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Signals of decline of flagship species *Ambystoma altamirani* Dugès, 1895 (Caudata, Ambystomatidae) in a Mexican natural protected area

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Abstract

Mexico is home to 18 species of salamanders in the family Ambystomidae. Endangered *Ambystoma altamirani* Dugès, 1895 is a flagship species for the Lagos de Zempoala National Park (LZNP) in central Mexico, a protected area subject to numerous anthropogenic threats. *Ambystoma altamirani* populations in the Park have been little studied. In 2016–2017, we surveyed four streams where populations of the species had been previously reported. Habitat variables did not differ amongst streams and three had invasive rainbow trout, but we were only able to locate one *A. altamirani* population in Quila, a small, cold water stream lacking fish. We captured an average of 88 individuals (total n = 354; range 53–109) across all samples in this stream, including larvae, juveniles and adults. Population estimates ranged between 53 and 127 individuals. The absence in other streams suggests reductions in the spatial extent of *A. altamirani* in the LZNP. We suggest rainbow trout presence in numerous streams have led to local extirpation of *A. altamirani* and that removal and blockage of the invasive fish and a planned re-introduction strategy might help in restoring this flagship species.

Key Words

mountain stream siredon, rainbow trout, salamander, Trans-Mexican Volcanic Belt, Zempoala

Introduction

Amphibians are the most threatened vertebrate group in the world with numerous populations experiencing severe declines (Young et al. 2001; Stuart et al. 2004; Hussain and Pandit 2012). Climate change, disease and habitat destruction are perhaps the most important causes of these declines (McCallum 2007; Wake and Vredenburg 2008). Mexico exhibits the fourth greatest diversity of amphibians in the world (~360 species, Sarukhán and Dirzo 1992; Flores-Villela and Canseco-Márquez 2004). Salamanders in the genus *Ambystoma* are amongst the most threatened groups in Mexico; 17 of 18 species are endemic and have relatively-small geographic ranges (SEMARNAT 2019; Frías-Álvarez et al. 2010; Parra-Olea et al. 2014; Frost 2020; IUCN 2020).

Ambystoma altamirani Dugès, 1895, an endangered salamander endemic to the Trans-Mexican Volcanic Belt, inhabits streams and lakes at altitudes of 2450 to 3487 m, in areas surrounded by grasses and temperate conifer forests (Lemos-Espinal et al. 1999; Uribe-Peña et al. 1999; Lemos-Espinal et al. 2016; Woolrich-Piña et al. 2017), especially stream areas dominated by mud substrate (Villareal-Hernández et al. 2020). It is a flagship species for the Lagos de Zempoala National Park (LZNP) (States of Morelos and Mexico). The Park, created in 1936, encompasses seven endorheic lakes and associated streams where *A. altamirani* were once abundant. The Park is exposed to a variety of anthropogenic stressors, including human use, illegal logging, stream capture for human consumption and invasive species introductions (CONANP 2008; Zambrano and Valiente 2008). Invasive fishes, such as rainbow trout, are particularly dangerous for amphibian populations (Estrella-Zamora et al. 2018).

Studies and monitoring data for *A. altamirani* in the LZNP are scarce. Initial descriptive and taxonomic studies for *A. altamirani* in the area date from the 1940s (Taylor and Smith 1945; Maldonado-Koerdell 1947), but its populations were only monitored between 2003 and 2008, when the species was reported from only four lakes and associated streams in the LNZP: Lake Zempoala, Lake Tonatiahua, Lake Quila and Lake Acoyotongo (CONANP 2009). Ten years after the last monitoring event, we initiated this study to 1) document the extent of the populations of *A. altamirani* in four streams where the species had been previously captured, 2) measure habitat and physical-chemical parameters for these streams, 3) quantify their abundance and 4) explore the potential effect of non-native rainbow trout on the presence of *A. altamirani*.

