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Ecology

Population density and sexual maturity of *Ambystoma lermaense*, an endangered species in central Mexico

Densidad poblacional y madurez sexual de Ambystoma lermaense*, una especie amenazada en el centro de México*

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Abstract

Ambystoma lermaense, an endangered salamander species endemic to central Mexico, faces significant threats from habitat degradation, pollution, and human activities. This study evaluates the population density, structure, and sexual maturity of *A. lermaense* in Capilla Vieja, Estado de México, over 3 time periods: 2009, 2010, and 2020. Using visual encounter surveys along a 730-m stream transect, we recorded population densities of 0.021 individuals/m² in 2009, 0.070 individuals/m² in 2010, and 0.075 individuals/m² in 2020, reflecting relative stability despite anthropogenic pressures. Juveniles accounted for 87% of the population, with sexual maturity observed at a snout-vent length of 60 mm, supported by histological evidence of vitellogenic oocytes in females and mature sperm in males. Our study provides important insights into the population dynamics and sexual maturity of *A. lermaense* in the sampling locality, where this species had not been previously studied.

Keywords: Ambystomatidae; Population structure; Paedomorphosis; Vitellogenic oocytes; Mature sperm; Decline

Resumen

Ambystoma lermaense, una especie de salamandra en peligro de extinción endémica del centro de México, enfrenta amenazas significativas debido a la degradación del hábitat, la contaminación y las actividades humanas. Este estudio evalúa la densidad poblacional, estructura y madurez sexual de *A. lermaense* en Capilla Vieja, Estado de México, durante 3 períodos: 2009, 2010 y 2020. Utilizando muestreos visuales a lo largo de un transecto de 730

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m en un arroyo, registramos densidades poblacionales de 0.021 individuos/m² en 2009, 0.070 individuos/m² en 2010 y 0.075 individuos/m² en 2020, lo cual refleja una estabilidad relativa a pesar de las presiones antropogénicas. Los juveniles representaron 87% de la población y se observó madurez sexual a un tamaño de 60 mm de longitud hocico-cloaca, sustentado por evidencia histológica de ovocitos vitelogénicos en hembras y espermatozoides maduros en machos. Nuestro estudio proporciona información importante sobre la dinámica poblacional y la madurez sexual de *A. lermaense* en la localidad de muestreo, donde esta especie no había sido estudiada previamente.

Palabras clave: Ambystomatidae; Estructura poblacional; Paedomorfosis; Ovocitos vitelogénicos; Espermatozoides maduros; Declive

Introduction

Amphibians worldwide are experiencing unprecedented declines, driven by a combination of habitat loss, pollution, climate change, invasive species, and disease (Grant et al., 2016; Stuart et al., 2004). These declines are particularly concerning for high-altitude amphibians, which often have restricted ranges and specialized habitat requirements (Green, 2003; Pounds, 2001; Stuart et al., 2004). As indicators of ecosystem health, amphibians play critical roles in maintaining ecological balance, yet they are disproportionately affected by anthropogenic pressures (Blaustein & Wake, 1990). Among these, *Ambystoma lermaense*, an endangered salamander species endemic to central Mexico, exemplifies the challenges faced by amphibians in fragmented and degraded habitats (IUCN SSC Amphibian Specialist Group, 2015).

Ambystoma lermaense, inhabits high-altitude streams at elevations ranging from 2,500 to 3,000 m asl (IUCN SSC Amphibian Specialist Group, 2015). This facultatively paedomorphic species is found in aquatic habitats such as lakes, ponds, marshes, and occasionally streams (Everson et al., 2021; IUCN SSC Amphibian Specialist Group, 2015), which are increasingly impacted by human activities (Reilly & Brandon, 1994). Agricultural expansion, livestock grazing, pollution, and urban development threaten the quality and connectivity of these aquatic habitats (Egea-Serrano et al., 2012; Robles-Mendoza et al., 2009). These pressures, combined with seasonal hydrological fluctuations and climate variability, pose significant risks to the long-term viability of *A. lermaense* populations (Dirzo et al., 2022; Huacuz-Elías, 2003).

