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Effects of nonnative Rainbow Trout on two species of endemic Mexican amphibians

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Abstract: Introduction of nonnative salmonids into fishless aquatic ecosystems often can have negative effects on these ecosystems. Amphibians appear to be particularly susceptible to the introduction of trout. Nonnative Rainbow Trout (*Oncorhynchus mykiss*) have been introduced to Mexico, including to streams in the mountains around Mexico City that are home to several endemic Mexican amphibians. We examined the effect of Rainbow Trout on the distributions of 2 endemic Mexican amphibians, *Ambystoma altamirani* and *Hyla plicata*, in the Sierra de Las Cruces. *Ambystoma altamirani* was never found at sites with Rainbow Trout, and *H. plicata* was observed only once at sites with Rainbow Trout. Stream site characteristics, for the most part, did not vary among streams occupied by different species, suggesting environmental variables were not primarily responsible for the species distributions. Our results provide strong circumstantial evidence that the introduction of Rainbow Trout in streams in the Sierra de Las Cruces has a negative effect on the distribution of native Mexican amphibians.
Key words: introduced species, trout farms, distribution, *Ambystoma altamirani*, *Hyla plicata*, *Oncorhynchus mykiss*

The introduction of nonnative salmonids, such as Rainbow Trout (*Oncorhynchus mykiss*), into fishless aquatic ecosystems can have myriad effects on the native aquatic community and often causes decline of at least some of the native species in these communities (Cambray 2003, Dunham et al. 2004, Crawford and Muir 2008). Introduced Rainbow Trout can alter the community composition or biomass of benthic invertebrates (Shelton et al. 2015a, 2016, Vimos et al. 2015), native fish (Shelton et al. 2015b, Turek et al. 2015, 2016), zooplankton (MacLennan et al. 2015, Loewen and Vinebrooke 2016), and periphyton and algae (Nyström et al. 2001, Buria et al. 2010, Shelton et al. 2015a, Vimos et al. 2015). Introduced trout can even affect terrestrial taxa, such as birds (Ortubay et al. 2006, Epanchin et al. 2010).

Amphibians are particularly susceptible to the negative effects of the presence of fish (e.g., Hecnar and M'Closkey 1997, Smith et al. 1999, Holbrook and Dorn 2016). In particular, the introduction of trout can decrease the abundance or occupancy of species of salamanders and frogs in the northern USA and Canada (Tyler et al. 1998, Matthews et al. 2001, Pilliod and Peterson 2001, Knapp 2005, Welsh et al. 2006, McGarvie Hirner and Cox 2007, Pearson and Goater 2008, Pilliod et al. 2010). Introduction of trout also has been associated with population declines of frogs in Australia (Gillespie and Hines 1999, Gillespie 2001) and South Africa (Karssing et al. 2012). These negative effects are thought to be primarily a consequence of predation by Rainbow Trout on the amphibians. Rainbow Trout will consume salamanders (Pearson and Goater 2009) and tadpoles of various species of anurans (Gillespie 2001). However, some species of anurans, particularly those thought to be unpalatable, are not affected or are positively affected by trout introductions (e.g., *Anaxyrus boreas*, Welsh et al. 2006, McGarvie Hirner and Cox 2007; *Taricha granulosa*, Welsh et al. 2006; but see Lanier et al. 2017). In addition to direct effects as predators, Rainbow Trout introductions can help spread emerging amphibian diseases, such as amphibian iridovirus (Jancovich et al. 2005) and the pathogenic oomy-

cete *Saprolegnia ferax* (Kiesecker et al. 2001), and could thus contribute to changes in amphibian distributions by serving as vectors of decline-inducing disease agents.

Despite well-documented negative effects of introducing nonnative trout into previously fishless habitats, nonnative Rainbow Trout have been introduced to Mexico for aquaculture (Hendrickson et al. 2002, Escalante et al. 2014, Sosa-Villalobos et al. 2016), including in mountain streams near Mexico City (López-García et al. 2014). Use of Rainbow Trout aquaculture has been suggested as a way to conserve native forests, such as the Monarch Butterfly Biosphere Reserve, because trout farms benefit from the retention or expansion of forest cover (López-García et al. 2014). In addition to intentional introduction, trout that escape from impoundments can affect natural waterways (Consuegra et al. 2011, Mercado Silva et al. 2012, Sepúlveda et al. 2013).