Methods

The LZNP (total area = 4790 ha) is in the headwaters of the Balsas River Basin (Pacific Slope) in Central Mexico (Fig. 1). The mean monthly average temperature in the area ranges from 5 (January) to 18 °C (May) with an average annual precipitation of 1550 mm (CONANP 2008). Until 1979, the LZNP had seven lakes, but three are currently dry and the other four experience fluctuating water levels (depending on seasonal rains) (Bonilla Barbosa and Novelo Retana 1995; Quiróz-Castelán 2008; Godínez-Ortega et al. 2017). The LZNP has numerous intermittent and permanent streams leading to endorheic permanent or ephemeral lakes. Streams are usually first to second order systems 50–200 cm wide, with clear, cold waters. Some streams have been tapped for water use in nearby towns. Non-native carp (*Cyprinus carpio* L., 1758), largemouth bass (*Micropterus salmoides* Lacépède, 1802), tilapia (*Oreochromis* sp.), grass carp (*Ctenopharyngodon idella* Valenciennes, 1844) and rainbow trout (*Onchorhynchus mykiss* Walbaum, 1792) have all been introduced at some point during the last 40 years into LZNP lakes. While some of these species have disappeared from the lakes, others have thrived. Rainbow trout were introduced to several LZNP lakes (Contreras-MacBeath and Urbina 1995) and expanded into LZNP streams.

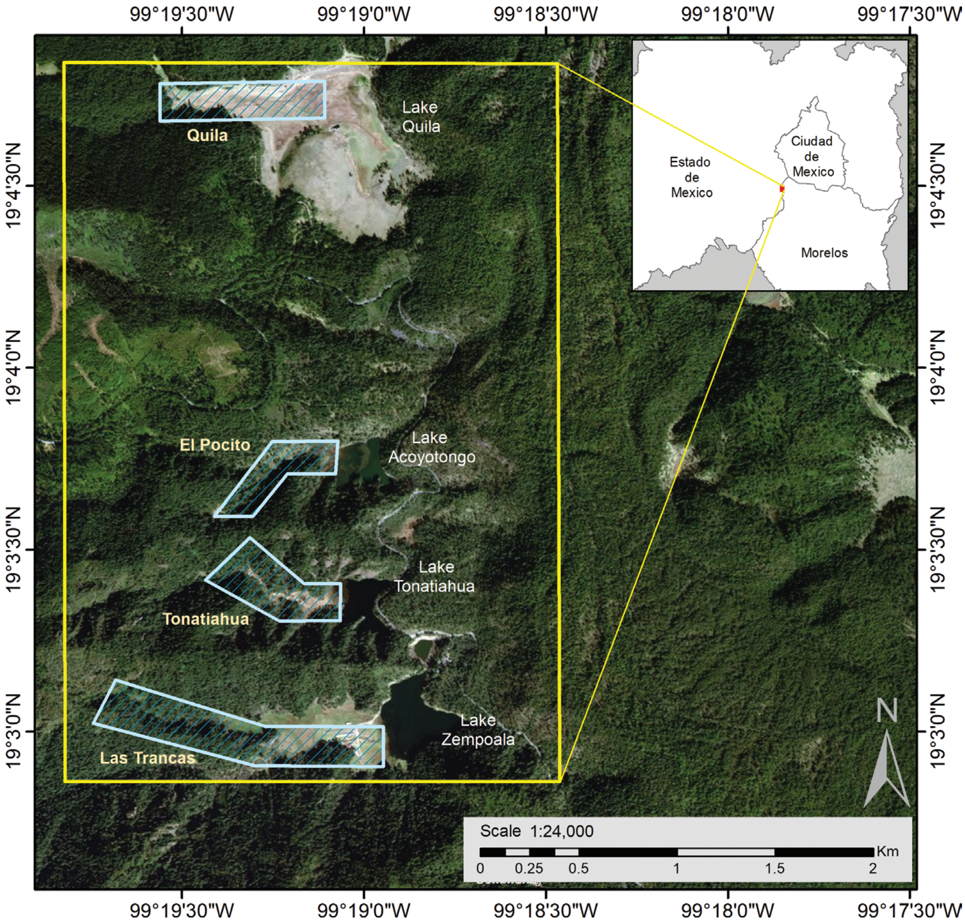

Figure 1. Streams Quila, Tonatiahua, las Trancas and El Pocito in the Parque Nacional Lagunas de Zempoala, in the States of Morelos and Mexico, Mexico. Polygons show approximate extent of area surveyed.