Understanding population dynamics is a cornerstone of effective conservation planning. Accurate data on population density, structure, and trends are essential for identifying populations at risk and prioritizing interventions (Pechmann et al., 1991). Previous studies on species of the genus *Ambystoma* have revealed a wide spectrum of population densities, ranging from critically low levels in urbanized environments to exceptionally high densities in protected areas. For instance, the Mexican axolotl (*A. mexicanum*) exhibits densities as low as 0.001 individuals per square meter in the urban canals of Xochimilco (Contreras et al., 2009), while *A. rivulare* densities reach 2.3 individuals per square meter in the protected natural area Reserva de la Biósfera Mariposa Monarca (Huacuz-Elías, 2003). These disparities underscore the profound impact of habitat quality and protection on amphibian populations.

Despite its resilience in suboptimal conditions, *A. lermaense* remains highly vulnerable to habitat degradation and fragmentation (Jacinto-Maldonado et al., 2023). In the Capilla Vieja region of Estado de México, semi-intensive livestock activities and seasonal stream drying compound the challenges faced by this species (previously recognized in this locality as *A. granulosum*; Everson et al., 2021). As part of a natural protected area, Capilla Vieja offers a unique opportunity to study how *A. lermaense* populations persist in a landscape that combines elements of protection and human impact. The site's connectivity to a broader watercourse system also provides insights into the role of habitat corridors in sustaining population viability.

This study examines the population density, structure, and sexual maturity of *A. lermaense* in Capilla Vieja over 3 time periods: 2009, 2010, and 2020. By situating these findings within the broader context of *Ambystoma* conservation, we aim to draw comparisons with other populations and species to better understand the factors driving population trends. The research also highlights the critical role of protected areas and localized conservation actions in addressing the multifaceted threats to amphibians. This work contributes to the growing body of knowledge needed to inform strategies for safeguarding *A. lermaense* and other endangered amphibians in Mexico's diverse ecosystems.

Materials and methods

The study was conducted in a section of a stream located in Capilla Vieja, municipality of Amanalco de Becerra, Estado de México, at coordinates 19º13'22" N, 99º59'12" W, with an elevation ranging from 2,790 to 2,870 m asl (Fig. 1). The predominant vegetation in this area is pine-oak-fir forest. The climate is temperate subhumid, with summer rains, an average annual temperature of 12 °C, and temperature extremes ranging from 0.5 °C to 29.7 °C (Rentería et al., 2005). Capilla Vieja is part of the sustainable ecosystem management subzone for agricultural areas within the Natural Protected Area known as Área de Protección de Recursos Naturales de las Cuencas de los Ríos Valle de Bravo, Malacatepec, Tilostoc y Temascaltepec (Semarnat, 2018).

The study stream crosses an extensive grassland plain that is used for semi-intensive livestock activities, including sheep, cattle, and horses. The stream during the rainy season features shallow areas with nearly stagnant water. In certain sections, the bottom is extremely soft, with mud depths ranging from 15 to 25 cm and exceeding 50 cm in some points. The stream width varies between 2 and 5 m, expanding during the rainy season and shrinking or disappearing during the dry season. This stream is part of a connected watercourse system that flows in a northeast-southwest direction, contributing to the water supply of the Capilla Vieja lagoon (Comisión Nacional de Áreas Naturales Protegidas, 2018).

Samplings were conducted once a month from July to October during 3 periods corresponding to the years 2009, 2010 and 2020. Each month, a 730 m transect with a width of 3 m along the stream was surveyed (starting coordinates: 19°13'10.81" N, 99°59'4.95" W; ending coordinates: 19°13'3.73" N, 99°59'15.13" W). Sampling was carried out using the visual encounter survey method between 09:00 and 14:00 hours (Heyer et al., 1994; Lips et al., 1999), recording the total number of individuals. Each individual was captured using hand nets and placed in 20-liter plastic containers filled with water from the stream.

Morphometric measurements were recorded for each specimen: snout-vent length (SVL), total length (TL), and weight (W). Individuals were classified into 2 categories: adult metamorphic (males and females) and larvae (juveniles), a category that included individuals with gills for whose sex could not be determined. Population density was calculated by dividing the total number of individuals (N) by the sampled area of the stream (N/m²) (Williams et al., 2002), and it was estimated for each sampling period. Males and females were identified based on the size and shape of the cloaca (Aguilar-Miguel et al., 2009; Taylor, 1940). In females, the cloaca is flat and smaller (Fig. 2a), whereas in males it is enlarged due to high glandular activity (Fig. 2b).

To estimate the size at sexual maturity, a sample of 52 individuals was collected from random points along the stream during the 2009 and 2010 sampling periods.

