UNIVERSIDAD MICHOACANA DE SAN NICOLÁS DE HIDALGO

INSTITUTO DE INVESTIGACIONES SOBRE LOS RECURSOS NATURALES

"EFECTO DE ESTRESORES AMBIENTALES SOBRE PARÁMETROS POBLACIONALES Y MORFOLÓGICOS DE UNA SALAMANDRA EN PELIGRO DE EXTINCIÓN *Ambystoma ordinarium*"

TESIS

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RESUMEN

La generación de información que permita entender cómo responden las especies a la pérdida y modificación de sus hábitats, es fundamental para el planteamiento adecuado de acciones de manejo y conservación. En el presente trabajo, evaluamos la distribución actual de una salamandra en peligro de extinción *Ambystoma ordinarium*, estimando el tamaño de sus poblaciones mediante la técnica de marcaje y recaptura, e identificando mediante un modelo jerárquico los efectos negativos de la calidad del hábitat y la presencia de un depredador introducido (trucha) sobre el tamaño de sus poblaciones. Asimismo, evaluamos la relación entre la calidad del hábitat y la prevalencia de anormalidades corporales en esta especie, mediante un modelo de regresión logística. La distribución de la especie disminuyó un 44.82% en relación con su registro histórico, mientras que la calidad de los hábitats y la presencia del depredador significativamente ejercieron un efecto negativo sobre el tamaño de las poblaciones, siendo menor en los sitios con presencia de truchas, mientras que los arroyos con altos niveles de calidad de hábitat presentaron mayores tamaños poblacionales. Significativamente la probabilidad de que un carácter sea anormal aumentó a medida que se perdió la calidad en los hábitats. Estos resultados presentan un panorama desalentador para la especie ya que de agudizarse los efectos negativos, la probabilidad de que la especie se extinga es eminente, por lo cual se recomienda monitorear permanentemente a la especie, así mismo es necesario realizar acciones encaminadas a su conservación *in situ* y la implementación de estudios dirigidos a la reproducción *ex situ*, que permitan garantizar la viabilidad de *A. ordinarium*.

Palabras clave: Peligro de extinción, marcaje y recaptura, modelo jerárquico, anormalidades corporales, depredador introducido (trucha).

ABSTRACT

The generation of information that allows us to understand how species respond to the loss and modification of their habitats, is fundamental for the proper management and conservation actions. In the present work, we evaluated the current distribution of an endangered salamander *Ambystoma ordinarium*, estimating the size of its population through the mark-recapture technique, and identifying through a hierarchical model the negative effects of habitat quality and presence of an introduced predator (trout) on the size of its populations. We also evaluated the relationship between habitat quality and the prevalence of body abnormalities in these species, using a logistic regression model. The distribution of the species decreased 44.82% in relation to its historical record, while the quality of the habitats and the presence of the predator significantly exerted a negative effect on the size of the populations; being smaller in those sites with the presence of trout, while the streams with high levels of habitat quality had higher population sizes. Significantly, the probability of a character being abnormal increased as habitat quality was lost. These results present a discouraging panorama for the species because of the worsening of the negative effects, the probability that the species is extinct is eminent, which is why it is recommended to permanently monitor the species. Likewise it is necessary to carry out actions aimed to *in situ* conservation of the species and the implementation of studies directed to *ex situ* reproduction, that allow to guarantee the viability of *A. ordinarium*.

Key words: Endangered, mark-recapture, hierarchical model, body abnormalities, introduced predator (trout).

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INTRODUCCIÓN

La pérdida de la biodiversidad en los últimos siglos se ha dado a una velocidad excepcionalmente rápida, se estima que en los últimos 515 años se ha extinguido una especie aproximadamente cada dos años (Ceballos et al. 2015). A nivel mundial, 11,939 especies de vertebrados de las 62,839 se encuentran amenazadas (19%), mientras que hasta un 30% de las poblaciones disminuyeron en cuarenta años (Baillie et al. 2010). Las causas más frecuentes se relacionan a efectos directos o indirectos generados por las actividades del hombre, como la pérdida y modificación de hábitats, la contaminación ambiental, la sobreexplotación de recursos naturales, la proliferación de patógenos, el aumento de radiación ultra violeta, el cambio climático y la invasión de especies exóticas, entre otras (Wright y Zamudio 2002, Funk et al. 2005, Schmidt et al. 2005, Urbina-Cardona 2008, Bennett y Saunders 2010).

La pérdida y modificación de los hábitats juega un papel preponderante ya que se modifican variables estructurales y ambientales, como la temperatura, humedad y la velocidad del viento, así como la disponibilidad de alimento, refugios y los patrones de presa-depredador, entre otros (Davies y Nelson 1994, Kiesecker et al. 2001, Hamer y McDonnell 2008), limitando la adecuación de las especies, forzando a los individuos a ocupar sitios subóptimos con baja calidad de hábitat (Bendik et al. 2014).

Los sistemas acuáticos son altamente vulnerables a las alteraciones del hábitat ya que sus características fisicoquímicas y estructurales dependen en gran medida de las condiciones del paisaje (Walsh et al. 2005, Ficetola et al. 2011, Campbell et al. 2014). En los arroyos la pérdida de la cubierta forestal y vegetal de las áreas aledañas, hace que la escorrentía permita un aporte de contaminantes y de material del suelo al fondo de los arroyos disminuyendo la profundidad, aumentando la temperatura del agua y alterando los patrones del flujo del agua (Davies y Nelson 1994, Hannah et al. 2008, Huang et al. 2013, Nadaï-Monoury et al. 2014).

