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Distribution of *Ambystoma altamirani* (Caudata: Ambystomatidae) in relation to biotic and abiotic factors in its habitat

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ABSTRACT

Introduction: The mountain stream axolotl (*Ambystoma altamirani*) is an endemic amphibian of Mexico cataloged as a priority species for conservation with a very restricted distribution in the Sierra de las Cruces. Anthropogenic activities currently endanger it.
Objective: To determine the habitat factors most associated with its presence to propose conservation strategies.
Methods: Four sites in Villa del Carbon, State of Mexico, were sampled monthly for one year (September 2022 to August 2023) with the presence and absence of *A. altamirani*. Environmental, physicochemical, bacteriological, physicochemical, and biotic parameters of the water were randomly analyzed.
Results: A total of 1 186 organisms were censused, and it was determined with a CCA that the variables most related to the populations were solar radiation, total coliforms, percentage of oxygen saturation, UV radiation, fecal coliforms, and temperature. It was observed that the habitat is not being adequately conserved. The populations studied showed a decline during the study. The sites with the highest population abundance showed an increase in the presence of adults, mainly females. It is essential to focus conservation actions on this umbrella species and maintain the variables mentioned above with little disturbance to allow the prevalence of the species.
Conclusion: This information can be useful in developing conservation actions for the habitat, which it shares with other endemic and endangered amphibians. 1) It is urgent to protect this species due to the fragility of its populations and rapid decline; 2) It is necessary to propose conservation strategies that are adapted to the localities studied and 3) This will help to prioritize the protection of microendemic species in local environments.

Key words: *Ambystoma altamirani*; endemic amphibian; water quality; wildlife conservation; extinction risk.

RESUMEN

Distribución de *Ambystoma altamirani* (Caudata: Ambystomatidae) en relación con factores bióticos y abióticos en su hábitat

Introducción: El ajolote arroyero de montaña (*Ambystoma altamirani*) un anfibio endémico de México es catalogado como una especie prioritaria para la conservación y tiene una distribución muy restringida en la Sierra de las Cruces. Actividades antropogénicas actualmente suponen un riesgo para la especie.
Objetivo: Determinar los factores de su hábitat más asociados a su presencia para plantear estrategias de conservación.

Métodos: Se muestrearon mensualmente por un año (septiembre 2022 a agosto 2023) cuatro sitios con presencia y ausencia de la especie *A. altamirani,* en Villa del Carbón, Estado de México. Se analizaron aleatoriamente parámetros ambientales, fisicoquímicos, bacteriológicos y bióticos del agua.
Resultados: Se censó un total de 1 186 organismos y se determinó mediante un ACC que las variables que más se relacionaron con las poblaciones fueron: radiación solar, coliformes totales, porcentaje de saturación de oxígeno, radiación UV, coliformes fecales y temperatura. Se observo que el hábitat de los organismos no se está conservando adecuadamente y las poblaciones estudiadas mostraron declive durante el estudio. Los sitios con mayor abundancia poblacional mostraron un incremento en la presencia de adultos, principalmente de hembras. Es fundamental enfocar las acciones de conservación en esta especie sombrilla, y mantener las variables antes mencionadas con poca perturbación, para permitir la prevalencia de la especie.
Conclusión: Esta información puede resultar útil para desarrollar acciones de conservación del hábitat, que además comparte con otros anfibios endémicos y en peligro. 1) Urge proteger esta especie debido a la fragilidad de sus poblaciones y rápido decrecimiento 2) Se necesita proponer estrategias de conservación que se adecuen a las localidades estudiadas; y 3) Esto ayudará a priorizar la protección de especies micro endémicas en ambientes locales.

Palabras clave: *Ambystoma altamirani*; anfibio endémico; calidad del agua; conservación de vida silvestre; riesgo de extinción.