The streams in the mountains of central Mexico are home to several species of endemic Mexican *Ambystoma* species, many of which are endangered or critically endangered (Frías-Alvarez et al. 2010, Wilson et al. 2013), and other amphibians. *Ambystoma altamirani* is a salamander endemic to Mexico, and is listed as Endangered globally (IUCN 2015) and Threatened by the Mexican government (SEMARNAT 2010). Frías-Alvarez et al. (2010) list alien species, overexploitation, landuse change, pollution, and emerging infectious diseases as conservation threats for *A. altamirani*. *Hyla plicata* is a frog endemic to Mexico and is classified as a species of Least Concern globally (IUCN 2015), but considered Threatened by the Mexican government (SEMARNAT 2010). The conservation threats to *H. plicata* were listed as "Lack of Information" by Frías-Alvarez et al. (2010).

We investigated the effects of introduced Rainbow Trout on the distribution of *H. plicata* and *A. altamirani* in the Sierra de Las Cruces. In particular, we were interested in knowing whether: 1) *A. altamirani* and *H. plicata* were found at the same stream sites as *O. mykiss*, 2) stream characteristics (i.e., width and depth, mud depth, and vegetation complexity) differed between sites with and without each taxon, and 3) the 3 species differed in their use of stream sites with different stream characteristics. Our goal was to better understand whether and how *O. mykiss* might affect the distributions of *A. altamirani* and *H. plicata* in the streams of Sierra de Las Cruces that are used for trout farms.

METHODS
Study species

Ambystoma altamirani is distributed in the central part of the Transvolcanic Belt of Mexico in the Distrito Federal, state of México, and Morelos. Its altitudinal distribution ranges from 2450 to 3487 m asl. It is found in small streams surrounded by pine and fir forests and grasslands of *Festuca* spp., *Stipa* spp., and *Mühlenbergia* spp. Breeding potentially takes place throughout the year (Campbell and Simmons

1962, Brandon and Altig 1973), with larvae taking 6 mo (Brandon and Altig 1973) to ≥1 y to metamorphose. Metamorphosed individuals stay in or near the aquatic habitat (Lemos-Espinal et al. 2016b). In the Arroyo Los Axolotes, a trout-free stream in the Sierra de Las Cruces, *A. altamirani* were found in all months of the year except December and January, with eggs laid in June (Lemos-Espinal et al. 2016b). Their diet consists primarily of ostracods and gastropods (Lemos-Espinal et al. 2015).

Hyla plicata is distributed along the edge of the Transvolcanic Belt in central Mexico, and in scattered localities of the Sierra Madre Oriental in Hidalgo, Puebla, and Veracruz. It inhabits humid pine and fir forests, where it is found in open meadows and slow meandering streams at elevations from 2400 to 3600 m asl. Adults of this species arrive at the trout-free Arroyo Los Axolotes in the Sierra de Las Cruces in March with egg laying taking place in June, tadpoles present in July and August, and metamorphs present in September and October (Lemos-Espinal et al. 2016a). *Hyla plicata* were not observed in the Arroyo Los Axolotes from November to February (Lemos-Espinal et al. 2016a). No studies on the diet of the adults of this species have been published, but it probably consists of insects (Lemos-Espinal and Dixon 2016).

Study area

The Sierra de Las Cruces is in the eastern part of the Neovolcanic Axis (lat 18°59′–19°43′ N, long 99°00′–99°40′W) and forms the boundary between the México (2220 m asl) and Toluca (2400 m asl) basins. It is 110 km long and 47 km wide at its northern part and 27 km wide at its southern part. Our study took place in the northwestern portion of the Sierra de Las Cruces (Fig. 1). We examined several streams along the following sections of road: San Luis Ayucán/Barrio Las Manzanas to Llano Las Navajas, Llano Las Navajas to Villa del Carbón, Llano Las Navajas to Centro Ceremonial Otomi, and Villa del Carbón to Cahuacán. These 4 sections are arranged in a U along highway México 3, from Barrio Las Manzanas to Cahuacán, all with an altitude >2400 m. Along these roads are extensive grasslands of *Bouteloua* spp., *Festuca* spp., *Mühlenbergia* spp., and *Stipa* spp., surrounded by woods of *Abies religiosa*, *Pinus hartwegii*, *Pinusmontezumae*, and *Quercus* spp. These grasslands and forests are crossed by a considerable number of streams formed by runoff from the higher elevations of the Sierra de Las Cruces. All of the studied streams are permanent and range from 1st- to 4th-order (although most are 3rd- and 4th-order).