Si los niveles de disturbio incrementan y persisten, los arroyos pierden gradualmente la capacidad de resiliencia, afectando la viabilidad y persistencia de las especies que los habitan (Angulo et al. 2006, Hamer y McDonnell 2008). Aunado a ello, la introducción de especies exóticas representa una de las amenazas antropogénicas más extendidas en los sistemas acuáticos (Hartel et al. 2007). Aunque poco evaluados, los efectos de las especies invasoras y decremento en calidad de hábitat, así como su interacción, pueden agudizar los efectos negativos sobre las poblaciones de vertebrados acuáticos (Hartel et al. 2007). Por lo tanto, la capacidad de los organismos para responder y el tiempo de respuesta a estos efectos podrá marcar la diferencia entre persistir o perecer.

La disminución del tamaño poblacional es uno de los efectos negativos relacionados a la pérdida y modificación de los hábitats (Lowe y Bolger 2002, Willson y Dorcas 2003, Denoël y Lehmann 2006, Barrett et al. 2010). Así, las poblaciones pequeñas y aisladas son más susceptibles a extinguirse (Vallan 2000, Armstrong 2005, Ferraz et al. 2007), al ser vulnerables a eventos estocásticos, ambientales y genéticos, además de que sus tasas de recuperación demográficas son bajas, lo que limita su resiliencia (Caughley 1994, Morris et al. 2002).

Algunas poblaciones de vertebrados pueden mostrar señales de alarma que no se reflejan inmediatamente en la disminución del tamaño poblacional, pero pueden ser afectaciones fisiológicas y conductuales que en un futuro pueden llegar a afectar negativamente la sobrevivencia de la especie. Reconocer este tipo de señales antes de que las poblaciones disminuyan o se extingan es un reto para la conservación y manejo de las especies (Soto-Rojas et al. 2017).

Las anomalías corporales pueden ser una señal de alarma y un reflejo del estado de salud de una población (Hegde y Krishnamurthy 2014, Martínez-Silvestre et al. 2014, Soto-Rojas et al. 2017), ya que pueden afectar directa o indirectamente la sobrevivencia de los individuos al limitar la capacidad para evadir depredadores, alimentarse y reproducirse (Johnson et al. 2006). En general, las causas que generan estas afectaciones corporales siguen siendo una interrogante (Johnson et al. 2006, Williams et al. 2008, Reeves et al. 2008, 2010, 2013).

La identificación de señales de alarma antes de que las poblaciones sean afectadas negativamente, además de la generación de información que permita entender cómo responden los individuos a la pérdida y la modificación del hábitat sigue siendo uno de los elementos clave para contribuir a la conservación de las especies de vertebrados.

Los anfibios son el grupo más amenazado de vertebrados, seguido de mamíferos y reptiles (Ceballos et al. 2015). Su piel altamente permeable, su ciclo de vida bifásico entre hábitats acuáticos y terrestres, su baja tasa de desplazamiento en comparación a otros vertebrados y su alta fidelidad a sus sitios de reproducción los hacen altamente vulnerables a las modificaciones en sus hábitats (Duellman y Trueb 1994, Orizaola y Braña 2006).

México es el segundo país a nivel mundial con mayor diversidad de anfibios del orden Caudata (Anfibios con cola), ya que cuenta con 137 de las 189 especies registradas. De este orden, la familia Ambystomatidae contribuye a que México sea considerado mega diverso por su alto grado de endemismo, ya que 16 de las 18 especie registradas para México son endémicas (88.8%) (Parra-Olea et al. 2014). Para los integrantes de esta familia el panorama es poco alentador ya que 15 de las 16 especies endémicas se encuentran enlistadas en alguna categoría de riesgo de la Unión Internacional para la Conservación (IUCN, por sus siglas en inglés).

En cuanto a la legislación nacional NOM-059-SEMARNAT-2010, 11 especies (73.5%) se encuentran listadas en la categoría de sujetas a protección especial (Pr), 3 (20%) como amenazadas y 1 (6.5%) en peligro de extinción (NOM-ECOL-059-ECOL). Para la mayoría de estas especies, las actividades humanas y sus efectos colaterales como el crecimiento urbano, el aclareo de bosques, la explotación de cuerpos acuíferos, entre otras, son las principales causas que amenazan su viabilidad y persistencia (Alcocer y Bernal-Brooks 2010, Clipp y Anderson 2014).

Si las amenazas persisten y los efectos se agudizan, la viabilidad de varias especies del género *Ambystoma* en México estarará amenazada. En este sentido, el reconocimiento de los efectos negativos de la perturbación del hábitat sobre las especies del género *Ambystoma* es fundamental para una adecuada conservación de este grupo de especies.

En el presente trabajo se evaluó el efecto de la modificación del hábitat y la presencia de una especie de pez exótica sobre el tamaño poblacional de una especie de *Ambystoma*. Se usó como modelo de respuesta a la salamandra de montaña *Ambystoma ordinarium*, catalogada por la IUCN como especie en peligro de extinción dado que su área de ocupación es menor 500 km^2, además de que su distribución se encuentra severamente fragmentada y existe un continuo declive en la extensión y calidad del hábitat forestal (Shaffer et al. 2004).

En particular, en varias zonas del área de distribución de ésta especie, existe una fuerte impacto sobre los arroyos que habita debido a presiones de deforestación, urbanización, cultivos, introducción de depredadores, entre otras (Soto-Rojas et al. 2017). Por lo cual, en el presente trabajo se plantearon las siguientes

Preguntas de investigación:

¿Cuál es la distribución actual de la especie?, ¿Cuáles son los niveles de calidad del hábitat en los arroyos ocupados por esta especie?, ¿La calidad del hábitat afecta el tamaño poblacional de la especie?, ¿Los depredadores exóticos como la trucha tienen algún efecto sobre el tamaño de las poblaciones de la especie?, ¿Las anomalías corporales se relacionan con la calidad del hábitat?.

