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Limb Deformities in *Ambystoma rivulare* (Caudata: Ambystomatidae), a Microendemic and Threatened Mexican Salamander

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Abstract.—**Recent global declines and disappearances of amphibian populations have increased the need for information concerning the adversities affecting amphibian populations worldwide. Some of the most alarming cases include the appearance of high incidences of deformities in amphibian populations. Although data about what causes body deformities are inconclusive and information about the frequency of deformities in natural populations is scarce, studying the incidence and characteristics of deformities in salamander populations could serve as an early indicator of environmental disturbance. In this study, we report limb deformities in a population of Toluca Axolotl (*Ambystoma rivulare*), from the municipality of Amanalco de Becerra, Estado de México, México. We sampled 144 individuals and found cases of ectrodactyly, syndactyly, polydactyly, brachydactyly, hypoplasia, and polymyelia. The percentage of deformed individuals (21.5%) far exceeded expectations for healthy populations. This information is vital for health assessments of amphibian populations and the increasing reports of amphibian diseases and habitat degradation.**

Key Words.—amphibian; brachydactyly; ectrodactyly; limbs; malformations; México; salamander

Introduction

Amphibians are useful indicators of habitat quality, including environmental alterations and the presence of some pollutants (Blaustein 1994; Blaustein and Wake 1995). Morpho-physiological features, such as highly permeable skin, shell-less eggs, and both aquatic and terrestrial life stages make them extremely susceptible to environmental changes, including fluctuations in temperature, precipitation, ultraviolet radiation, chemical disturbances, and anthropogenic wastes (Blaustein and Johnson 2003a,b), which can lead to deformities. Deformities in amphibians have worried specialists since detection and reports of limb abnormalities increased in the 1990s (Helgen 1997). Although deformities are not the result of a single cause and potentially are the result of a combination of environmental and anthropogenic factors, studies have associated deformities with predatory attempts by native and introduced species (Bohl 1997; Ballengée and Sessions 2009; Bowerman et al. 2010), parasitic infections (Sessions and Ruth 1990; Johnson et al. 2001a; Sessions et al. 1999), chemical contamination derived from anthropogenic practices (Bonin et al. 1997; Burkhart et al. 1998; Bridges 2000; Taylor et al. 2005; Gurushankara et al. 2007), exposure to ultraviolet radiation (Blaustein et al. 1997; Broomhall et al. 2000; Anzalone et al. 1998; Ankley et al. 2000, 2002), and cannibalism (Crump et al. 1992).

Incidence of deformities is suggested to be < 5% in a healthy population (Meyer-Rochow and Asashima 1988; Read and Tyler 1994; Tyler 1998) but reports of amphibians with deformities have increased significantly during the last three decades, and it has become increasingly common to observe large numbers of deformed organisms within a population (Ouellet 2000; Lannoo 2008; Johnson et al. 2010). In diverse studies, an increase in the number and severity of deformities in amphibian populations has been observed compared to past decades (Helgen et al. 2000; Hoppe 2000). In some populations, > 80% of individuals have deformities, including abnormal limb morphologies (including digit abnormalities), extra limbs, and reduced limbs (Alford and Richards 1999; Houlahan et al. 2000; Johnson et al. 2002).

Some deformities that occur during embryonic development might induce low hatching success and egg mortality (Blaustein et al. 1997; Robles-Mendoza et al. 2009; Frías-Alvarez et al. 2010). The reported abnormalities include head (Hopkins et al. 2013; Guerra and Araóz 2016), tail (Henle et al. 2012; Hopkins et al. 2013; Romano et al. 2017), and numerous cases of malformed limbs in anuran and caudate species (Helgen et al. 2000; Williams et al. 2008; Ballengée and Sessions 2009; Hopkins et al. 2013; Romano et al. 2017). The most commonly observed deformities in salamanders are ectrodactyly (lost digits), brachydactyly (partial digits), and polydactyly (extra digits), all of which have been reported worldwide in numerous studies and affecting many species (e.g., Worthington 1974; Johnson et al. 2001b, 2006; Williams et al. 2008; Romano et al. 2017). Deformed organisms potentially have a lower rate of success capturing prey, avoiding predators, and mating (Blaustein et al. 1997; Johnson et al. 2006). Ultimately, deformities can lead to decreased survival; consequently, there is a need to determine the prevalence of deformities in amphibian populations and the potential impact on population declines (Sparling et al. 2003; Johnson et al. 2010).