INTRODUCTION

Mexico is positioned as the fifth country in amphibian richness with a very high level of endemism; however, amphibians are the most endangered vertebrates worldwide (Hoffmann et al., 2010) and are currently suffering the worst extinction crisis in their history; 43 % of Mexican species are considered threatened or critically endangered (Parra-Olea et al., 2014), as is the case of amphibians of the genus Ambystoma (Parra-Olea et al., 2011). In an analysis carried out by the IUCN, it was determined that the most important factor for the decrease in Mexican amphibian populations is deforestation and transformation of vegetation; this problem is affecting areas such as the Villa del Carbón mountain range, which is the current habitat of *Ambystoma altamirani* (Demetrio & Martínez, 2020). However, more studies are needed to have the necessary information to qualify the degree of threat to many species of Ambystomidae in Mexico and propose appropriate conservation strategies (Frías-Álvarez et al., 2010; Heredia-Bobadilla & Sunny, 2021).

Amphibians, specifically ambystomas, play an important role in the stability of all the ecosystems they inhabit. It has been pointed out that they can act as emblematic species and bioindicators of environmental changes, especially in aquatic environments since they require these spaces for reproduction and development of early life stages (Monroy-Vilchis, 2015; Oropeza-Sánchez et al., 2022). The aquatic habitat inhabited by ambystomas may appear identical, but it certainly presents characteristics that influence the presence of specific species (Franco et al., 2022; Rohman et al., 2020; Venâncio et al., 2022). Water quality is among the essential parameters for the adequate maintenance of amphibians (Odum & Zippel, 2008), as are feeding and ecological interactions with their prey (Arribas et al., 2014). The causes of amphibian population decline in the genus Ambystoma are complex and are generally shared by all aquatic animal taxa (Chaparro-Herrera et al., 2013; Grant et al., 2019). While the global decline of amphibians is alarming, ambystomids are not alone in facing an uncertain future unless we are able to conduct appropriate conservation efforts at local scales rather than pursuing generalities and increasing capacity to test and implement management strategies at appropriate levels, especially for vulnerable and endemic species with small geographic distributions (Grant et al., 2019; Heredia-Bobadilla & Sunny, 2021).

Very few natural history studies have been developed for wild species in Mexico. For some groups they are practically nonexistent, as is the case for the caudates in general and more specifically for the family Ambystomatidae (Lemos-Espinal et al., 2016). This family is represented by a single genus comprising 33 species, of which 18 are distributed in Mexico, 17 are endemic, and are listed in NOM-059-SEMARNAT-2010 (Frías-Álvarez et al., 2010). We studied the mountain stream axolotl (*Ambystoma altamirani*); due to its high level of endemism, it is listed as threatened in the official Mexican standard (Diario Oficial [DOF], 2010) and in the endangered category according to the IUCN (Shaffer et al., 2008). In addition, its distribution is limited to the west and south of the Valley of Mexico in the Sierra de las Cruces in the states of Morelos, State of Mexico, and Mexico City (Fig. 1) (Lemos-Espinal et al., 2016). Currently, this species shows a drastic decrease in its population (González-Martínez et al., 2014; Shaffer et al., 2008), and it has been shown that this species is susceptible to disturbance (Cosentino et al., 2011; Lemos-Espinal et al., 2016) and there is limited ecological information on it.

Because this species presents very particular characteristics for its presence, the objective of this study was to determine the biotic and abiotic factors that are associated with the distribution of *A. altamirani* in the wild, contrasting them with sites with historical presence and local extinction of the species. These populations present different characteristics of human disturbance, which can help us determine what factors are influencing the distribution of this species in its natural habitat in contrast to the monitored sites of absence. This study is relevant since there are no clear conservation strategies to be applied for this group of endemic amphibians, and it is important to propose strategies applicable at a local scale.

Fig. 1. A juvenile axolotl is shown on the left side and on the right side a usual site where this species is present in the Sierra de las Cruces.

MATERIALS AND METHODS

Study area: Villa del Carbon is located in the Sierra de las Cruces, a mountain range with a maximum elevation of 3 800 meters above sea level that is part of the Trans-Mexican Volcanic Belt and is the border between the Mexico Basin and the Toluca Basin. In this Sierra, there are abundant altitudinal contrasts over relatively short distances, which is associated with the development of abundant ravines and valleys (García-Palomo et al., 2008). This area is vital for the recharge of other aquifers; there are more than 50 wells that supply water to various populations, and this represents a growing threat (Mejía, 2012).