Nuestra hipótesis es que el rango de distribución y el tamaño de las poblaciones de esta especie disminuirán a medida que se pierda la calidad del hábitat, además de que la presencia del depredador afectará negativamente el tamaño de la población de *Ambystoma ordinarium*. Así mismo se plantea que la baja calidad del hábitat incrementará la probabilidad de que los individuos de *A. ordinarium* presenten anomalías corporales.

BIBLIOGRAFÍA

Alcocer, J. y F. W. Bernal-Brook. 2010. Limnology in Mexico. *Hydrobiologia*. 644 (1), 1-54.

Angulo, A., J. V. Rueda-Almonacid, J. V. Rodríguez-Mahecha y E. La Marca . 2006. Técnicas de inventario y monitoreo para los anfibios de la región tropical andina. Conservación Internacional. Serie Manuales de Campo Nº 2. Panamericana Formas e Impresos S.A., Bogotá D.C. 298 pp.

Armstrong, D.P. 2005. Integrating the metapopulation and habitat paradigms for understanding broad-scale declines of species. *Conservation Biology*. 19 (5), 1402–1420.

Baillie, J. E. M., J. Griffiths, S. T. Turvey, J. Loh, y B. Collen. 2010. Evolution Lost. Secretary 1-72.

Barrett, K., B.S. Helms, C. Guyer, y J.E. Schoonover. 2010. Linking process to pattern: causes of stream- breeding amphibian decline in urbanized watersheds. *Biological Conservation*. 143 (9), 1998–2005.

Bendik, N. F., B.N. Sissel, J.R. Fields, L.J. O'Donnell, M.S. Sanders. 2014.Effects of urbanization on abundance of jollyville plateau salamanders (*Eurycea tonkawe*). *Hepetological Conservation and Biology*. 9 (1), 206-222.

Bennett, A.F. y D.A. Saunders. 2010. Hábitat fragmentation and landscape. Oxford University Press. 88-106.

Campbell, E.H.G., N.M. Amber y K.C. Rice. 2014. Stream-water temperature limits occupancy of salamanders in mid-atlantic protected areas. *Journal of Herpetology*. 48 (1), 1-7.

Caughley, G.1994. Directions in conservation biology. *Journal of Animal Ecology*. 63 (2), 215-244.

Ceballos, G., P. R. Ehrlich., A. D. Barnosky., A. García., R. M. Pringle., y T. M. Palmer. 2015. Accelerated modern human–induced species losses: Entering the sixth mass extinction. *Science advances*. 1-5.

Clipp, H. L. y J. T. Anderson. 2014. Environmental and anthropogenic factors influencing salamanders in riparian forests: a review. *Forests*. 5, 2679-2702.

Davies, P., y M. Nelson. 1994. Relationships between riparian buffer widths and the effects of logging on stream habitat, invertebrate community composition and fish abundance. Marine and Freshwater Research. 45 (7), 1289-1305.

Denoël, M. y A. Lehmann. 2006. Multi-scale effect of land- scape processes and habitat quality on newt abundance: Implications for conservation. *Biological Conservation*. 130 (4), 495- 504.

Duellman,W. E., y L. Trueb. 1994. Biology of Amphibians. The Johns Hopkins University Press, Baltimore, MD. 1-696

Ferraz, G., J.D. Nichols, J.E. Hines, P.C. Stouffer, R.O. Bierregaard Jr. y T.E Lovejoy. 2007.A large-scale deforestation experiment: Effects of patch area and isolation on Amazon birds. *Science*. 315, 238–241.

Ficetola, F.G., L. Marziali., B. Rossaro., F. Bernardi De., E. Padoa-Schioppa. 2011. Landscape-stream interactions and habitat conservation for amphibians. *Ecological*. 21 (4), 1272–1282.

Funk, W. C., A. E. Greene, P. S. Corn y F. W. Allendorf. 2005. High dispersal in a frog species suggests that it is vulnerable to hábitat fragmentation. *Biology Letters*. 1,13–16.

Hamer, A. J., y M. J. McDonnell. 2008. Amphibian ecology and conservation in the urbanising world: A review. *Biological Conservation*. 141 (10), 2432–2449.

Hannah, D. M., I. A. Malcolm, C. Soulsby, y A. F. Youngson. 2008. A comparison of forest and moorland stream microclimate, heat exchanges and thermal dynamics. *Hydrological Processes*. 22 (7), 919–940.

Hartel, T., S. Nemes., D. Cogalniceanu, K. Ollere., O. Schweiger C.I. Moga y L. Demeter. 2007. The effect of fish and aquatic habitat complexity on amphibians. *Hydrobiologica*. 583, 173–182.

Hegde, G., and S.V. Krishnamurthy. 2014. Analyusis of the health status of common frog *Fejervarya limnocharis* (Anura: Ranidae) in rice-paddy agroecosystems of Western Ghats, India. *American-Eurasian Journal of Agricultural and Environmental Sciences*. 14,1492−1497.

Huang, J., J. Zhan, H. Yan, F. Wu, y X. Deng. 2013. Evaluation of the impacts of land use on water quality: a case study in the Chaohu Lake Basin. *The Scientific World Journal*. 2013, 1-7.

Johnson, P.T., E.R. Preu., D.R. Sutherland., J.M. Romansic., B. Han., y A.R. Blaustein. 2006. Adding infection to injury: synergistic effects of predation and parasitism on amphibian malformations. *Ecology*. 87 (9), 2227-2235.

Kiesecker, J.M., A.R. Blaustein y L.K. Belden. 2001. Complex causes of amphibian population declines. *Nature.* 410 (6820), 681- 684.