México has a large diversity of amphibians and is ranked fifth among all nations for overall amphibian diversity, and second for diversity of species of salamanders (158 species of salamanders in its territory; http://amphibiaweb.org/ [Accessed 10 February 2020]): 18 species of the genus *Ambystoma*, 16 of which are endemic (Parra-Olea et al. 2014), and 12 currently listed in the Red List of the International Union for Conservation of Nature (IUCN 2021). In México, relatively few studies report deformities in salamanders of the genus *Ambystoma*, especially the species distributed in the central states where habitat is increasingly threatened by anthropogenic activities, such as agriculture and clandestine logging (Frías-Álvarez et al. 2010). Cruz-Pérez et al. (2009) describe Tiger Salamanders (*A. tigrinum*) in the state of Querétaro, México, with polydactyly and either brachydactyly or ectrodactyly, and they suggest the use of agrochemicals, and possibly trematode infections, as possible causes for observed deformities. Soto-Rojas et al. (2017) observed Michoacan Stream Salamanders (*A. ordinarium*) with ectrodactyly, polydactyly, brachydactyly, and gill lesions. Furthermore, they reported that the probability of *A. ordinarium* presenting some morphological abnormality is inversely proportional to the quality of the habitat in which the organism develops (Soto-Rojas et al. 2017).

Barriga-Vallejo et al. (2015) analyzed the leukocyte profile (white blood cell count) of adult Toluca Axolotl (*A. rivulare*) at Amanalco de Becerra, Estado de México, México (the same population studied in this work). They reported that the population was in optimal health because the leukocyte profile was similar to those reported for other species within the genus. Additionally, the authors indicated that they did not observe deformities in the population while the study was conducted; however, during a subsequent sexual dimorphism study in the same population, numerous cases of limb deformities were observed (Sánchez-Manjarrez 2017). In this study, we analyzed an *A. rivulare* population at Amanalco de Becerra, Estado de México, México, to evaluate the incidence of limb deformities, and further compared deformity rates between sexes. In addition, we compared our results to known incidences and types of deformities reported in other Ambystomatid salamander species.

Materials and Methods

Site description.—The population studied is located in the municipality of Amanalco de Becerra, situated west of the Estado de México, México. The locality presents a temperate sub-humid climate with an average annual temperature of 14.5° C and an annual precipitation of 1140 mm (Comisión Nacional del Agua 2010). We sampled in the stream known as Los Hoyos, which is part of the Corral de Piedra micro basin and is adjacent to a trout (*Salmo* sp.) farm.

Study species.—*Ambystoma rivulare* is a Mexican microendemic species distributed in Michoacán, Estado de México and Guerrero at elevations above 2,800 m. The species is listed as Threatened by the Mexican Secretary of Environment and Natural Resources (http://dof.gob.mx/nota_detalle.php?codigo=5173091&fecha=30/12/2010) and is categorized as Endangered by the IUCN (IUCN 2021). Additionally, it is considered a species with medium (13 points) vulnerability to environmental degradation according to the Environmental Vulnerability Score (EVS; Wilson et al. 2013). *Ambystoma rivulare* inhabits lotic water bodies and is present in Pine, Pine-oak, and Fir forests (Huacuz 2001; Casas-Andreu et al. 2004). Its restricted distribution is aggravated by the growth of human settlements and habitat disappearance (Woolrich-Piña et al. 2017), and populations are further impacted by emerging infectious diseases, such as that caused by *Batrachochytrium dendrobatidis* (Frías-Álvarez et al. 2008).