The trout farms in the Sierra de Las Cruces are small-scale operations. The farms consist of pools ~3 × 3 × 1 m deep that are dug by the farmers. Farms receive trout (*O. mykiss*) for free from the government based on the number of pools created. The government teaches farmers how to produce their own fish, but exerts no subsequent over-
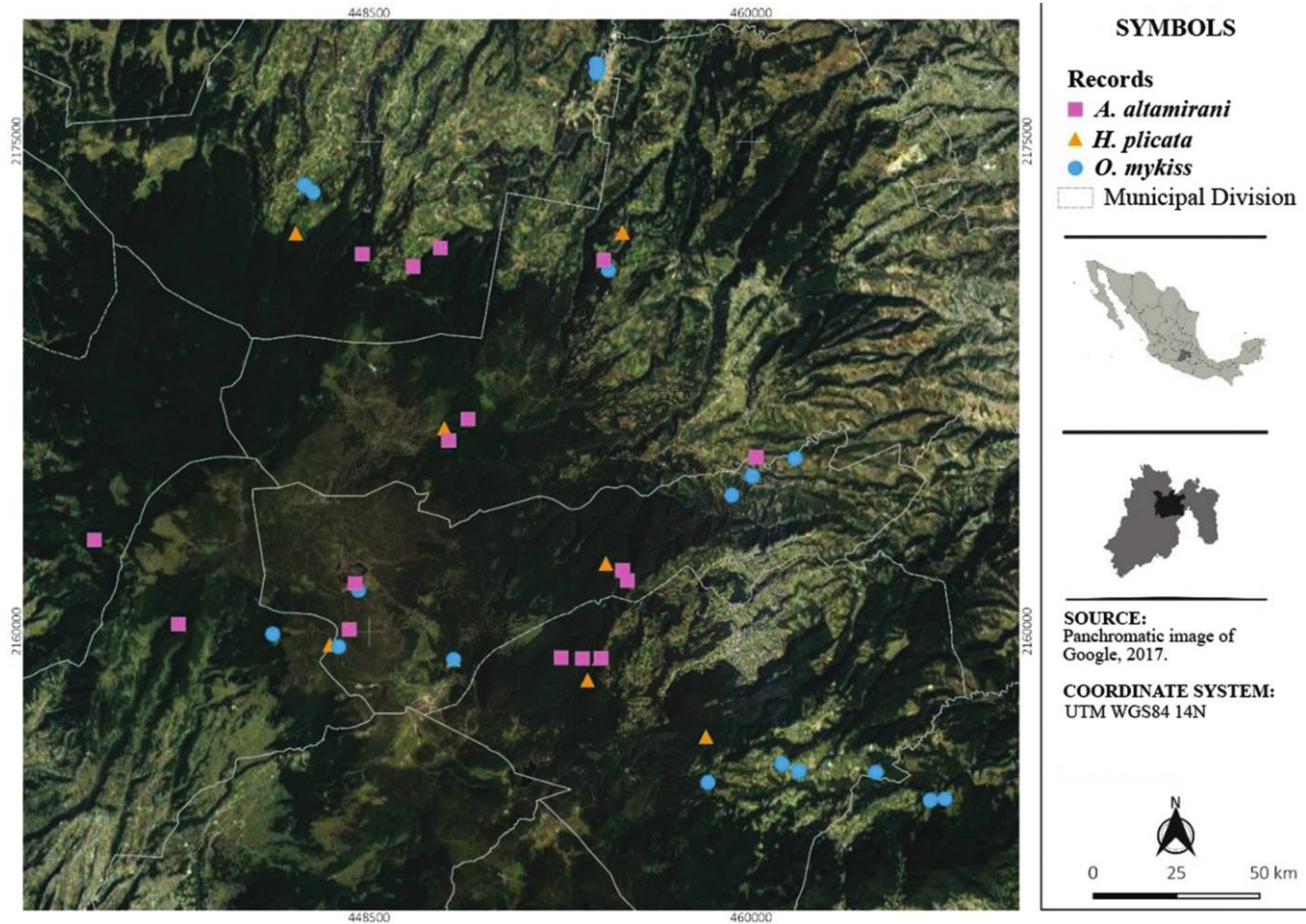
Figure 1. Map of study area in the Sierra de Las Cruces, México with locations of the sites containing *Ambystoma altamirani*, *Hyla plicata*, and *Oncorhynchus mykiss*.