Lowe, W. H., y D. T. Bolger. 2002. Local and landscape-scale predictors of salamander abundance in New Hampshire headwater streams. *Conservation Biology*. 16 (1),183–1.

Martínez-Silvestre A., F. Amat., y S. Carranza. 2014. Natural incidence of body abnormalities in the montseny newt, *Calotriton arnoldi. Herpetology Notes*. 7, 277-279.

Morris, W., P. Bloch., B. Hudgens., L. Moyle, y J. Stinch-combe. 2002. Population viability analysis in endan-gered species recovery plans: past use and future improvements. *Ecological Applications*. 12 (3), 708–712.

Nadaï - Monoury, E., F.Gilbert, y A. Lecerf . 2014. Forest canopy cover determines invertebrate diversity and ecosystem process rates in depositional zones of headwater streams. *Freshwater biology*. 59 (7), 1532-1545.

Orizaola, G., y F. Braña. 2006. Effect of salmonid introduction and other environmental characteristics on amphibian distribution and abun-dance in mountain lakes of northern Spain. *Animal Conservation*. 9 (2), 171–178.

Parra-Olea, G., Flores-Villela, O., Mendoza-Almeralla, C. 2014. Biodiversidad de anfibios en México. *Revista Mexicana de Biodiversidad*. 85, 460-466.

Reeves, M.K., C.L. Dolph., H .Zimmer., R.S. Tjeerdeman, y A. Trust. 2008. Road proximity increases risk of skeletal abnormalities in Wood Frogs from national wildlife refuges in Alaska. *Environ Health Perspect.* 116 (8), 1009-1040.

Reeves, K.M., P. Jensen., C.L. Dolph., M. Holyoak., y K.A. Trust. 2010. Multiple stressors and the cause of amphibian abnormalities. *Ecological Monographs*. 80 (3), 423-440.

Reeves, M.K., K.A.Medley., A.E. Pinkney., M. Holyoak., P.T. Johnson., y M.J. Lannoo. 2013. Localized hotspots drive continental geography of abnormal amphibians on US Wildlife Refuges. *PLOS ONE*. 8, e77467.

Schmidt, B. R., R. Feldmann., y M. Schaub. 2005. Demographic processes underlying population growth and decline in Salamandra salamandra. *Conservation biology*. 19 (4), 1149-1156.

Shaffer, B., O. Flores-Villela, G. Parra-Olea y D. Wake. 2004. *Ambystoma ordinarium*. In: IUCN 2013. IUCN Red List of Threatened Species. http://dx.doi.org/10.2305/IUCN.UK.2015-4.RLTS.T59066A53974247.en. Downloaded on september 2017.

Soto-Rojas, C., I. Suazo-Ortuño., J. A. M. Laos y J. Alvarado-Diaz. 2017. Habitat quality affects the incidence of morphological abnormalities in the endangered salamander *Ambystoma ordinarium*. *PLOS ONE*. 12 (8),1-15.

Urbina-Cardona, J. N. 2008. Conservation of neotropical herpetofauna: research trends and challenges. *Tropical Conservation Science*. 1 (4),359–375.

Vallan, D. 2000. Influence of forest fragmentation on amphibian diversity in the nature reserve of Ambohit- antely, highland Madagascar. *Biological Conservation.* 96, 31–43.

Walsh, C.J., A.H.Roy., J.W. Feminella., P.D. Cottingham., P.M. Groffman., y R.P. Morgan II. 2005. The urban stream syndrome: current knowledge and the search for a cure. *Journal of the North American Benthological Society*. 24 (3), 706–723.

Williams, R., D. Bos, D.Gopurenko, y A. Dewoody. 2008. Amphibian malformations and inbreeding. *Biology Letters*. 4 (5), 549–552.

Willson, J.D., y M.E. Dorcas. 2003. Effects of habitat disturbance on stream salamanders: Implications for buffer zones and watershed management. *Conservation Biology*. 17 (3), 763–771.

Wright, A. N. y K. R. Zamudio. 2002. Color pattern asymmetry as a correlate of habitat disturbance in spotted salamanders (*Ambystoma maculatum*). *Journal of Herpetology.* 36 (1), 129–133.

CAPÍTULO I. RESPUESTA DE LA POBLACIÓN A LA CALIDAD DEL HÁBITAT Y LA PRESENCIA DE UN DEPREDADOR SOBRE LA SALAMANDRA MEXICANA EN PELIGRO DE EXTINCIÓN (*Ambystoma ordinarium*)

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POPULATION RESPONSES TO HABITAT QUALITY AND PREDATOR PRESENCE OF AN ENDANGERED MEXICAN SALAMANDER (*AMBYSTOMA ORDINARIUM)*

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Abstract

Knowledge about how habitat quality modulates populations'size is crucial for ecology and conservation planning. In a time span of 43 years, the endangered salamander *Ambystoma ordinarium* has disappeared from 44.82% of historicaly registered localities. Using mark-recapture we estimated population size of this species, sampling all localities where this species is presently known to exist. Additionally, using a hierarchical model, we evaluated the sinergic effect of habitat quality and the presence of an exotic predator (trout, *Oncorhynchus mykiss*) on the salamander's population size. During a period of five months we captured a total of 506 individuals, of which 115 (22.72%) were recaptured. The model adjusted to 87% of the sampling sites, and the results showed that quality of the habitat and the presence of the predator had a significant effect on population size. Salamander's population size was lower in sites with presence of trouts, whereas streamss with high levels of habitat quality presented higher population sizes. These results suggest that effects of anthropogenic disturbance resulting in the reduction of habitat quality and in the presence of alien predators may impact negatively populations' size and consequently increase the risk of extinction of this species.