Sampling methods.—We sampled twice a year, once during the rainy season (February-April) and again during the drought season (June-September) across four consecutive years (2015–2019). We visually located individuals along the river and searched the hollows on the riverbanks and captured salamanders with an aquatic dip net. Although sampling was intended to be unbiased, our sampling methods may have resulted in higher capture rates of deformed individuals with

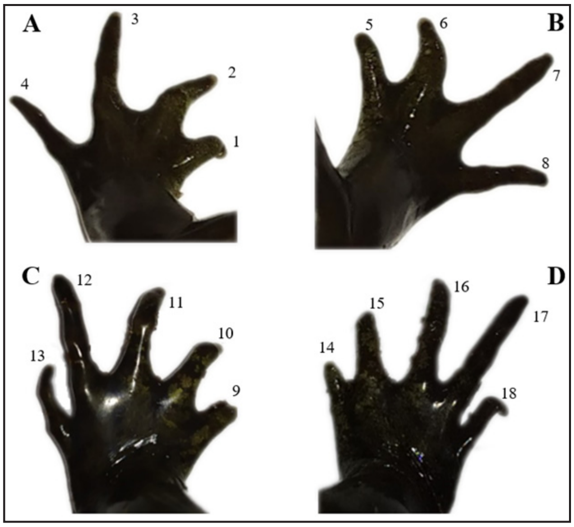

FIGURE 1. Numbers assigned to each digit of Toluca Axolotls (*Ambystoma rivulare*) limbs. (A) Left Forelimb, (B) Right Forelimb, (C) Left Hindlimb, (D) Right Hindlimb.

reduced mobility. We placed salamanders in 20-L plastic containers, previously filled with water from the stream, and then we recorded the following data for every individual: time of capture, sex, weight, snout-vent length (SVL), cloacal length (CLL), and cloacal width (CLW). We recorded all visible deformities of the organisms checking the dorsal, ventral, head, tail, and limb areas. We recorded specific limb deformity by assigning a number to each digit following an ascending order starting with the forelimbs (left to right) and then the hind limbs (left to right). We considered the digit closest to the body of the left forelimb (dorsal view) as digit number one and the digit furthest from the body of the right hindlimb as digit number 18 (Fig. 1). We classified deformities according to the studies carried out by Meteyer et al. (2000), Johnson et al. (2001b), and Thomson et al. (2014). Subsequently, we photographed dorsal, lateral, and ventral portions of all captured salamanders.

We used APHIS photo identification software to create a photographic database of the individuals collected based on the dorsal pigmentation to avoid repeated sampling (Moya et al. 2015; Romano et al. 2017). When we could not classify deformities by external observation, we transported the salamanders with inconclusive diagnoses to the laboratory where we x-rayed them to scrutinize the underlying bones to correctly classify the deformity. With data obtained from the deformity registry, we calculated the incidence of deformities in the population by the percentage of cases found. We also determined which limbs presented the greatest number of deformities and which were the most commonly affected digits. We calculated deformities per limb; if a salamander presented two deformed limbs, we recorded two abnormalities, one for each affected limb.

Statistical analyses.—Prior to the statistical comparisons, we tested the homogeneity of variances with Levene's test, as well as normality (Shapiro-Wilk) and goodness-of-fit (Kolmogorov-Smirnov). Because data were normally distributed and variances did not differ from equality, we used the Student's *t*-test to determine if there were significant size differences between males and females, and between organisms affected by deformities and organisms that did not show deformities. We tested the interaction between sex and the presence of deformities using Chi-square with fourfold table. We performed the statistical analyses with the Statgraphics Centurion XVI software, and the software for scientific data analysis Past 3 (version 3.26). We used $\alpha = 0.05$ for all statistical tests.