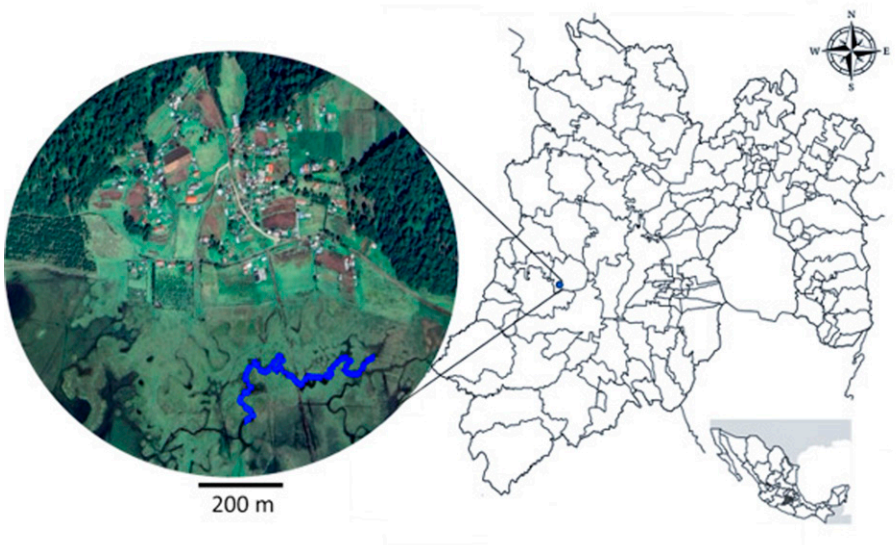

Figure 1. Location of the Capilla Vieja locality, Capilla Vieja, Amanalco municipality, Estado de México. The blue section indicates the sampled stream during the 3 sampling periods (2009, 2010, and 2020).

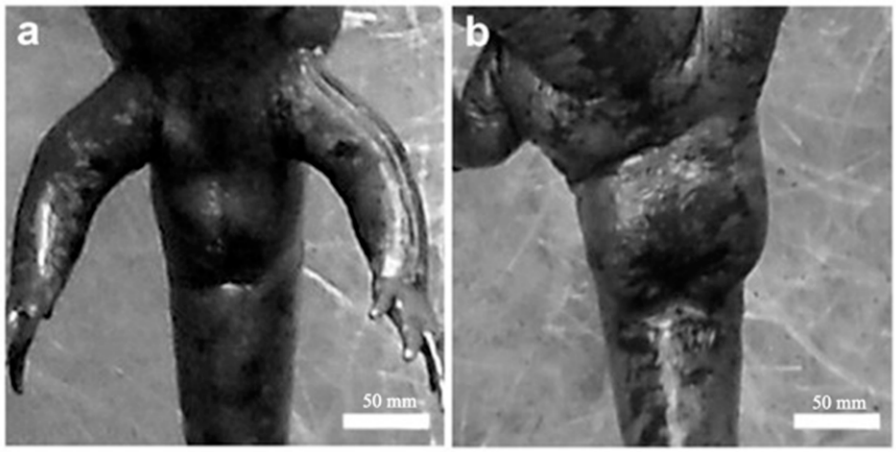

Figure 2. Sex determination in *Ambystoma lermaense* in females (a), and males (b), based on cloacal size and shape.

The sample included individuals ranging in size from 50 to 115 mm in SVL. An ANOVA was used to assess the relationship between body size and sexual maturity.

To euthanize the specimens, they were anesthetized using a 0.1% solution of MS-222 (tricaine methanesulfonate) for 20 min (Mitchell, 2009; Rubio-Limonta & Silveira-Coffigny, 2009). A longitudinal ventral incision was made to expose the ovaries and testes. The gonads were fixed in 4% paraformaldehyde for 24 h at 4 °C. Standard histological techniques for light microscopy were applied: dehydration in graded alcohols, clearing with xylene, embedding in paraplast, sectioning at 5 µm, and Hematoxylin-Eosin staining (Aguilar-Miguel et al., 2009). Digital images of spermatogenesis and oogenesis stages were captured using a Nikon Coolpix 4300 (4 MP) digital camera mounted on a Nikon Optiphot-2 upright microscope and calibrated with ImageJ software.

Results

A total of 366 individuals were recorded across the 2,190 m² of the sampled stream during the 3 periods (Table 1). The period with the lowest abundance was 2009, with 46 individuals, while the highest was 2020, with 165 individuals. The population density of *A. lermaense* increased over the sampling periods. In 2009, the density was 0.021 ind/m², in 2010, it increased to 0.070 ind/m², and finally, in 2020, it reached 0.075 ind/m².