sight of the trout farms. Typically, the pools are 3 to 5 m from the stream and receive flowing water from the stream through a channel dug between the stream and the pool. To allow the needed flow of water, an exit channel is dug from the pool back to the stream. All trout farms have barriers to prevent fish migration upstream. The barrier consists of metal screening placed in the channels to and from the stream. However, these barriers do not appear to be very effective, and many trout escape from farm pools into the stream, especially during the rainy season when it is easier for the trout to jump over the barriers. Given the ubiquity of trout farms along these streams and the apparent ease of escape, all sites along these streams are probably equally likely to contain trout.

Fieldwork

We visited the study area monthly from October 2015 to October 2016. During each visit, we walked up to 1 km along a stream and randomly selected up to 10 sites along the stream depending on its length ($n = 13$ streams; total

number of sites surveyed = 66). Each site was 100 m from the previous site. All sites in a stream were connected by flowing water. We visited each stream only once during the study period in an effort to sample all available streams in the study area. Each randomly selected site was a 5-m-long stretch of the stream. At each site, we recorded the presence of individuals (larvae or adults) or egg masses of *A. altamirani* and *H. plicata* and the presence of Rainbow Trout. At other locations in the Sierra de Las Cruces (see above), *A. altamirani* and *H. plicata* are found in most months of the year (Lemos-Espinal et al. 2016a, b).

Our study design does not allow us to explicitly estimate detectability of each species because we visited each site only once during the study (Mazerolle et al. 2007). Thus, our results might be subject to biases if detectabilities of the different species varied or if the detectability of a species depended on the presence (or absence) of another species (see Mazerolle et al. 2007 and Guimarães et al. 2014 for a discussion of these issues). As a point of reference, previous investigators have estimated detection probabilities ranging from 0.459 to 0.89 for pond and wetland *Ambystoma*

(Corn et al. 2005, Hossack and Corn 2007, Gorman et al. 2009, Peterman et al. 2013), from 0.39 to 0.96 for stream-dwelling, nonambystomatid salamanders (Jung et al. 2005, Kroll et al. 2010), and from 0.14 to 0.94 for hylid frogs (Pellet and Schmidt 2005, Smith et al. 2006, Gómez-Rodríguez et al. 2010, DiRenzo et al. 2017). However, the characteristics of our study system give us confidence that we were able to detect each species effectively and that detectability of any species was unlikely to be affected by the presence of other species.

We carefully searched the stream section visually and by using a snake hook along the bottom of the stream and in depressions in the side of the stream to induce movement by any organisms, thereby making them obvious. These 3 species are easy to detect during most of the year and the probability of detecting them might be regarded as the same for the 10 mo in which the water flow is low. These streams are generally <1 m deep with very clear, slow moving water with good visibility. *Oncorhynchus mykiss* and adult *H. plicata* are easy to detect because of their size. Tadpoles of *H. plicata* and larval and adult *A. altamirani* are harder to detect because their body color tends to be close to the substrate color. However, because we searched relatively small stream sites physically and visually, hidden individuals probably moved making them easy to detect. Eggs of *H. plicata* and *A. altamirani* are conspicuous and easy to detect. However, heavy summer storms in the area (June, July, August) can decrease detectability of individuals of these species. During all surveys, we were careful to spend substantial time searching each site to avoid missing individuals in the stream. We also looked under all rocks or other objects in the stream section. We are confident that our use of these methods to search every site thoroughly enabled us to detect any amphibians or fish at each site.

At each site, we measured the width and depth of the stream and the depth of mud at the site. We classified aquatic vegetation based on the following complexity categories: none = no aquatic vegetation of any kind observed at site; low complexity = simple, low aquatic vegetation, such as moss or algae present; medium complexity = aquatic vegetation with some vertical structure, such as grasses or ferns, present; high complexity = more complex structure in the form of fallen tree trunks or branches present in addition to components of other categories.