The oak-pine forest in Villa del Carbon is well preserved, covering 57 % of the municipality's surface, of which 25 % of the flora has some type of use, including timber (Rubio-Licona et al., 2011). Villa del Carbon is a municipality that still has a significant natural value, and there is a recent interest in seeking alternatives to help reduce environmental impact levels, as well as to conserve and contribute to the permanence of natural resources (Hernández-Ramírez & Rosas-Roa, 2010).

For these reasons, Villa del Carbon was selected to study the distribution of *Ambystoma altamirani*; for this purpose, we selected four sites that represent different populations of this species with particular characteristics and different degrees of disturbance, as well as sites with local extinction of the species to contrast them. A small tributary of water represents site one in a sparsely populated rural area. Livestock farming activities are carried out on the slopes of this body of water. It was observed that human and domestic waste from these settlements could reach the said body of water through leachates. Site two is a river located in an ecotourism park where tourist activities such as fishing, camping, outdoor grills, and swimming pools are carried out with a constant flow of tourists. Site three is located near human settlements and cattle and sheep grazing was observed, as well as people visiting the site in order to wash clothes in that body of water. Finally, site four is a body of water close to a mine that is no longer active and there is also cattle grazing. Occasional vehicular traffic was also identified near all sites (Fig. 2).

Habitat characterization and sampling design: One visit per month during one year (September 2022 to August 2023) was made for each site, alternating the time and day of

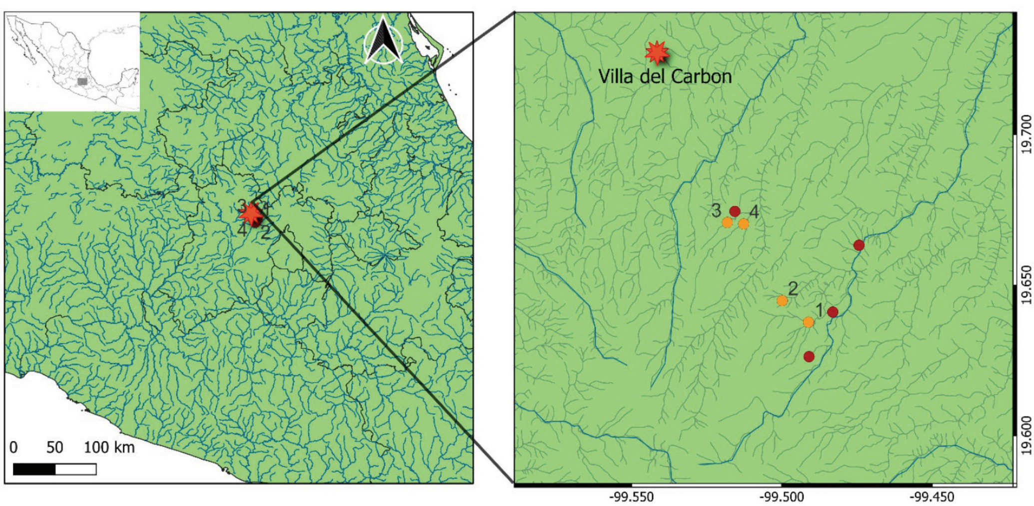

Fig. 2. Map showing the main rivers and the four sites located to study the mountain stream siredon in the Sierra de las Cruces; in yellow are the sites where the species is present, and in red are the sites where it is absent in the locality of Villa del Carbon. The red star represents the municipal seat of the municipality of Villa del Carbon.

sampling, for which a 1 000 m^2 transect, was drawn parallel to the river. Each transect was sampled by direct search and visual encounter in the opposite direction to the river current to avoid counting the same individual twice for approximately one hour (Pineda & Rodríguez-Mendoza, 2010). The stage of each specimen was recorded, considering gilled organisms as adults, and the sex was determined when possible.