Our study had two independent but complementary phases. In phase one (accomplished between September and October 2016), we carried out sampling in the four streams where the species was reported in a 2003–2008 survey (CONANP 2009). We sampled 1–4 km reaches in each of four permanent streams: Las Trancas (19°2.96'N, 99°19.05'W, emptying into Lake Zempoala), Tonatiahua (19°3.7'N, 99°19.06'W, emptying into Lake Tonatiahua), El Pocito (19°3.76'N, 99°19.08'W, emptying into Lake Acoyotongo) and Quila (19°4.75'N, 99°19.15'W, which used to feed Lake Quila, now just a marsh during the rainy season) (Fig. 1). In each reach, we haphazardly established between 3 and 20 different sampling points or transects where we obtained environmental data and carried out sampling activities. In each sampling point, we used funnel traps to sample for *A. altamirani* (Sparling et al. 2001; Wilson and Dorcas 2003). A trap was set in a preselected pool in a stream for 16–24 hours and then checked for *A. altamirani.* When transects were implemented, a 150–200 m stretch of stream was sampled visually, with hand dip nets or using a backpack electrofisher (ETS Electrofishing Systems) with reduced voltage (Brown and May 2007; Dgebuadze and Bashinskiy 2016). During visual samples, two surveyors walked along the stream banks searching for *A. altamirani*, taking note of the presence of fish. When hand dip-netting, two surveyors walked upstream in the creek, scooping rocks, undercuts and submerged vegetation at depths 24–52 cm with 1 cm-mesh hand dip nets. Electrofishing was implemented by sampling in all available habitats (i.e. undercuts, amongst rocks, in submerged vegetation) in a transect. All individual *A. altamirani* or fish captured were placed in buckets with water prior to processing. Rainbow trout presence (via observation or by individuals captured during electrofishing or hand dip net surveys) was recorded for each stream. In each sampling point or transect, we obtained habitat and water physical-chemical data. We used a Hanna multimeter (HI9829) to obtain information on water temperature (°C), pH (standard units), dissolved oxygen (DO) (% and mg/l), conductivity (μS/cm) and total dissolved solids (mg/l). Average depth and water velocity (m per second) were measured (Global Waters flow meter) at three points in a site or transect (Table 1).

The second phase of the study consisted in estimating *A. altamirani* abundance. During the initial survey in 2016, we were only able to locate *A. altamirani* in Quila Stream. Thus, the following procedures describe sampling and individual processing only in Quila Stream from January to December 2017. The Quila Stream segment we surveyed using hand dip nets consists of a 1.2 km stream stretch with abundant undercuts running through a meadow with no tree cover. The stream meanders down from a water extraction facility to a marshy area that used to form Quila Lake. The 2017 sampling period encompassed three sampling events during the dry-cold (Jan – Mar 2017), rainy (Jul – Sept 2017) and the wet-cold (Oct – Dec 2017) seasons. From each captured individual, we obtained snout-vent length (SVL), tail width, head width (all in mm) and body mass (g) using a Vernier and weight scale (Ohaus Scout). Sex and stage (larva, juvenile, adult) were obtained following Semlitsch and Wilbur (1988). Individuals ≥ 55 mm SVL were marked in all but the last sampling event with visible implant elastomer (VIE) tags (Northwest Marine Technology) and then released back to the same stream section where they were captured. VIE tags are a common, safe and effective method used in salamander population estimate studies (Ralston Marold 2001; Heemeyer et al. 2007). Identification of previously marked individuals was carried out in surveys 2–9. Two different easy-to-detect-under-UV-light elastomer colours, green and pink, were used for tagging and identifying captured individuals. Following Donnelly et al. (1994), we developed a coding system where the location of differently coloured marks in different areas of the body (dorsal area, the anterior part of front and hind legs or abdomen) led to a given individual-specific number which was used for tracking capture events. After marking or being recaptured, every individual was returned, unharmed, to the same area of the stream where it was captured.

We tested for differences in each physical-chemical and habitat variable across streams using analyses of variance (ANOVA) (JASP ver. 0.10.2.0). From *A. altamirani* collection data, we obtained the total number of individuals captured and recaptured per sampling event and calculated descriptive statistics on the total number of individuals for all sampling events. Further, we tested for differences in abundance between the three sampling seasons (Kruskall-Wallis test, JASP ver. 0.10.2.0). We used the Cormack-Jolly-Seber model (Jolly 1965, Manly 1984) to estimate *A. altamirani* population in Quila Stream in all but the first and last sampling events.