Population structure. Of the total number of individuals found across the 3 periods, 87% were juveniles, 6% were metamorphic females, and 7% were metamorphic males. Throughout the 3 sampling periods, the months with the highest number of larvae were August and September, for metamorphic females, July and September, and for metamorphic males, August and September (Fig. 3).

Sexual maturity. Of the 52 dissected individuals, 24 were males, 14 females and 14 larvae. Males had an SVL ranging from 60 to 115 mm, females from 60 to 100 mm, and larvae from 50 to 85 mm (Table 2). According to the ANOVA, a significant association was found between size and the different age categories ($p < 0.05$). Through histological analysis, 38 individuals exhibited sexual development (Table 2). We determined that sexual maturity occurs at an SVL of 60 mm, as vitellogenic oocytes in females and mature sperm in males were observed at this size (Fig. 4).

Table 1
Total number of individuals recorded during the sampling periods.

Period	July	August	September	October	Total
2009	4	23	14	5	46
2010	4	56	70	25	155
2020	7	130	23	5	165
Total	15	209	107	35	366

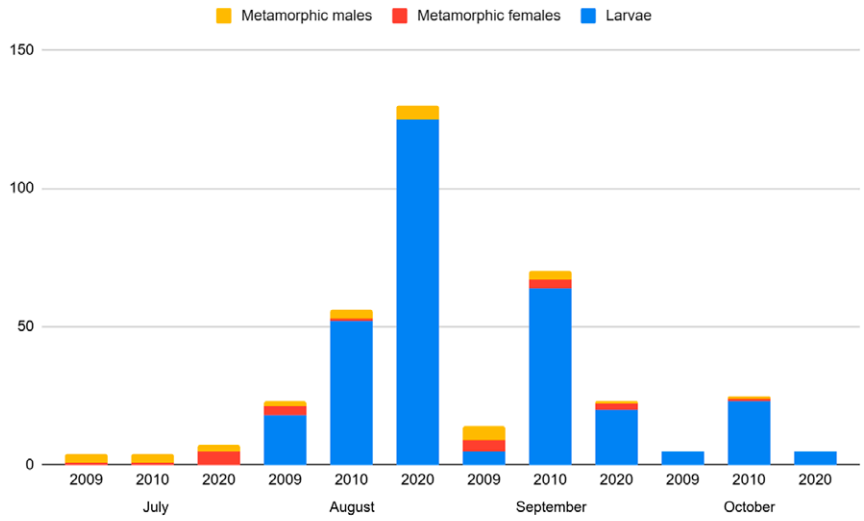

Figure 3. Number of *Ambystoma lermaense* individuals grouped by categories, recorded during the 3 sampling periods (2009, 2010, and 2020).

Table 2

Morphometric data (average, minimum, and maximum) of individuals analyzed histologically to determine sexual maturity. SVL = Snout-vent length, TL = total length (mm), and weight (g).

Category	Metamorphic females	Metamorphic males	Larvae
Avg. SVL	81.4	90.8	62.5
Min. SVL	60	60	50
Max. SVL	100	115	85
Avg. TL	156.1	167.9	114.6
Min. TL	130	110	85
Max. TL	180	220	155
Avg. weight	23.53	20.13	9.57
Min. weight	10	8	4
Max. weight	42	36	20
Gonadal development (Yes/No)	Yes	Yes	No
Number of individuals	14	24	14

Discussion

Our study on *Ambystoma lermaense* in Capilla Vieja provides critical insights into its population dynamics, sexual maturity, and conservation status. By situating these findings within a broader ecological and conservation context, we can deepen our understanding of the factors influencing the persistence of this species and its relatives. The gradual increase in population density from 0.021 individuals/m² in 2009 to 0.075 individuals/m²

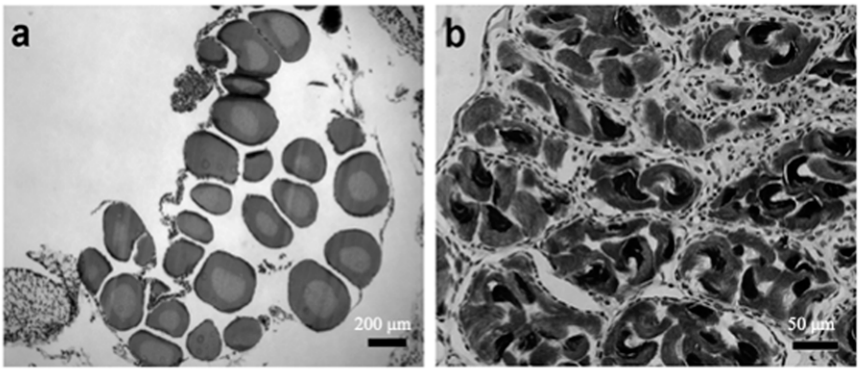

Figure 4. a) Vitellogenic oocytes in females; b) mature sperm in males observed in individuals with an SVL of ≥ 60 mm.