Transparency was determined in situ as well as physicochemical factors of the water: pH, temperature, dissolved oxygen, and percentage of oxygen saturation using a HANNA HI98194 multiparametric probe. Solar and UV radiation were also recorded at the water level using APOGEE MQ-500 and MU-200, respectively.

A surface water sample was taken with 500 ml amber bottles to determine nutrients (nitrates, phosphates, and ammonium) and heavy metals (copper, arsenic, and chromium) in the laboratory using colorimetric tests in a YSI EcoSense 9 500 Photometer.

A bacteriological sample was taken in NASCO WHIRL-PAK sterile bags and transferred to the laboratory to determine fecal and total coliforms using the most probable number technique according to NMX-AA-042-SCFI-2015 (DOF, 2015).

Using a fine mesh fish net number five Azul brand, a trawl was performed at each site, and the zooplankton organisms captured were fixed in 5 % formalin (Guerly, 2022). Samples were processed by identifying each organism to a taxa level using QUASAR stereo microscopy Science Qm15 80X. Subsequently, monthly zooplankton abundance data were analyzed by obtaining the Shannon-Weaver index using the PAST program version 4.03 (Chávez-Veintemilla et al., 2021).

Statistical analyses: One-way ANOVA tests were performed for each factor evaluated, and Tukey's test was used to determine differences between sites using SigmaPlot software V. 13 3.1. p = 0.05 was used to determine differences between groups (D'Cruze & Kumar, 2011).

To analyze the relationship between the parameters mentioned above and axolotl populations, a canonical correspondence analysis (CCA) was performed using CANOCO 5.0 software, which allows us to show the relationship in terms of environmental characteristics (physicochemical parameters, temperature, etc.) and biological variables (species), only that in this case it will be adapted considering populations as species for the purposes of this study (Kostov, 2008).

RESULTS

Four localities with different characteristics were sampled with the presence of the species in Villa del Carbon from September 2022 to August 2023. Populations one and three are close to rural towns; population two is associated with a place with ecotourism activity close to axolotls. On the other hand, the population four are associated with a site with abandoned mining activity. A total of 1 199 salamanders were recorded at these sites, of which sites one and two had the greatest number of organisms, with 639 and 374, respectively, and showed the greatest number of adult specimens. Sites three and four showed the lowest numbers of specimens with 166 and 20, respectively. No eggs were recorded at site four during the year of sampling (Fig. 3). Similarly, the proportion of females and males was higher in sites one and two than in the others (Fig. 4).

The environmental, physicochemical, bacteriological, and biotic parameters of the water showed differences in the sites with the presence and absence of *Ambystoma altamirani* (Table 1). Of the 16 parameters analyzed, eight showed significant differences: water temperature, transparency, UV radiation, fecal coliforms, total coliforms, nitrates, phosphates, and zooplankton diversity.

The biotic parameters of the water, such as zooplankton, were composed of numerous taxa, of which the most numerous were copepods, followed by larvae of Ephemeroptera, Diptera, Ostracoda, and Coleoptera, among others. Predation by *A. altamirani* on anuran

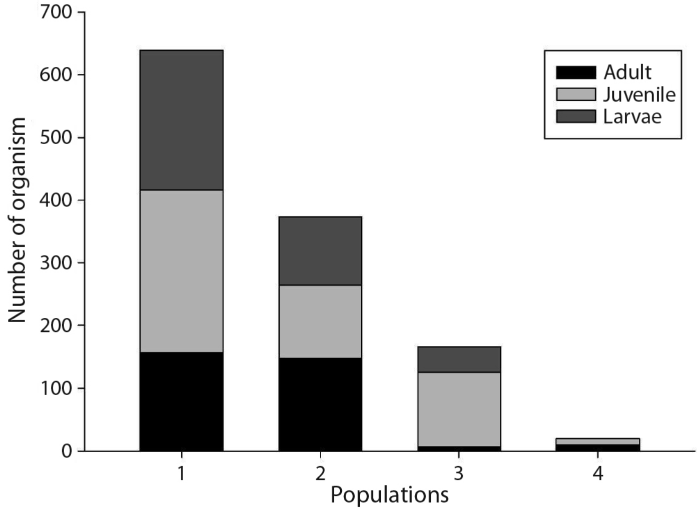

Fig. 3. The number of organisms sampled by life stage, categorized into adults, juveniles, larvae, and eggs in each of the four populations.

