7).

Discussion

An important aspect of this study is that it sheds light on the abundance of *A. velasci* (368 individuals) a number similar (or higher) to other species of ambystomatids. For example, 136 ajolotes were reported for *A. andersoni*

Table 1. Kruskal-Wallis analysis and mean value ± standard error of thermal and environmental variables for age classes by season. Tb = body temperature, Tmh = microhabitat temperature, and Mh = microhabitat. Activity (Actv) refers to the time of the day when individuals are active, and * denotes statistical significance.

	Rainy					
	Hatchling	Juvenile	Subadult	Adult	*H*	*P*
Tb	19.5±0.60 (14-22)	19.1±0.33 (14-25)	19.1±0.3 (14-27)	19.4±0.54 (14.5-25)	0.86	0.82
Tmh	18.2±0.39 (16-22)	18.9±0.31 (16-26)	19.4±0.28 (16-26)	19.7±0.48 (16-24)	2.18	0.41
*Mh	2.35±0.32 (1-4)	2.2±0.12 (1-4)	2.15±0.08 (1-5)	2±0.13 (1-6)	11.8	0.01
*Actv	1955±48.6 (1020-2245)	1739±48.6 (940-2300)	1456±26.1 (1020-2245)	1434±44.5 (915-2155)	45.5	0.001
	Dry					
	Hatchling	Juvenile	Subadult	Adult	*H*	*P*
Tb	14	14.7±1.23 (10-22)	13.9±043 (9-23)	15.3±0.81 (10.5-23)	4.95	0.17
Tmh	17	14±1.01 (10-18)	14.3±0.58 (10-29)	15.2±0.84 (10-21)	2.20	0.51
Mh	2	1.36±0.15 (1-2)	**2.36±0.17 (1-6)**	1.76±0.10 (1-2)	3.11	0.66
Actv	1700	1501±07.7 (1022-2203)	1754±34.6 (1030-2230)	1719±58.4 (1200-2300)	5.74	0.12

Note. Only one hatchling was recorded during the dry season; therefore, it was not included in the mean value.

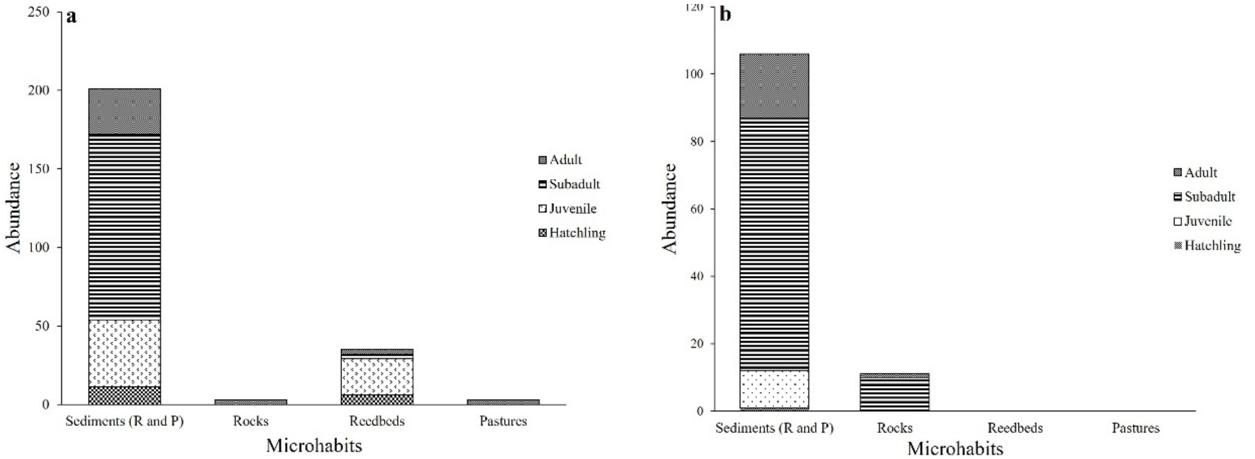

Fig. 6. Seasonal microhabitat preference of *A. velasci* by sex and age class. **a** = rainy season, and **b** = dry season.

Table 2. PCA, correlation between ajolote body temperature (Tb), activity (Actv) ambient temperature (Ta, Tmh), and age classes in each season.