in 2020 is a positive indicator of stability. However, these densities remain substantially lower than those reported by Huacuz (2003) in the Reserva de la Biosfera Mariposa Monarca (2.3 individuals/m²). This stark contrast underscores the influence of habitat quality and protection on population viability. The Capilla Vieja site, despite being part of a protected natural area, is exposed to semi-intensive livestock activities and seasonal hydrological fluctuations, both of which could limit carrying capacity for *A. lermaense*.

Examining densities and threats across 3 *Ambystoma* species provides valuable comparative insights: *Ambystoma mexicanum*, critically low densities in Xochimilco lake (0.001 individuals/m²) highlight severe impacts from urbanization and habitat degradation (Contreras et al., 2009). *Ambystoma altamirani*, densities ranging from 0.016 to 0.027 individuals/m² in trout-free streams in Estado de México suggest that invasive species exacerbate population declines (Lemos-Espinal et al., 1999). *Ambystoma ordinarium*, densities of 0.030-0.050 individuals/m² in Michoacán State highlight the challenges posed by deforestation and agricultural expansion (Aguilar-Miguel, 2005). These comparisons reinforce the role of protected areas in supporting higher population densities and the importance of mitigating threats in unprotected habitats.

The discovery that sexual maturity in *A. lermaense* occurs at a SVL of 60 mm aligns with findings for other facultatively paedomorphic *Ambystoma* species. Paedomorphosis, the retention of larval traits in adults, allows these salamanders to reproduce in aquatic habitats, potentially enhancing resilience in fragmented or fluctuating environments. However, the high proportion of juveniles (87%) in Capilla Vieja suggests a reliance on consistent recruitment, which could be disrupted by environmental changes.

The Capilla Vieja site is part of a connected watercourse system, but its fragmentation due to agricultural activities and seasonal stream drying limits habitat availability. Maintaining connectivity between breeding and non-breeding habitats is critical for sustaining gene flow and reducing local extinction risks. Lessons from the Reserva de la Biosfera Mariposa Monarca, where robust habitat connectivity supports high densities of this species, should guide management strategies for fragmented landscapes.

High-altitude amphibians like *A. lermaense* are particularly vulnerable to climate change. Rising temperatures and altered precipitation patterns could shrink suitable habitat ranges and exacerbate seasonal drying of streams. Proactive measures, such as restoring vegetation cover and monitoring hydrological changes, are necessary to mitigate these effects. While protected areas like the Reserva de la Biosfera Mariposa Monarca demonstrate the effectiveness of large-scale conservation, localized efforts are equally vital. Capilla Vieja's inclusion in a protected area offers an opportunity to implement targeted interventions, such as: enhancing water quality through pollution control; reducing the impact of livestock on stream habitats; and introducing community-based conservation programs to engage local stakeholders.

The findings on *A. lermaense* reflect broader trends in amphibian conservation. Amphibians worldwide face similar threats of habitat degradation, invasive species, and climate change. This study contributes to the growing body of evidence that localized conservation actions, combined with broader protections, can stabilize and even enhance populations of threatened species. Future studies could explore: genetic diversity, assessing genetic variation in *A. lermaense* populations to evaluate the effects of fragmentation on genetic health. Behavioral ecology, investigating breeding behavior, dispersal patterns, and predator-prey dynamics to inform conservation strategies.

Impact of invasive species, examining whether invasive species, such as trout, are encroaching on nearby *A. lermaense* habitats.

The relatively stable population density of *Ambystoma lermaense* in Capilla Vieja reflects its resilience but highlights the challenges posed by habitat degradation and fragmentation. Comparisons with other *Ambystoma* species and populations in protected areas underscore the importance of habitat quality and connectivity. To ensure the long-term survival of *A. lermaense* and its relatives, conservation efforts must integrate habitat restoration, community engagement, and climate adaptation strategies. As amphibians are critical indicators of ecosystem health, their protection benefits biodiversity and ecological integrity on a broader scale.

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