RESULTS

We captured 144 adult *Ambystoma rivulare* from Amanalco de Becerra locality, 81 males and 63 females. We found significant differences in SVL (t = 3.85; df = 143, $P < 0.001$) between males (n = 81, mean SVL ± standard deviation = 95.43 ± 6.31 mm; range, 80–114 mm) and females (n = 63, mean SVL = 99.42 ± 6.02 mm; range, 84–115 mm) with females being larger on average. We only observed deformities in the limbs and not in the head, tail, or the ventral and dorsal areas of the sampled individuals. We observed limb deformities in 31 individuals (21.5%), including 18 males (12.6%) and 13 females (8.9%); deformities were documented in 33 limbs belonging to 31 adult individuals, including two females with more than one limb affected (Table 1). The limb deformities identified included brachydactyly, ectrodactyly, polydactyly, hypoplasia, syndactyly, and polymelia (Figs. 2 and 3). The most commonly found deformities in this population were brachydactyly (41.9%) and ectrodactyly (29%; percentages of deformities found among the affected organisms). Brachydactyly was the most frequent deformity in males (44.4%) and females (38.5%). In males, the second most-commonly observed deformity was ectrodactyly (33.3%); however, in females, ectrodactyly, polydactyly, and hypoplasia were found in the same proportion (23.1%; Table 1). Additionally, 21 (14.5%) of the sampled individuals presented scars on the tail.

The SVL of males did not differ significantly between those with or without deformities (t = 1.61; df = 80, P = 0.112), or for females (t = -1.48; df = 62, P = 0.146) with and without deformities. The incidence of deformities between males and females also did not differ significantly (X^2 =0.07, df = 1, P = 0.781). The

Table 1. Proportion of organisms with deformations and differential incidences of each type of deformity found in the population of Toluca Axolotls (*Ambystoma rivulare*) located in the municipality of Amanalco de Becerra, Estado de Mexico. Proportion of organisms with normal limbs and proportions of organisms with one and two affected limbs in the population of *A. rivulare* located in the municipality of Amanalco de Becerra, State of Mexico. Proportion of deformities found in each limb in the population of *A. rivulare* located in the municipality of Amanalco de Becerra. The abbreviation N/A = not affected.

	Males		Females		Total	
	n	%	n	%	n	%
Deformities						
Brachydactyly (partial digits)	8	44.4	5	38.5	13	41.9
Ectrodactyly (lost digits)	6	33.3	3	23.1	9	29
Polydactyly (extra digits)	2	11.1	3	23.1	5	16.1
Hypoplasia (reduced digits)	1	5.6	3	23.1	4	12.9
Syndactyly (merged digits)	2	11.1	0	0	2	6.5
Polymelia (extra limb)	0	0	1	7.7	1	3.2
Total Affected (Malformed)	18	22.2	13	20.6	31	21.5
Without Deformities	63	77.8	50	79.4	113	78.5
Total	81		63		144	
Number of Affected Limbs						
Normal Limbs	63	77.8	50	79.4	113	78.5
One Affected Limb	18	22.2	11	17.5	29	20.1
Two Affected Limbs	0	0	2	3.2	2	1.4
Total	81		63		144	
Affected Limbs						
Right Forelimb	6	33.3	3	23.1	7	22.6
Left Forelimb	1	5.6	3	23.1	4	12.9
Right Hindlimb	11	61.1	7	53.8	17	54.8
Left Hindlimb	N/A	N/A	1	7.7	1	3.2
Total	18		13		31	

right hind limb was most prone to deformities; more than half (54.8%) of the affected salamanders had some deformity of this limb (Table 1). Digit 16 was absent, presented a deformity, or loss of one or more phalanges in most cases (24.3%).

Figure 2. Deformities found in the extremities of Toluca Axolotls (*Ambystoma rivulare*) in the population of Amanalco de Becerra, México. (A) Brachydactyly (partial digits), (B) Ectrodactyly (lost digits), (C) Polydactyly (extra digits), (D) Hypoplasia (reduced digits), (E) Syndactyly (merged digits), (F) Polymelia (extra limb).