	Rain		Dry	
Variables	CP1	CP2	CP1	CP2
Tb	0.89	0.27	0.88	0.09
Actv	0.61	0.56	0.75	0.15
Ta	0.89	0.19	0.95	0.17
Tmh	0.94	0.10	0.85	0.27
Mh	-0.19	0.88	-0.42	0.88
Correlation	0.960		0.967	

(Valencia-Vargas and Escalera-Vázquez 2021), 190 for *A. ordinarium* (Calderón et al. 2011), 161 for *A. leorae* (Sunny et al. 2014), 247 and 306 for *A. altamirani* (Guerrero de la Paz et al. 2020; Lemos-Espinal et al. 2016, respectively); however, the data in these studies are also influenced by their specific sampling methods, therefore, a standardization would be required for a more in-depth analysis. While sex ratios in the populations of *A. velasci* are 1:1 as predicted by Fisher (1930; see Vitt and Caldwell 2014), future studies along its geographic distribution, employing improved sex identification techniques, are needed for greater accuracy.

In relation to physiological aspects such as thermoregulation, ambystomatids are organisms well adapted to bodies of water with typically low temperatures (Ávila-Akerberg et al. 2021); this is the case for *A. velasci* in Sierra Gorda de Guanajuato, where individuals optimize their thermoregulation according to the season (Fig. 4). Both males and females from Charco Azul did not show significant differences with respect to body temperature, which indicate they are not thermoconformers; that is, their body temperature is not influenced so much by the water column, but more by the microhabitat type, such as the substrate and/or