Key words: estimated population, habitat quality, mark-recapture, hierarchical model, predator, endangered salamander.

1. Introduction

In recent centuries, loss of biodiversity has ocurred at an exceptionally high rate (Ceballos et al., 2015). Since 1970, an average reduction of 30% in population size has been recorded worldwide for wild vertebrates, and 19% of 62,839 vertebrate species are threatened (Baillie et al., 2010). Habitat loss and habitat modification have played a leading role in the reduction of populations'size (Anciães and Marini, 2000; Baillie et al., 2010; Smith and Green, 2005) as a result of negative effects of these threats on availability of food resources and refuges, as well as prey-predator's interactions (Davies and Nelson, 1994; Hamer and McDonnell, 2008; Kiesecker et al., 2001). These changes might force individuals to occupy sites with suboptimal habitat conditions, limiting the ability to meet their biological requirements (Bendik et al., 2014). Freshwater ecosystems are highly vulnerable to habitat alterations as their physicochemical and structural characteristics are highly dependent on landscape's conditions (Ficetola et al., 2011; Grant et al., 2014; Walsh et al., 2005). Loss of forests and other vegetation cover in streams'surrounding areas frequently results in a reduction in water depth, an increment in water temeparture, as well as an alteration in flowing patterns (Davies and Nelson, 1994; Hannah et al., 2008; Huang et al., 2013; Nadaï-Monoury et al., 2014; Rouse et al., 1999). If alterations persist, streams gradually lose resilience, affecting the viability and permanence of species inhabiting these ecosystems (Hamer and McDonnell, 2008). In addition, one of the most widespread anthropogenic threats in aquatic systems is the introduction of exotic species (Hartel et al., 2007). Although poorly evaluated, the interaction between invasive species and habitat quality may exacerbate the negative effects on aquatic vertebrate populations (Hartel et al., 2007). Reduction of population size of wild vertebrates is one of the effects that has been reported in relation to habitat loss and modification (Barret et al., 2010; Denoël and Lehmann, 2006;

Lowe and Bolger, 2002; Willson and Dorcas, 2003). In general, species with small populations are more susceptible to extinction (Armstrong, 2005; Ferraz et al., 2007; Vallan, 2000), as they are highly vulnerable to stochastic, genetic and environmental events (Caughley, 1994; Morris et al., 2002). Therefore, information on the response of population size to reduction of habitat quality is a necessary step to delineate conservation strategies for wild species.

The mexican salamander, *Ambystoma ordinarium*, is a facultative paedomorphic species inhabiting mountain streams in the middle section of the Trans-mexican volcanic axis (state of Michoacán), classified as endangered according to the IUCN and under the category of special protection in local legislation NOM-ECOL-059-2010. It is also considered an evolutionary distinct species (EDGE score 5.86;http://www.edgeofexistence.org/amphibians/species_info.php?id=586). Its distribution is limited and strongly fragmented in an area of less than 500 km2 of occupation (IUCN accessed March 2017). The streams inhabited by this species present a markedly environmental deterioration, product of anthropogenic activities such as immoderate logging, livestock farming and vehicular traffic, among others (Alvarado-Diaz et al., 2003; Ruiz-Martínez et al., 2014). The exploitation for medicinal purposes (C. Soto-Rojas, Pers. Comm.) is an additional threat for this species, as it has been registered for other *Ambystoma* species (Zambrano et al., 2007). In addition, the presence of the exotic rainbow trout (*Oncorhynchus mykiss*), a predator that can consume salamanders (Manentli et al., 2009) has been reported in some of these streams (Soto-Rojas, 2012).

Our main goal in this study was to evaluate how the habitat quality and the presence of an exotic predator affects the population size of *A. ordinarium*. Our hypothesis is that the populations size of *A. ordinarium* will be larger in streams with high levels of habitat quality, whereas in the presence of trouts (alien predator) population size will be small regardless of habitat quality.

2. Methodology

2.1. Field surveys.

Of the 29 streams where *A. ordinarium* had been historically registered (Anderson and Worthington, 1971; Weisrock et al., 2006) we sampled 16 streams where this species was still present by 2014 (Soto-Rojas et al., 2017) (Fig. 1). Salamanders were visually searched along a 100 m long transect established at the middle section of each of the 16 streams. Each transect was sampled upstream three times (once each day in three consecutive days). The sampling effort was standardized to 18 person-hours per transect. Procedures for salamanders'field surveys, such as sampling protocol, capture technique, and anesthetizing methods are described in detail in Soto-Rojas et al. (2017). Adult paedomorphic individuals (> 60 mm snout-vent length, Anderson and Worthington, 1971), previously anesthetized, were marked with a PIT tag (Trovan ID-100A), following the technique of Winandy and Denoël (2011). One tag per individual was inserted in the caudal musculature, about 1 cm posterior to the vent. After recovering from the anesthesia, tagged individuals were released at the capture site. From March to July 2014, each of the 16 streams were sampled along the established transect for three consecutive days, marking new individuals and recapturing tagged salamanders. Recaptured individuals were identified with a field microchip reader (Trovan GR-250). During the search for salamanders the presence of trouts in the stream was visually evaluated.