Data analysis

For *A. altamirani* and *H. plicata*, we used separate χ^2 tests to compare the number of sites with and without each species in the presence and absence of Rainbow Trout. We used nonparametric Mann–Whitney *U* tests to compare stream width, stream depth, and mud depth between sites with and without each species because of violations of the assumptions of normality for ANOVA. Stream depth and stream width were significantly correlated ($r = 0.39$, $p =$

0.0011), but no other correlations among the independent variables were significant ($p > 0.05$ in all cases). We used Fisher's Exact Tests to compare vegetation complexity of sites used by each species to sites without those species. For *A. altamirani* and *H. plicata*, we repeated these analyses based on only sites without *O. mykiss*. We used Kruskal–Wallis tests to compare stream width and depth and mud depth among species. We used Fisher's Exact Tests to compare vegetation complexity among species. All statistical analyses were conducted using JMP (version 12.0; SAS Institute, Cary, North Carolina). Means are given ±SE.

RESULTS
Ambystoma altamirani

We found no *A. altamirani* at sites that had Rainbow Trout, but we found *A. altamirani* in nearly 45% of sites without Rainbow Trout ($\chi^2_1 = 12.11$, $p = 0.0005$; Fig. 2A). Sites with *A. altamirani* were narrower than sites without
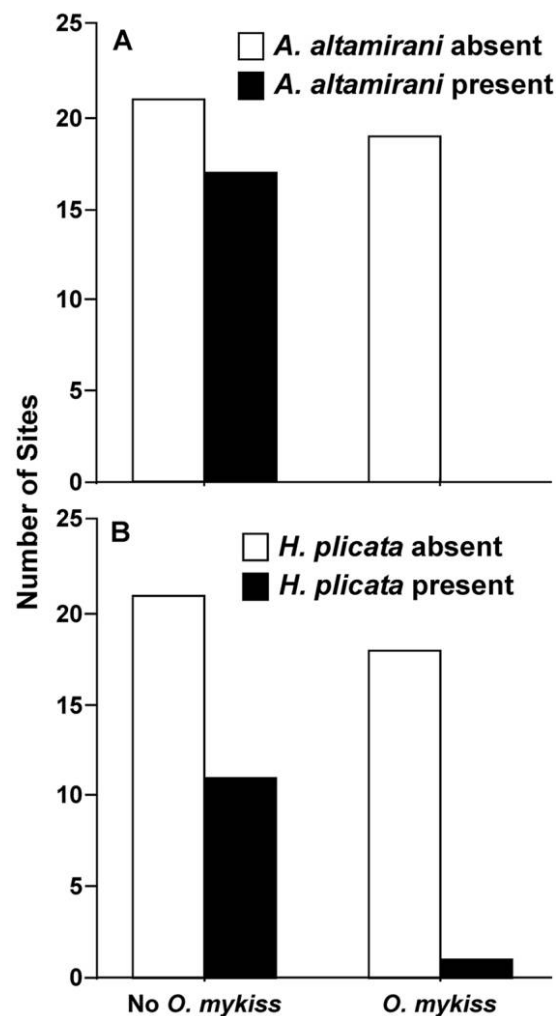

Figure 2. Number of sites with and without Rainbow Trout (*Oncorhynchus mykiss*) that have *Ambystoma altamirani* (A) and *Hyla plicata* (B) present or absent.

A. altamirani ($U_1 = -2.21$, $p = 0.03$; Table 1). Sites with and without *A. altamirani* did not differ in depth ($U_1 = -0.85$, $p = 0.39$; Table 1) or depth of mud ($U_1 = 1.23$, $p = 0.22$; Table 1). Sites with and without *A. altamirani* did not differ in vegetation complexity (Fisher's Exact Test: $p = 0.55$; Table 2).

When we considered only sites without *O. mykiss*, stream sites with *A. altamirani* were narrower (121.2 ± 14.8 cm, $n = 17$) than sites without (222.0 ± 31.4 cm, $n = 30$) ($U_1 = -2.03$, $p = 0.042$). Sites with and without *A. altamirani* did not differ in stream depth (75.5 ± 14.6 cm, $n = 17$ vs 74.3 ± 10.4 cm, $n = 30$; $U_1 = -0.14$, $p = 0.88$) or mud depth (8.9 ± 1.4 cm vs 10.1 ± 1.8 cm, $n = 30$; $U_1 = 0.067$, $p = 0.95$). Vegetation complexity did not differ between sites with and without *A. altamirani* for sites without *O. mykiss* (Fisher's Exact Test, $p = 0.65$). Fifteen sites without *O. mykiss* had only *A. altamirani* and 2 had both *A. altamirani* and *H. plicata*.