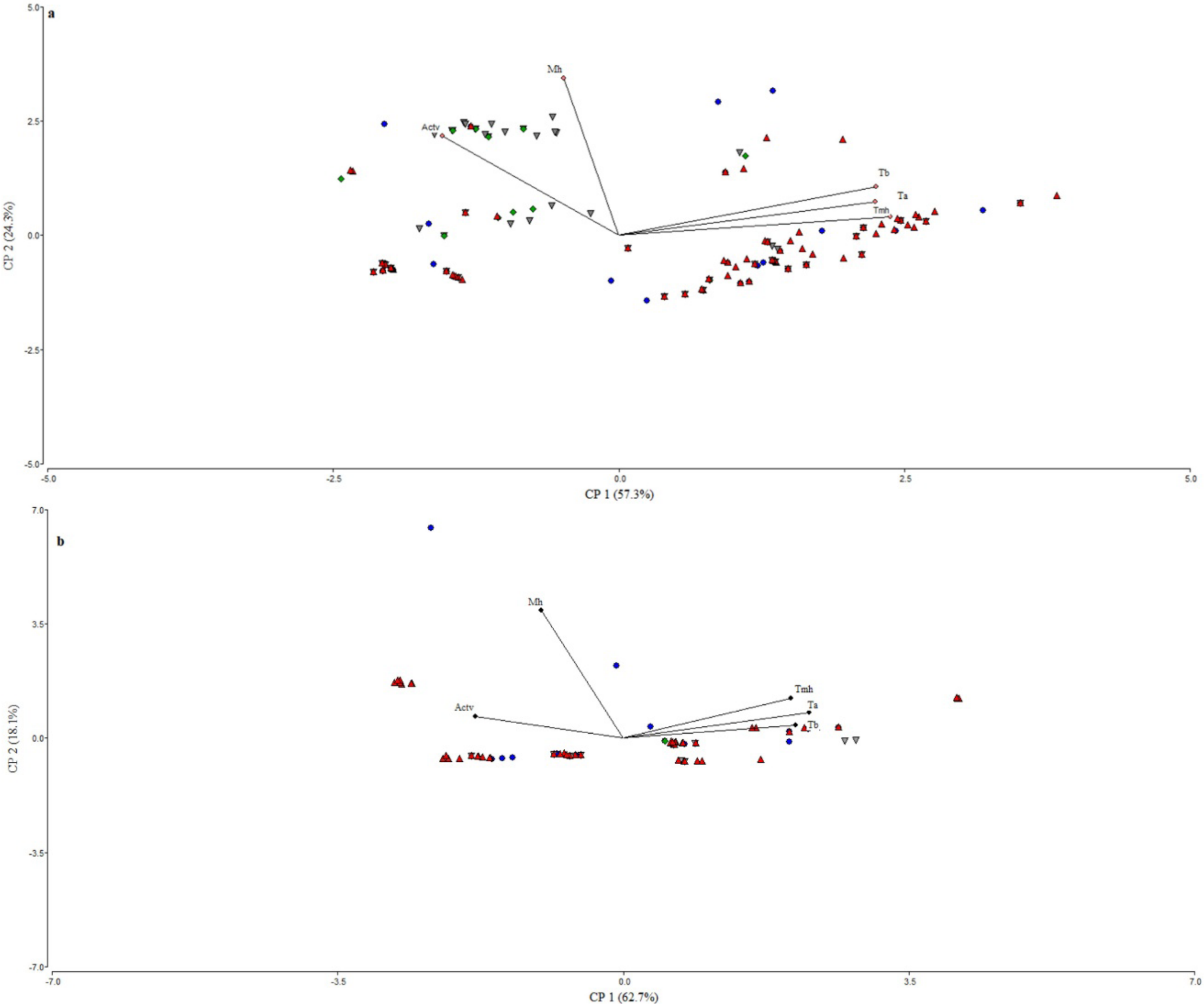

Fig. 7. Principal Component Analysis of variables associated with microhabitat with respect to age classes of *A. velasci*. Adults = blue circle, Subadults = red triangle, juveniles = gray triangle, and hatchlings = green diamond. **a** = Rainy; **b** = Dry.

rocks, and consequently being rather thigmothermic (Sanabria et al. 2003a and b; Vitt and Caldwell 2014). As one could expect in *A. velasci*, the body thermal pattern follows the environmental one as does other ectothermic vertebrates (Pough et al. 2022; Fig. 4). This brings up the concerns of how these salamanders as well as the rest of the amphibians will be able to cope with rising global temperatures, which presumably affect reproduction, development, and the ability to battle diseases that benefit under warm conditions (Bosch et al. 2018; Hof et al. 2011; Luedtke et al. 2023). This warning goes beyond simple assumptions, since after the depauperating and complete loss of suitable habitats, the effects of global climate change are the next threat, particularly in the neotropics given their notable biological richness (Luedtke et al. 2023).

On the other hand, the four age classes suggested for *A. velasci* represent a less arbitrary designation and delimitation of the development stages since they are associated with size and sexual maturity. These designations also allow for a more practical interpretation of the population structure even though somatic features are absent in paedomorphic species such as *A. velasci*. In this regard, it is not suitable to use the term "larva" to delimit specific stages in the ontogeny of these ajolotes. In the animal kingdom "larva" and "adult" are distinct morphs, and in this case with their own life habits (Kardog 2012). However, "larva", in amphibians paedomorphic lineages refers to a stage, in this case to a larval stage, with the understanding that the juveniles features will remain at sexual maturity, and therefore, without the adult morph according to the designations by Vitt and Caldwell (2014), Kardog (2012), Pough et al. (2016), and Pough et al. (2022). Nonetheless, we can keep the term "adult" as a reproductive stage, but not necessarily as a morph. Currently, we cannot confirm that the proportions observed in the age classes are ecological constants at the study site, since data for February and April are still lacking. Overall, our data on population structure showed proportions (4.89% hatchlings, 21.19% juveniles, 57.06% subadults, 16.86% adults) that are different to other species in the genus. For example, in *A. altamirani*, Guerrero de la Paz et al. (2020) reported 64.7% of adults and 35.22% distributed in other classes resulting in almost opposite proportions. However, data from this study is the first reference for *A. velasci*. On the other hand, as seen in many species, *A. velasci* is more abundant in summer, the season with more resources and favorable thermal conditions (Figure 5), when biomass, water flow, and food availability increase concurrently in the ecosystem (SEMARNAT 2018; Vitt and Caldwell 2014).

Since *A. velasci* is a species that tend to be found in ponds, streams, and lakes of temperate ecosystems, the microhabitats that it uses are rocks, sandy substrate, and aquatic vegetation (Morales-García et al 2021; SEMARNAT 2018). For Charco Azul, Xichú, four microhabitats were identified, being rocks and sandy sediments the most frequently used in both dry and wet seasons, and reedbed and grass also were used in the wet season. Consequently, we would expect that the ontogenetic and seasonal factors would reveal differences regarding microhabitat use between sexes and between age classes; however, that was not the case likely due to the relatively homogeneous habitat where ajolotes use the few available microhabitats. This pattern was generally observed in other species of the genus, such as *A. mexicanum* (Morales-Valdes and Serna-Lagunes 2021), and *A. ordinarium* (Soto-Rojas 2012); however, the latter species can use a wider array of microhabitats. In this regard, it is important to consider that the microhabitats used by ambystomatids will depend on the particular conditions of the bodies of water that they inhabit, as well as the seasonal changes that these habitats experience. Furthermore, an additional factor is the ability of individuals to move among microhabitats, such as *A. velasci*, that move away from water and use grass and cavities as shelters but always in proximity with water sources (Leyte-Manrique and Alejo-Iturvide 2024).