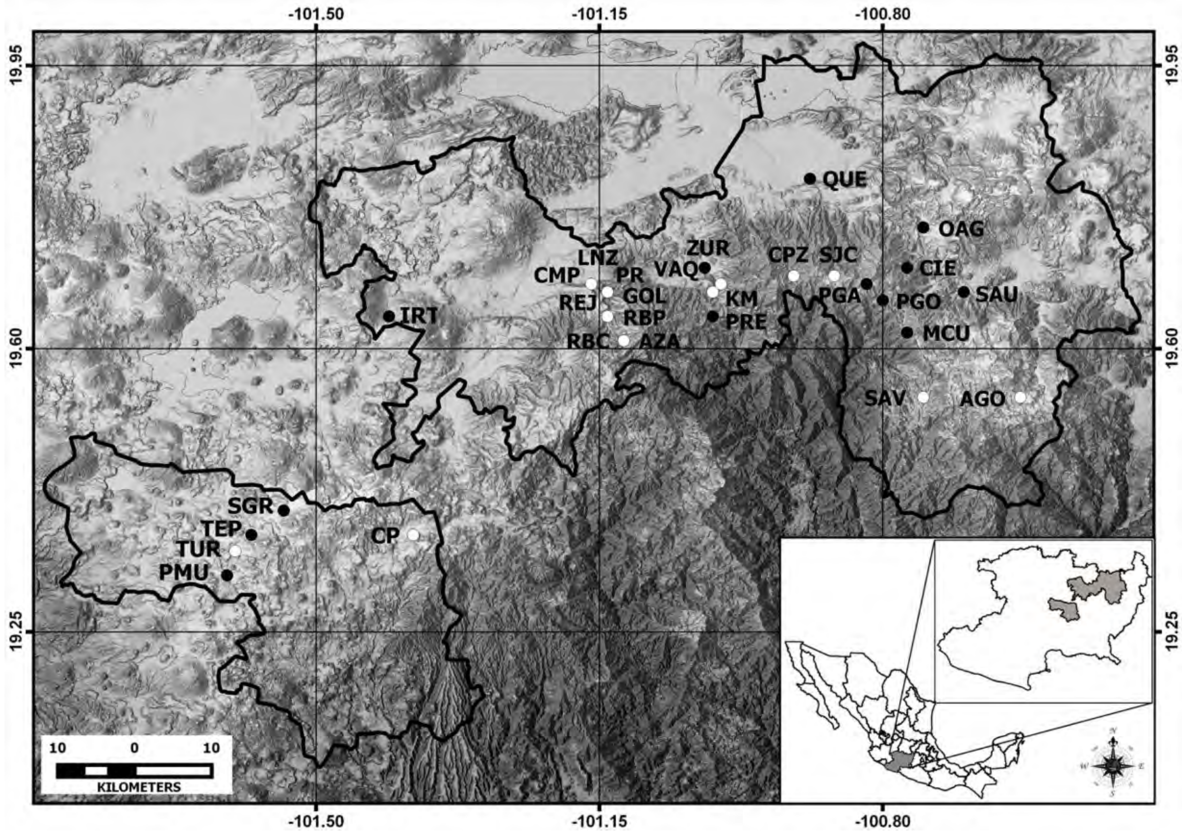

Fig.1. Locations where historically *Ambystoma ordinarium* was registered in Michoacán, México (black dots). White dots represent current distribution: Paso del muerto PMU, Turirán TUR, Tepetate TEP, San Gregorio SGR, Cruz del plato CP, Iratzio IRT, Río bello conservado RBC, Agua zarca AZA, Río bello perturbado RBP, Golondrinas GOL, Reja REJ, Piedra redonda PR, Campestre CMP, Lienzo LNZ, Pino real PRE, Kilómetro 23 KM, Vaquerito VAQ, Zurumbeneo ZUR, San Antonio Villalongín SAV. Puerto Garnica PGA, Carindapaz CPZ, San José de la Cumbre SJC, Queréndaro QUE, Agostitlán AGO, Mil cumbres MCU, Pino Gordo PGO, Los Sauces SAU, Cieneguillas CIE, Ojo de Agua OAG.

2.2. Habitat quality

We used the Rapid Bioassessment Protocols (RBPs) of Barbour et al. (1999) to assess habitat quality of the 16 surveyed streams. This protocol is a visually based method that evaluates habitat condition using a categorical scoring system to appraise habitat condition (Barbour et al., 1999). The evaluated variables to assess habitat quality were vegetative protection of stream-bank, type and embededss of substrate, channel flow status, patterns of velocity and depth, sediment deposition, riffle frequency, and human alterations. These variables were recorded along the 100 m long transect laid along the middle section of each stream (see Soto-Rojas et al., 2017 for a detailed description of measurement of variables). Scored values of RBPs range from 0 (indicating a markedly disturbed condition) to 200 (indicating a habitat in pristine condition) (Barbour et al., 1999). The evaluated streams were assigned to four habitat condition categories according to RBPs scores: poor (0-59), marginal (60-112), sub-optimal (113-165) and optimal (166-200). RBPs have been widely used to evaluate habitat condition of wadeable streams (e.g. Boonsoong et al., 2009; 2010; Manentli et al., 2009; Petty et al., 2013; Soto-Rojas et al., 2017; Sweeten and Ford, 2016).