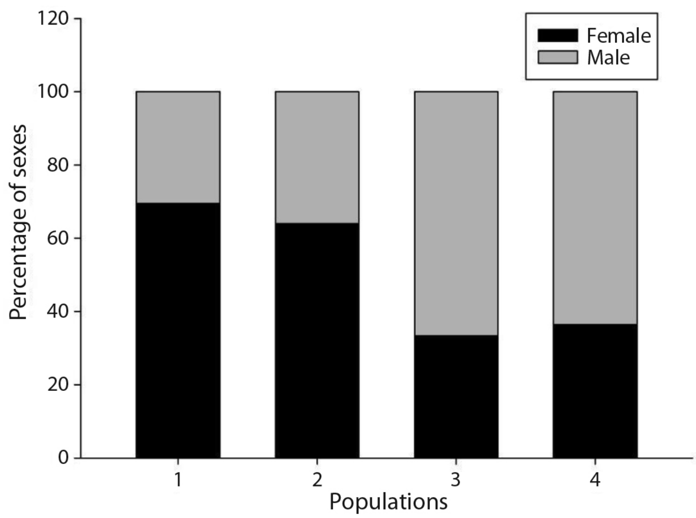

Fig. 4. The proportion of females and males in each of the four populations.

larvae and various larvae and insects such as Coleoptera was observed.

The canonical correspondence analysis showed that the variables most associated with the presence of the populations were solar radiation (64.61 %), followed by total coliforms, oxygen saturation, fecal coliforms, and UV radiation, which together explained 97.76 % of the variability of the data (Fig. 5). Each population showed different associations with environmental, physicochemical, bacteriological, and biotic parameters of the water; population one showed an inverse correlation with zooplankton and fecal coliforms, as well as a close relationship with ammonium and solar radiation. Population two showed a strong interaction with dissolved oxygen, as well as an inverse relationship with temperature and

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Table 1
The environmental, physicochemical, bacteriological, and biotic parameters of the water of each population of *Ambystoma altamirani*.

Parameter	Population				Absence	Min-	Max+	p
	1	2	3	4				
pH	6.945+	6.973-	7.191+	6.945	7.295	6.10	7.92	0.334
Dissolved oxygen (mg/l)	5.400-	6.852+-	6.030	6.295	5.540	3.44	8.22	0.298
Oxygen saturation (%)	88.713-	92.100+	87.450	92.500	74.000	44.7	103.2	0.075
Temperature (°C) *	15.479	12.093-	16.004	16.704+	14.625	8.1	21	0.012
Transparency (cm)*	2.917	0.000	3.333+	0.000	23.000	0	40	< 0.001
Solar radiation (µmol $m^{-2} s^{-1}$)	1 152.50+	575.60	212.900	410.500	625.000	0	1 725	0.147
UV radiation (µmol)*	35.250+	6.250	21.200	26.400	30.000	0	189	0.020
Fecal coliforms (NMP/100 ml) *	12.050	6.050	13.500+	4.500	38.500	0	290	0.001
Total coliforms (NMP/100 ml) *	115 000	13 000	58 500	27 000+	185 000	0	1 100	0.023
Nitrates (mg/l) *	0.0910	0.0960	0.0635+	0.0625	0.400	0	0.840	0.009
Phosphates (mg/l) *	0.0390+	0.01000	0.01000	0.000	0.650	0	1.8	0.002
Ammonium (mg/l)	0.0300+	0.0250	0.0200	0.0250	0.0350	0	0.07	0.703
Arsenic (PPB)	10.000	0.000+	10.000	10.000	10.000	0	30	0.600
Chromium (mg/l)	0.0450+	0.0150	0.0390	0.0300	0.000	0	0.65	0.444
Copper (mg/l)	0.000	0.0750	0.000+	0.000	0.000	0	0.4	0.092
Zooplankton diversity (H') *	0.912-	1.126+	1.338	0.983	0.791	0.4826	1.852	0.002

Parameters that showed a significant difference with respect to the control are shown with the symbol *. The + symbol represents the site with the highest value recorded for the parameter, and the - symbol represents the lowest value recorded in the year of sampling.