Discussion

The prevalence of deformities in *A. rivulare* inhabiting the Los Hoyos stream in the municipality of Amanalco de Becerra, Estado de México, México, is more than four times higher than the expected 5% for a healthy population (Meyer-Rochow and Asashima 1988; Read and Tyler 1994). High prevalence of deformities also occurred in populations of *A. ordinarium* inhabiting the central portion of the Trans-Mexican Volcanic Belt in México (Soto-Rojas et al. 2017). In this region, 224 salamanders (44.6% of the sample) from 29 streams exhibited at least one abnormality, including partial gills (26%), ectrodactyly (23%), and shortened limbs (8.9%; Soto-Rojas et al. 2017). In comparison, deformities, including missing digits on the hind limbs (n = 2) and extra digits on the right hind limb (n = 1) were observed in only three of 60 (5%) Tiger Salamanders (*Ambystoma tigrinum*) examined from the state of Querétaro, México (Cruz-Pérez et al. 2009).

The most common limb deformities we found in *A. rivulare* were brachydactyly and ectrodactyly. Previous studies suggest that total or partial loss of digits can be caused by either predation attempts (Johnson et al. 2006; Ballengée and Sessions 2009) or cannibalism (Johnson

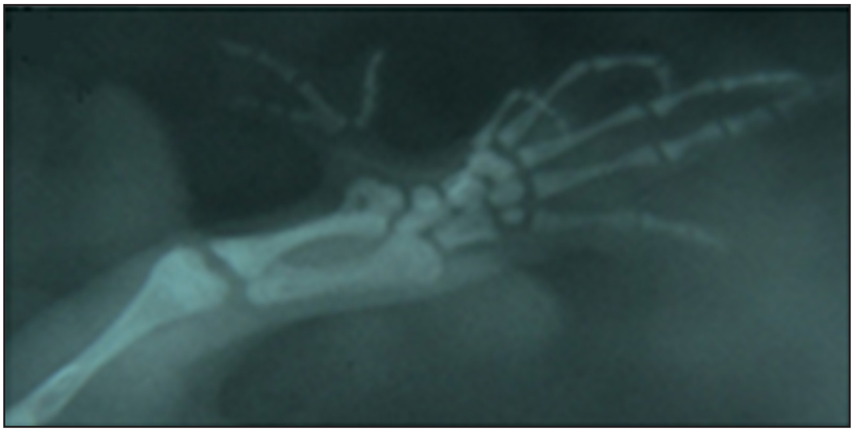

Figure 3. Radiograph showing the bone structures of the Polymelia case observed on the extremities of Toluca Axolotls (*Ambystoma rivulare*) in the population of Amanalco de Becerra, México. (Photographed by Daniel Sánchez-Manjarrez).

et al. 2006). The frequency of cannibalism can increase if a large number of young larvae coexist and feed in a small area (Thomson et al. 2014), either because of loss of suitable habitat or because of population isolation. High adult deformity rates (approximately 50%) also occur in the Mexican Axolotl (*Ambystoma mexicanum*) cultured in ex-situ breeding facilities where grouping individuals during the early stages of development favors cannibalism and biting episodes (Thompson et al. 2014). Also, limb injuries do not successfully regenerate in these conditions, which can mimic the appearance of deformities (Thompson et al. 2014). Potential predators of salamander larvae (e.g., odonata nymphs, belostomatids, and fish from the adjacent trout farm) are frequently observed in our study area. Additionally, *A. rivulare* is a microendemic species with a restricted distribution (Woolrich-Piña et al. 2017), increasing the likelihood of cannibalism in isolated populations with high larval densities, a notion supported by past reports of larger individuals consuming smaller ones (Lemos-Espinal et al. 1999). Observations of non-lethal cannibalistic episodes and bite lesions during feeding in various species of *Ambystoma* (Lemos-Espinal et al. 1999; Johnson et al. 2006; Thomson et al. 2014) suggest that population density in early stages of development play an important role in the prevalence of deformities in adult organisms among populations.