2.3. Estimation of population size

Considering that spatial movements of *A. ordinarium* are markedly limited (Montes-Calderón et al., 2011), populations at each of the sampling stream were considered as close units, without the ocurrence of mortality, natality, migration or inmigration during the sampling period. We used a protocol that maximizes sampling homogeneity, assuming that probabilty of detection is constant (Ficetola et al., 2011). To estimate population size at each of the streams'section where the sampling transect was laid we used a hierarchichal model, assuming that tagging does not affect probability of recapture, that tags are not lost, that captured salamanders represent a random sample of the population and that all individuals have the same probability of capture. In this hierarchichal model, site-specific population size N_i ($i = 1, \dots, R$) is regarded as latent variable following a discrete Poisson distribution with intensity parameter $\lambda_i = exp(\alpha + \beta X_{1i} + \gamma X_{2i})$, where β and γ are unknown parmeters that quantify the effect of the covariables X_{1i} and X_{2i} on variability of abundance, where X_{1i} are the RBPs scores in the i-th site and X_{2i} is a binary covariable that indicates presence (value of 1) or absence (value of 0) of the predator (trout) in the survey at the i-th locality. Additionaly, it is assumed that the vector of capture frequency in the i-th locality, $\vec{y}_i = (y_{i0}, y_{i1}, \dots, y_{im})$ follows a multinomial distribution $(N_i, \vec{\pi})$, where the elements of the vector of probabilities $\vec{\pi}$ are given by $\pi_j = \binom{m}{j} p^j (1-p)^{m-j}$, where m represents the number of visits to each locality ($m = 3$) and p is an unknown parameter that represents the probability of capture. It is assumed that observations $\vec{y}_1, \dots, \vec{y}_R$ are independent. The point estimation of parameters was made using the maximum likelihood method. We used a parametric bootstrap method to estimate confidence intervals. The parameter of main interest is the expected size of the population in the i-th locality, given the data set and the model.

3. Results

3.1. Habitat quality.

We surveyed a total of 16 000 lineal meters of streams with a total sampling effort of 288 person-hours (2 persons x 3 hours x 3 sampling days x 16 sites). RBPs scores indicated that two streams (12.5%) were in the optimal category of habitat condition, nine (56.25%) were suboptimal, and five (31.25%) were in the marginal category.

3.2. Population size.

We tagged a total of 506 salamanders in the 16 surveyed streams. The model of population size estimation satisfactorily adjusted in 14 of the 16 sampled streams (Fig. 2).

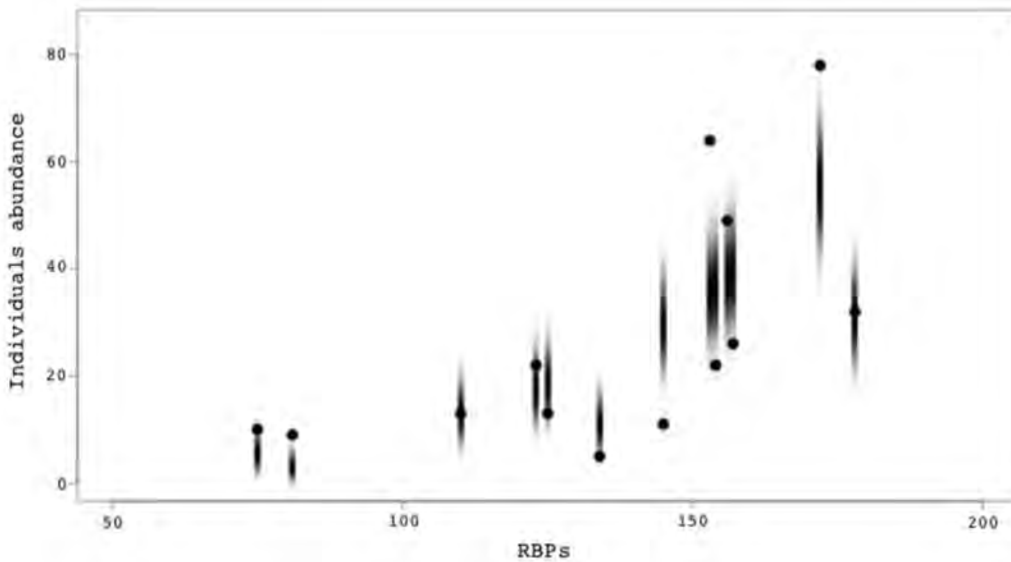

Fig. 2. Observed and estimated captures of *A. ordinarium* in relation to scores of Rapid Biassessemnet Protocols RBPs, in each of the sampling streams. Grey-scale shows the estimated probability density function for the number of individuals caught at the i-th locality. Observed captures are marked with a black dot.

Therefore, for the model of population size estimation we considered 376 salamanders that were tagged in these 14 streams. Of the 376 tagged salamanders we recaptured 85 (22.6%). SJC was the stream with the highest number of salamanders observed more than once (recaptured once or twice), whereas KM was the stream with the lowest number of salamanders observed more than once (Table 1). Trouts were observed in three of the 14 sampled streams.

Table 1. Number of individuals of *A. ordinarium* observed one time (Capture 1), two times (Capture 2) and three times (Capture 3), and population size estimates (CL = central limit, LL = lower limit, HL = higher limit) for each of the sampled sections of the 14 selected streams along the Trans-mexican volcanic axis. Also shown are RBPs scores (Scores), category of habitat condition (HC) and presence (+) of trout for each stream (Trout).

Locality	Capture 1	Capture 2	Capture 3	Total Captures	CL	LL	HL	Scores	(HC)	Trout
AGO	40	8	1	49	79	71	89	156	Suboptimal	
SJC	49	18	11	78	121	110	137	172	Optimal	
SAV	2	1	2	5	14	11	18	134	Suboptimal	+
CPZ	7	8	7	22	50	43	60	154	Suboptimal	
KM	7	2	0	9	12	10	13	81	Marginal	+
CP	19	3	0	22	50	43	60	154	Suboptimal	
AGZ	45	17	2	64	92	85	101	153	Suboptimal	
RBC	27	5	0	32	56	48	67	178	Optimal	+
TUR	26	0	0	26	56	49	67	157	Suboptimal	
PR	13	0	0	13	28	24	33	125	Suboptimal	
LNZ	10	0	0	10	15	13	17	75	Marginal	
GOL	22	0	0	22	36	32	41	123	Suboptimal	
VAQ	11	0	0	11	34	28	42	145	Suboptimal	
REJ	13	0	0	13	23	20	28	110	Marginal	

The model of population size estimation indicated that population size responded significantly to both the level of habitat quality and the presence of trouts ($p < 0.00001$). As the level of habitat quality of streams increased, population size also increased. However, in streams where trouts resided, population size of salamanders was small regradless of the level of habitat quality (Fig. 3).