Hyla plicata

We found *H. plicata* at only 1 of 19 sites with *O. mykiss*, whereas we found *H. plicata* at 34% of sites without *O. mykiss* ($\chi^2_1 = 5.62$, $p = 0.018$; Fig. 2B). Stream width ($U_1 = 0.50$, $p = 0.62$), depth ($U_1 = -1.11$, $p = 0.26$), and vegetation complexity (Fisher's Exact Test: $p = 0.13$) Tables 1, 2) did not differ between sites with and without *H. plicata*. Sites with *H. plicata* had deeper mud than sites without *H. plicata* ($U_1 = 3.20$, $p = 0.0014$; Table 1).

At sites without *O. mykiss*, stream width (243.2 ± 72.9 cm, $n = 11$ vs 167.9 ± 17.7 cm, $n = 36$; $U_1 = 0.16$, $p = 0.87$), depth (60.7 ± 18.3, $n = 11$ vs 79.0 ± 9.5 cm, $n = 36$; $U_1 = -1.08$, $p = 0.28$), and vegetation complexity (Fisher's Exact Test: $p = 0.18$) did not differ between sites with and without *H. plicata*. Sites with *H. plicata* (15.6 ± 3.0 cm, $n = 11$) had deeper mud than sites without *H. plicata* (7.8 ± 1.2 cm, $n = 36$) ($U_1 = 2.43$, $p = 0.015$). Eleven sites without *O. mykiss* had only *H. plicata* and 2 had both *H. plicata* and *A. altamirani*.

Oncorhynchus mykiss

Sites with or without *O. mykiss* did not differ in width ($U_1 = 0.37$, $p = 0.71$; Table 1) or vegetation complexity (Fisher's Exact Test: $p = 0.39$; Table 2). Sites with *O. mykiss* were deeper than sites without *O. mykiss* ($U_1 = 2.08$, $p = 0.037$), whereas sites without *O. mykiss* had deeper mud than sites with *O. mykiss* ($U_1 = -2.32$, $p = 0.020$) (Table 1).

Species comparisons

Mean stream width ($H_2 = 3.28$, $p = 0.19$; Table 1), depth ($H_2 = 4.28$, $p = 0.12$; Table 1), and vegetation complexity (Fisher's Exact Test: $p = 0.11$; Table 2) did not differ among sites with respect to occurrence of the 3 species. Mud depth was greater at sites where *H. plicata* were found compared to sites where *A. altamirani* and *O. mykiss* occurred ($H_2 = 12.34$, $p = 0.0021$; Table 1).

DISCUSSION

In the streams in the Sierra de Las Cruces, *A. altamirani* never occurred at sites with *O. mykiss*, and *H. plicata* were observed only once at sites with *O. mykiss*. These observations suggest a strong negative association between nonnative *O. mykiss* and *A. altamirani* and *H. plicata*. Our observations concur with those of previous investigators who found that the presence or introduction of trout reduces or eliminates populations of native *Ambystoma* (Tyler et al. 1998, Pilliod and Peterson 2001, Welsh et al. 2006, Pearson and Goater 2008, Pilliod et al. 2010). Other investigators have demonstrated that introduced trout reduce or eliminate populations of hylids (Matthews et al. 2001, Knapp 2005, Welsh et al. 2006).

Stream site characteristics overlapped among sites and did not vary significantly among the 3 species (except mud depth) suggesting that these environmental variables were not primarily responsible for the distributions of each species. In addition, when we repeated our analyses for *A. altamirani* and *H. plicata* including only sites without *O. mykiss*, we found no change in the results compared to when we included all sites. This set of results suggests that the pattern of distributions probably reflects the presence or absence of *O. mykiss*, not the distribution of stream characteristics. However, *O. mykiss* were found at deeper sites, a finding suggesting that amphibians may be able to find refuge in shal-

Table 1. Mean (±SE) stream characteristics of sites with and without *Ambystoma altamirani*, *Hyla plicata*, and Rainbow Trout (*Oncorhynchus mykiss*) in the Sierra de Las Cruces, Mexico. Number of sites where each taxon was present or absent is given in parentheses. Total number of sites surveyed = 66. * indicates a significant difference between sites with and without that taxon.