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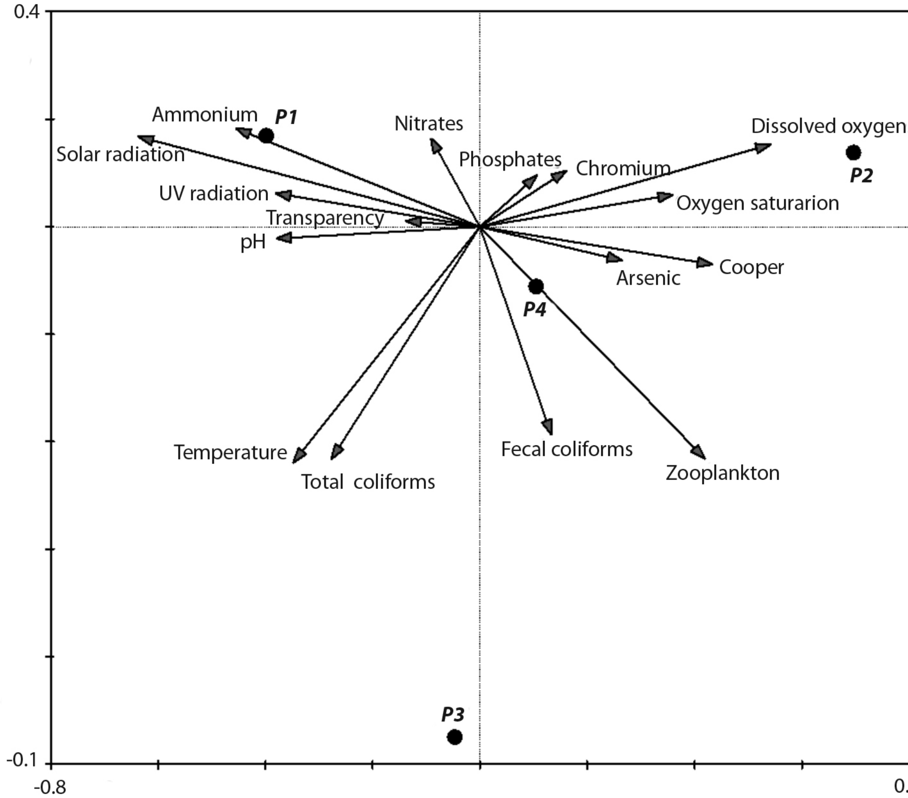

Fig. 5. CCA biplot of the *Ambystoma altamirani* sites sampled in which the dots represent the four populations studied.

pH. Population three did not show a close association with the variables. Population four maintained a relationship with fecal coliforms, zooplankton, and some heavy metals.

DISCUSSION

Several studies on *A. altamirani* and other ambystomatids have reported a higher abundance of females (1.0 versus 0.6 males) (Guerrero de la Paz et al., 2020); in our study, we found that in populations one and two, the proportion of females was higher than 60 %. Likewise, these sampling points have a higher proportion of eggs and the presence of adults, so we could consider them more stable and healthier compared to the other sampling sites.

A. altamirani has been reported under very particular habitat characteristics for its persistence; abundance has been associated mainly with physicochemical factors, with limited influence of terrestrial factors (Franco et al., 2022). Although each population from the different sampling sites showed different associations to environmental, physicochemical, bacteriological, and biotic parameters of the water, the CCA showed that among the most important characteristics that we should maintain in the environments to maintain populations with the highest number of organisms include terrestrial factors, such as solar and UV radiation in the ranges found.