Polydactyly and polymyelia are deformities commonly associated with parasitosis in populations, mainly by trematodes of the genus *Riberoia* (Johnson et al. 2006). The incidence of these types of deformities in our study population is low, however, with only one case of polymelia observed (Fig. 3). Thus, we doubt that parasitic infection is a major cause of the deformities in *A. rivulare* in Amanalco de Becerra, although studies examining the incidence of parasitic infection in the population are necessary to substantiate our claim.

Although organisms with limb deformities may experience loss of mobility that impacts prey capture, dietary analyses of *A. rivulare* at this site reveal that salamanders with and without deformities do not differ in type of prey consumed (Sánchez-Manjarrez 2020). Additionally, the relative volume and the relative abundance of prey consumed does not differ between individuals with limb deformities compared to those without deformities (Sánchez-Manjarrez 2020). Thus, we suggest that the ability to capture prey is not negatively affected by the presence of deformities in our study population.

Water quality is a factor correlated with the presence of deformities in aquatic and semi-aquatic organisms (Soto-Rojas et al. 2017). We doubt, however, that the high prevalence of deformities in *A. rivulare* is the result of poor water quality. The Los Hoyos supports populations of Amphipoda, Trichoptera, and Ephemeroptera, which are bioindicators used to evaluate and monitor water quality because they require clean water (Stephenson and Mackie 1986; Dohet 2002; Pereira et al. 2012). We did not analyze water chemistry, however, and the presence of contaminants in the stream cannot be discounted, especially because of a trout farm near our study population. Pipes from the trout farm drain towards the stream and could be releasing contaminants that negatively affect water quality on-site and negatively impact embryonic development of *A. rivulare*. Thus, analyses of water quality in the Los Hoyos stream are warranted to determine if the high incidence of deformities in the population is, indeed, associated with chemical contamination. Future investigations could also address the potential for predation by fish that manage to escape from the farm. Introduction of trout in rivers inhabited by salamanders can reduce their growth rate by up to 85% and can reduce the proportion of organisms that complete metamorphosis by up to 65% (Kenison et al. 2016). Additionally, the introduction of trout can increase the natural concentrations of nitrogen in rivers, and this may have a negative correlation with the number of salamanders present at the sites, a situation also linked to the predation of salamander larvae by fish (Tyler et al. 1998).

The possibility that organisms with deformities are easier to detect or observe, given their potentially reduced mobility (and associated restrictions to flight or concealment behaviors) is worth noting. Deformities leading to abnormal behavior can result in higher capture probabilities for deformed individuals and, thus, unusually high incidences of these abnormal salamanders in our samples. Most of the salamanders captured during our study, however, were found within cavities or vegetation on the riverbanks, where the observation of each organism before capture was not possible.

Further studies on other *A. rivulare* populations may be needed to understand if the percentage of limb deformities we detected is common in this species (i.e., the natural baseline rate) or if our findings are specific to the population and time studied. Also, comparative studies regarding *Ambystoma* deformity rates and water quality rates at the study site are necessary to understand the real impact that water quality can have on the appearance of deformities. This research represents one of the few studies conducted in México concerning the presence of deformities in salamander populations. Data obtained during this study can be used as a baseline for comparison for future studies concerning the types of deformities as well as the incidence of each deformity in salamanders of the genus *Ambystoma* in México. Also, this information may serve as an indication of declining environmental quality in the region. Our findings will further contribute information on what leads to increases in the incidence of deformities in endemic amphibian populations.

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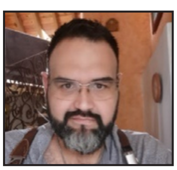

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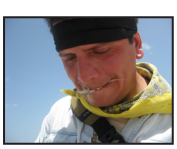

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