Species	Presence/absence	Stream width (cm)	Stream depth (cm)	Mud depth (cm)
A. altamirani	Present (17)	121.2 ± 14.8*	75.5 ± 14.6	8.9 ± 1.4
	Absent (49)	204.8 ± 21.5	90.1 ± 9.7	9.1 ± 1.7
H. plicata	Present (12)	247.9 ± 66.7	72.1 ± 20.2	18.3 ± 3.8*
	Absent (54)	168.9 ± 14.3	89.5 ± 8.9	7.0 ± 1.2
O. mykiss	Present (19)	177.6 ± 24.6	115.1 ± 17.8*	7.6 ± 3.5*
	Absent (47)	185.5 ± 21.8	74.7 ± 8.4	9.6 ± 1.2

Table 2. Aquatic vegetation complexity at sites with *Ambystoma altamirani*, *Hyla plicata*, and Rainbow Trout (*Oncorhynchus mykiss*), and sites with none of the 3 species found. See Methods for definition of each category.

Vegetation complexity	A. altamirani	H. plicata	O. mykiss	None
None	0	0	1	2
Low complexity	7	4	12	9
Medium complexity	5	8	5	5
High complexity	5	1	2	5

lower sites. We found no significant differences in the depth of sites where the amphibians were and were not found, but both *A. altamirani* and *H. plicata* tended to occupy sites that were shallower than unoccupied sites. Of particular concern is that Rainbow Trout were found in sites in all vegetation complexity categories. Vegetative complexity might increase the ability of amphibians to coexist with trout. For example, *Ambystoma macrodactylum* were found in the more vegetated areas of lakes with trout (*Oncorhynchus* spp.), and the chance of detecting salamanders in lakes with trout was greater at sites with more vegetation (Kenison et al. 2016). Nevertheless, we did not observe coexistence of Rainbow Trout and *A. altamirani* in any stream site, regardless of vegetative complexity.

In our study, *A. altamirani* and *H. plicata* rarely occurred together in the same sites. Similarly, in the Arroyo Los Axolotes, a trout-free stream in the Sierra de Las Cruces, *H. plicata* and *A. altamirani* occurred in the same stream sites less frequently than expected by chance (Lemos-Espinal et al. 2016a). The cause of these patterns is not clear, and further work is necessary to determine whether it arises from competition, predation, or some other factor.

Our results for *A. altamirani* suggest that stream width might be associated with their distribution in streams. Lemos-Espinal et al. (2016b) found they tended to be in highly oxygenated, large, and deep stream sites with fast moving water in a previous study of the natural history of *A. altamirani* from the Arroyo Los Axolotes, a trout-free stream in the Sierra de Las Cruces. Individuals also were found in sites with grassy vegetation and mud and sandy substrates (Lemos-Espinal et al. 2016b). The depth of the mud in a stream site was important for *H. plicata*. In the trout-free Arroyo Los Axolotes, *H. plicata* tended to be found in stream sites with more water (deeper and wider), high dissolved O_2 levels, and slow water speeds (Lemos-Espinal et al. 2016a). The presence of vegetation and the type of substrate did not appear to affect the distribution of *H. plicata* in the stream (Lemos-Espinal et al. 2016a). Thus, stream characteristics are important in the distribution of *A. altamirani* and *H. plicata*, but the presence of *O. mykiss* probably limits the number of sites these amphibians can successfully choose.

In conclusion, our results provide circumstantial evidence that the establishment of Rainbow Trout farms in and near streams in the Sierra de Las Cruces might negatively affect the distribution of native amphibians in these streams. Given the conservation status of *A. altamirani* and *H. plicata*, consideration needs to be given to mitigating the negative effects of Rainbow Trout introductions in these streams. Trout farms have been encouraged by Mexican governmental agencies and recommended as a potential conservation tool for native forests (López-García et al. 2014, Sosa-Villalobos et al. 2016), so the best strategies may include isolating trout farms from streams containing native amphibians, increasing efforts to prevent escapes of fish from trout farms, and attempting to eradicate populations of escaped trout from streams, thereby balancing the economic and conservation value of trout farms with their potential negative effects on native amphibians.

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