On the other hand, coliforms in low amounts (fecal and total) are the preferred sites for these amphibians in relation to the absence sites. The presence sites showed values below 13.5 and 115 NMP/100 mL, respectively (Table 1). Fecal and total coliforms are below the permissible limits for rivers, lakes, and lagoons, according to NOM-001-SEMARNAT-2021 (DOF, 2022).

Otherwise, according to the CCA, high oxygen saturation (6.852 mg/l) is also fundamental to maintaining the species populations (Table 1). It has been reported that higher levels of dissolved oxygen have been shown to be preferable for organisms (Lemos-Espinal et al., 2016). In this case, population two showed the highest levels of dissolved oxygen; However, population one could be defined as the healthiest, and it also presented the lowest levels of dissolved oxygen saturation. We know that dissolved oxygen can be reduced by increasing temperature (Bello et al., 2017), and based on the data, the temperature of population one is higher than population two. On the other hand, fecal coliforms, total coliforms, phosphates, ammonium, and chromium presented higher levels at this site compared to population two and even other sites. It has been reported that the decomposition of nutrients also consumes dissolved oxygen (Liu et al., 2022). These factors cause the reduced reading of oxygen saturation and dissolved oxygen in population one compared to population two, which is also more stable compared to the others.

Although it has generally been reported that high oxygen concentration is preferable for axolotls, it has also been seen that the species is usually found at levels of 5.44 ± 0.44 mg/l (Lemos-Espinal et al., 2016). It should be noted that levels above 5 mg/L of dissolved oxygen are healthy for aquatic life (Prasad et al., 2014).

Transparency was among the values that showed the difference between presence and absence sites, highlighting that organisms prefer good water transparency. This may be explained by the fact that the genus Ambystoma has low visibility compared to other aquatic organisms such as fish; therefore, environments with greater transparency may benefit it in its hunting behavior and competition for food against other organisms (Chaparro-Herrera et al., 2020). On the other hand, it cannot be ruled out that low transparency may be a factor interfering with the observation of this species, and this aspect should be studied with other types of population censuses.

Nutrients in the water, such as nitrates and phosphates, were also different in the absence and presence of amphibians. However, all sites are well below the permissible limits according to NOM-001-SEMARNAT-2021 to not affect aquatic fauna (DOF, 2022).

One of the most striking parameters has been UV radiation since it has recently been identified as one of the stressors that most threaten amphibians at all stages of their lives and can have sublethal effects, as well as interact with pollutants to enhance their effects (Blaustein et al., 2003). However, the population with the highest number of organisms and the sites of absence also showed the highest value of UV radiation, which could indicate that the effects of UV radiation may be due to the sum of several related factors, such as synergy with pollutants, or may be evidence of the tolerance of these organisms to the rays. Tolerance in amphibians to UV radiation has been documented as a local adaptation, and it has been inferred that organisms at high altitudes may show greater tolerance (Pahkala et al., 2002). It has also been shown that exposure to UV radiation is reaching lethal levels in conjunction with cold temperatures, increasing the thermal limits of organisms as plasticity (Kern et al., 2015), which is consistent with our data.

Although the temperature of population one was not the lowest, in general, these organisms are characterized by preferring cold temperatures, even showing activity close to the freezing point (Sánchez-Sánchez et al., 2022). The temperature difference between sites is due to factors such as flow volume, flow speed, riparian vegetation, and human use of the aquatic system. This species, like other ambystomatids, presents high genetic variability, finding genetic subpopulations with a significant level of genetic structure and variability within the same population (Heredia-Bobadilla et al., 2017). Due to this condition, the four populations studied showed different relationships with the variables analyzed. Each population is behaving differently to the variables which

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makes it very difficult to define conservation actions applicable to the species and even more so to the genus *Ambystoma*.