Table 1. Physical-chemical and habitat variable range (min. – max.) for sites and transects in four streams of the Parque Nacional Lagunas de Zempoala (Mexico) in 2016–2017.

Stream	DO (%)	DO (mg/l)	pH	T (°C)	Cond. (μS/cm)	TDS (mg/l)	Vel. (cm/s)	Depth (cm)
Trancas	65–67	5.0–5.23	7.7–8.4	10.0–12.5	69–74	40–49	0.2–0.6	15–45
Tonatiahua	63–70	5.16–5.24	7.2–8.3	10.5–12.0	70–75	40–45	0.2–0.4	19–37
Pocito	65–69	5.3–5.17	7.5–8.0	10.0–12.5	68–72	40–47	0.1–0.6	16–43
Quila	65–70	5.2–5.15	7.2–8.4	10.5–13.0	60–73	45–49	0.3–0.5	15–47

Results

Ambystoma altamirani was captured only in Quila Stream in 2016. Sampling methods implemented in the other three streams rendered no *A. altamirani*. Rainbow trout were observed or captured in the Trancas, Tonatiahua and El Pocito streams. Trout were not captured or seen in Quila Stream. Physical-chemical and habitat variables were similar throughout sites, transects and sampling periods (all $p > 0.05$). Generally, all sites and transects had, on average, 65 mg/l (67%) DO, slightly basic pH, relatively cold water (11 °C), with low conductivity (68 μS/cm) and total dissolved solids (45 mg/l). Sites were relatively shallow (15–47 cm) and had water velocities 0.1–0.6 mps (Table 1).

From January to December 2017, we captured 354 individuals of *A. altamirani* in Quila Stream, 247 individuals were marked and 210 individuals were recaptured at least once. We captured (recaptures in parentheses) 109 (0), 66 (38) and 86 (64) individuals in January, February and March 2017, respectively. We captured 53 (25), 84 (68) and 88 (82) individuals in July, August and September 2017, respectively. We captured 104 (78), 95 (83) and 106 (73) individuals in October, November and December 2017, respectively. Over the course of all months, we captured an average 87.8 (SD = 18.7, range = 53–109) individuals. We found no statistical difference in abundance between samples obtained in different seasons ($H = 3.2$, $p = 0.202$). Population estimates for the Quila population were 72 (lower and upper C.I. = 58 and 77), 107 (99, 113), 53 (54, 55), 98 (92, 103), 107 (100, 112), 128 (116, 139) and 127 (108, 146), for sampling periods 2 to 8, respectively.

Considering all 354 individuals captured, the SVL range was 9.0 – 182 mm (Fig. 2); tail and head width range were 0.5 – 23 and 5.0 – 25 mm, respectively; and body mass range was 0.5 – 59 g. Of 247 marked individuals, 160 were adults (100 female; 60 male) and 87 juvenile, when first encountered. A total of 107 larvae were collected.

Figure 2. *Ambystoma altamirani* from Quila Stream in the Parque Nacional Lagunas de Zempoala, in the States of Morelos and Mexico, Mexico. Individual shown (SVL = 182 mm) was captured in November 2017.

Discussion

Our results illustrate a potentially dire situation for *A. altamirani* populations in the LZNP and provide information that should alert and help managers throughout its range. This area is undergoing fast environmental deterioration (Young et al. 2001; Collins and Storfer 2003) which, in addition to synergistic effects from climate change, emerging diseases and exotic (alien) species, will continue to increase pressure on remnant populations (Villareal-Hernández et al. 2020).