With respect to niche overlap between age classes, this was low for the wet season, likely caused by ample availability of diverse microhabitats. Additionally, sediments at the bottom, rocks, reedbed, and grass provided important refuges, resulting in high overlap for the use of rocks between age class and between sexes, especially in the dry season. In this regard, Morales-Valdes and Serna-Lagunes (2021) found a similar pattern for *A. mexicanum*, revealing a high niche overlap, likely influenced by the homogeneous characteristics of the ponds and streams, inferring that perhaps the use of microhabitat likely depends on the water thermal factor and the ability of these organisms to thermoregulate (Vitt y Caldwell 2014).

The Principal Component Analysis indicated that the relation of the variables with the body temperature and microhabitat temperature explains the behavior between the age classes in the wet and dry seasons. Adults and subadults showed a remarkable relationship with their thermal capacity, for both the wet and dry season. In this regard, while thermal conditions strongly determine activity patterns and microhabitat selection, their effect on resource availability, such as food and shelter, appears to be minor (Leyte-Manrique et al. 2018).

Conclusions

The results presented herein offer a novel and more comprehensive interpretation of key biological parameters defining the life history of *A. velasci*. This species, despite its extensive geographic range, remains relatively poorly characterized. Additionally, we have established population structure categorization criteria that can be applied to other ambystomatid taxa.

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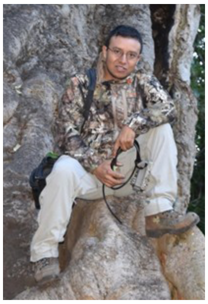
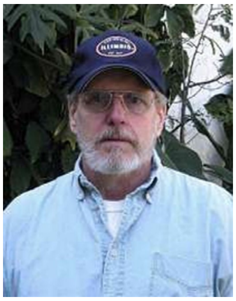


Oscar Báez-Montes earned a B.S. and M.S. from the Universidad de Guadalajara. His interests include the ecology and conservation of terrestrial vertebrates and their relationship to human communities. He has worked with diverse groups of fauna in areas of conservation importance such as priority terrestrial regions, Ramsar sites, and natural protected areas in the Mexican Plateau and Western Mexico. He is currently a full-time professor at Universidad Autónoma de Guadalajara and also is President of the Board of Directors of the College of Professionals in Biological and Environmental Sciences of Jalisco, A.C.



Larry David Wilson was a renowned herpetologist with lengthy experience in Mesoamerica who passed away from leukemia on 28 April 2024. He was born in Taylorsville, Illinois, USA, and received his university education at the University of Illinois at Champaign-Urbana (B.S. degree) and at Louisiana State University in Baton Rouge (M.S. and Ph.D. degrees). He authored or co-authored 488 peer-reviewed papers and books on herpetology. Larry was the senior editor of *Conservation of Mesoamerican Amphibians and Reptiles* (2010) and a co-author of seven of its chapters. His other books include *The Snakes of Honduras* (1985), *Middle American Herpetology* (1988), *The Amphibians of Honduras* (2002), *Amphibians & Reptiles of the Bay Islands and Cayos Cochinos, Honduras* (2005), *The Amphibians and Reptiles of the Honduran Mosquitia* (2006), and *Guide to the Amphibians & Reptiles of Cusuco National Park, Honduras* (2008). He was also the co-author of 16 entries in the Mexican Conservation series, which dealt with the herpetofauna of the states of Michoacán, Oaxaca, Chiapas, Tamaulipas, Nayarit, Nuevo León, Jalisco, Puebla Coahuila, Hidalgo, Veracruz, Querétaro, Tabasco, Guanajuato, and the Baja California Peninsula, as well as the tri-state Mexican Yucatan Peninsula. In addition, he was a co-author of several significant publications on the development and extensive application of the EVS measure and on conservation issues related to the Mexican and Central American herpetofaunas. He authored or co-authored the descriptions of 76 currently recognized herpetofaunal species, and six species have been named in his honor, including the anuran *Craugastor lauraster*, the lizard *Norops wilsoni*, as well as coccidian parasite *Isospora wilsoni.* In 2005 he was designated a Distinguished Scholar in the Field of Herpetology at the Kendall Campus of Miami-Dade College by the then-campus president Dr. Wasim Shomar. Larry also served as a Co-chair of the Taxonomic Board for the website Mesoamerican Herpetology.