This species, like other ambystomatids, presents high values of heterozygosity, inbreeding, and reduction of the effective population size probably caused by bottlenecks, which may be reducing its genetic variability, which is fundamental for the species to adapt to environmental changes (Heredia-Bobadilla et al., 2017). The reduction of genetic quality can be a factor that leads species to extinction since it reduces their plasticity; in ambystomas, there is a low flow, and genetic knowledge is insufficient, which greatly hinders their conservation (Parra-Olea et al., 2011). We know that amphibians and ambystomes are susceptible to water quality and the characteristics of their habitat, so we should focus conservation efforts on both (Piñon-Flores et al., 2021), in addition to further study of site contamination, which may be presenting effects that are not yet visible in the organisms and although they do not yet show alarming values.

This species of salamander does not show a clear relationship with zooplankton diversity, according to the CCA, but we could assume that they prefer environments with greater diversity (Table 1). There needs to be more information on the feeding of these organisms; in the case of larvae, it has been reported mainly ostracods, gastropods, and insects (Lemos-Espinal et al., 2015), although, in the adult stage, there is no precise information. A greater abundance of prey could benefit organisms in all stages of their life.

Although some studies have suggested that habitat variables show no influence on the presence or absence of this species (Guerrero de la Paz et al., 2020), the CCA shows the variables that we can pay attention to for the conservation of amphibians in these environments. Several studies on this species have proposed conservation strategies to maintain favorable water conditions for its persistence, among which are good water flow and volume, low temperature, good stream width, shallow water depth, high dissolved oxygen level (Franco et al., 2022; Lemos-Espinal et al., 2016) suggesting that critical stream characteristics for *A. altamirani* include sites that do not dry out, presence of mud substrates and absence of fish (Hernández et al., 2020a). It is also highlighted that it is crucial to minimize the expansion of human influence on aquatic habitats where *A. altamirani* specimens are found (Franco et al., 2022) as desiccation due to the likely increased water use by Mexico City, which may be a crucial factor for the decline of this species (Franco et al., 2022; Lemos-Espinal et al., 2016).

The favorable water temperature for this species has been reported in low ranges, between 12.32 °C and 17.44 °C (Camacho et al., 2020), even observing organisms with water temperature activity at 6.4 °C (Sánchez-Sánchez et al., 2022). The temperature at sites one and two, which had the highest number of organisms, was between 12.093 and 15.497 °C, and at the sites with the lowest number of organisms, it was higher than 16 °C. Water temperature has been one of the most relevant factors for the study and maintenance of Ambystoma; it has also been shown that high temperatures (27 °C) represent a higher risk of mortality than cool and dry conditions (Rohr & Palmer, 2013). Temperature is a factor that cannot be ruled out, as the synergistic effects of climate change, emerging diseases, and exotic species will continue to increase pressure on remnant populations (Hernández et al., 2020b).

This species responds differently to seasonal environmental variations at different stages of development (Martínez-Ugalde et al., 2022), which should be studied further, and each population may respond differently to the environment, which complicates the establishment of generalized conservation actions. The causes of amphibian population declines are multifaceted and context-dependent; they include complex effects of abiotic and biotic factors that can be stressful and lead to population losses (Blaustein et al., 2018). We conclude that it is urgent to protect this species due to the fragility of its populations and rapid decline; in addition, we know that the organisms of this group are emblematic species of which conserving their habitat can be beneficial to preserve numerous species (Oropeza-Sánchez et al., 2022). We propose conservation strategies to avoid contamination by heavy metals and other compounds to maintain water quality in good condition as reported in this study, maintain water transparency to not interfere with the behavior of organisms, as mentioned above, avoid reducing the water level to avoid reducing dissolved oxygen, which is important for these organisms (Oropeza-Sánchez et al., 2022).

We know that the fragility of populations and rapid decline of amphibians is alarming; we need more specialized and possibly experimental studies to propose conservation strategies that are appropriate to the localities studied, although each population may act differently to the factors of their environment to generalize some vital aspects that could ensure the conservation of the species at least in a particular area. It is essential to prioritize the protection of microendemic species in local environments, as this ensures the persistence of the habitat of all the species and the community associated with these habitats.

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