In addition to the LZNP populations, *A. altamirani* has been recorded from the Las Cruces mountains (States of Mexico and Mexico City) and other areas in the upper Lerma River basin (Reilly and Brandon 1994; Lemos-Espinal et al. 1999; Lemos-Espinal et al. 2015; Woolrich-Piña et al. 2017; Monroy-Vilchis et al. 2019). Despite having some level of federal or state protection, other freshwater ecosystems in the area face similar threats as they do in the LZNP. These threats, relatively small range and declining populations have led to *A. altamirani* being listed in both international (Shaffer et al. 2008) and national (SEMARNAT 2019) Red Lists. Recent studies have further identified possible genetic bottlenecks and small effective population sizes in *A. altamirani* populations in nearby areas (Heredia-Bobadilla et al. 2017; Monroy-Vilchis et al. 2019).

Absence of previously known *A. altamirani* populations in three of four LZNP streams we surveyed suggests the species might be extirpated from these systems. Our study revisited lotic sites where a 2003–2008 survey still reported the species from four lakes and their streams (CONANP 2009). This report did not clearly specify the number of individuals captured in each stream or sampling event or provided population estimates for studied sites. However, it does report that between 22 and 118 individuals were collected in aquatic systems of the LZNP in the period of study. We believe our evidence should prompt further efforts for surveys, attempting better quantification of *A. altamirani* populations in the protected area.

Our abundance and population estimates for Quila Stream suggest the species is relatively stable in this system. Our estimates are similar to those reported for similar species in Mexico; samples of 190, 161 and 306 individuals of *A. ordinarium*, *A. leorae* and *A. altamirani*, respectively, have been reported in other recent studies (Calderón et al. 2011; Sunny et al. 2014; Lemos-Espinal et al. 2016). However, further analyses should identify the effective population size as there was asymmetry in adult sex proportion (1.0 females vs. 0.6 males) and we observed temporal variation in abundance.

The 2003–2008 study did not report on water physical-chemical or other habitat variables, but our 2016–2017 surveys indicated little difference in parameters amongst systems. Thus, habitat variables do not seem to factor heavily in the presence and absence of *A. altamirani* in our study. Presence of the carnivore rainbow trout, however, seems to be key to the absence of *A. altamirani* in lotic habitats of the LZNP. Alien species are known to have a negative impact on native amphibian communities (Larson et al. 2002; Bosch et al. 2006). *Ambystoma mexicanum* and *A. dumerillii* have experienced impacts from common carp and tilapia, especially as larvae or juveniles (Huacuz-Elías 2002; Valiente 2006; Zambrano et al. 2010). Rainbow trout was introduced into Central Mexican aquatic ecosystems in the early 1900s and expanded its range. Once established, it affected native fauna via foodweb interactions and disease spread (Consuegra et al. 2011; Mercado Silva et al. 2012; Sepúlveda et al. 2013; Estrella-Zamora 2018). While efforts for trout removal and for impeding their access to novel stream reaches have had success elsewhere (Contreras-MacBeath et al. 2016; Meyer et al. 2017; Shelton et al. 2017), the practice is relatively novel in Mexico. Such efforts, however, would help protection of remnant *A. altamirani* populations in the LZNP. We suggest that Quila Stream being disconnected from a lentic water body where trout could be planted and its being isolated from human use comparatively to the other lakes, might be reasons why the species remains.

While our study identified potential declines of *A. altamirani* populations in the LZNP streams, we note that these amphibians have also been reported in the Park´s lakes, that we did not sample lentic systems due to logistics limitations and that several other permanent and ephemeral streams remain unsampled. It is thus possible that Lakes Acoyotongo, Tonatiahua and Zempoala hold *A. altamiran*i populations. However, these Lakes are known to contain trout and, in the case of Tonatiahua and Zempoala, also grass carp *Ctenopharyngodon idella* and other invasives (Contreras-MacBeath and Urbina 1995). If these Lakes contain *A. altamirani*, we believe it is unlikely they can re-populate streams if trout are not first removed. Further, even when these salamanders might have a wider variety of habitats in lentic systems, they will also be subject to competition from non-natives. This study was carried out over a short period of time; a longer term study with intensified sampling efforts throughout the LZNP might render additional populations of *A. altamirani*.

Despite the above, our work is one of few for *A. altamirani* in the LZNP, reports perhaps the largest individuals known for the species and has identified some of the threats faced by this flagship species. Our results should inform park managers about the importance of initiating non-native species removals and blockages and the protection of viable *A. altamirani* populations. These efforts should be adopted soon, as synergistic threats to the species might further threaten its viability in the wild.

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