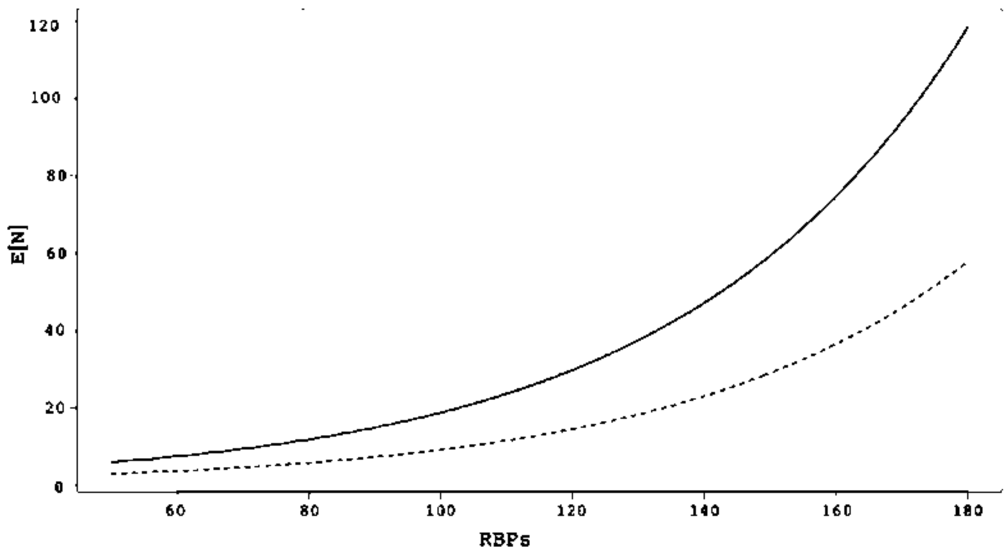

Fig. 3. Model of population estimation of *Ambystoma ordinarium* using as covariable scores of RBPs and the presence of trouts. Solid line corresponds to expected abundance estimation in the absence of trouts and dotted line corresponds to expected abundance estimation in persence of trouts.

Values of parameters used for the population size estimation model are shown in Table 2. The highest estimated population size was 121 salamanders in one of the optimal habitat quality streams (SJC) without presence of trouts (Table 1). Trouts were registered in the only other stream ranked in the optimal level of habitat quality (RBC) and the estimated population of salamanders was of only 56 individuals (Table 1).

Table 2. Values of the parameters used for the model of population size estimate with a confidence interval of 95%.

Parameter	Estimator	Lower limit	Upper limit
α	0.63001736	-0.1720	1.3606
β	0.02303161	0.0183	0.0283
γ	-0.71687454	-1.0511	-0.4296
p	0.24278943	0.2045	0.2790

4. Discussion

Habitat loss and modification associated to human activities frequently results in a general reduction in distribution ranges and population size of wild vertebrate species (Baillie et al., 2010; Grant et al., 2014; Pike et al., 2011). Species with restricted distributions and small population size are at higher risk of extinction than species with larger population size since they are more vulnerable to demographic and environmental changes as well as loss of genetic variability (Caughley, 1994; Cushman, 2006; Feeley and Terborgh, 2008; Morris and Doak, 2002; Petranka et al., 2004; Sunny et al., 2014). For wild populations of *A. ordinarium,* survival and long-term persistence is a difficult challenge considering that in the last 43 years its occupation range has diminished 44.82 % (Soto-Rojas et al., 2017). In the present study, we found that at all of streams'sampled sections, population size of *A. ordinarium* was relatively small. Reductions in the size of *A. ordinarium*'s populations closely followed the gradient from high to low levels of habitat quality of streams, except for those streams with resident trouts. The three streams in which trouts were observed presented different levels of habitat quality (optimum, suboptimal and marginal) and had population sizes lower than expected by the model of population size estimation (Table 1).

It has been reported that exotic fish predators, such as trouts, tilapias and carps negatively impact population size of the ambystomatid salamanders *A. mexicanum* (Zambrano et al., 2007) and *A. macrodactylum* (Funk and Dunlap, 1999), sometimes even extirpating populations. In the case of trouts, Manentli et al. (2009) described them as generalist predators that can consume larvae and neotenic adults of ambystomatids. Although it is likely that *A. ordinarium* is preyed upon by a variety of native predators, only the snake *Thamnophis cyrtopsis* has been registered as predator of this species (García-Garrido et al., 2016). Effects of natural predators on population size of *A.*

ordinarium have not been evaluated, but it is possible that these effects are negligible, as it occurs in other ambystomatid species (Gall and Mathis, 2010).

Negative effects of habitat disturbance on other salamander species have been documented (e.g. *Ambystoma mexicanum* [Zambrano et al., 2007], *Eurycea cirrigera* [Barret et al., 2010; Wilson and Dorcas, 2003], *Desmognathus fuscus* [Price et al., 2012], *Cryptobranchus alleganiensis alleganiensis* [Burgmeier et al., 2011], *Plethodon cinereus* [Hocking et al., 2013], *Ensatina eschscholtzii* [Welsh. Jr. et al., 2015], *A. maculatum* [Strojny and Hunter Jr., 2010]). Some anthropogenic activities implicated in the decline of salamander populations include deforestation, agriculture, pollution, urbanization and introduction of exotic species (Ethier and Fahring, 2011; Ficetola et al., 2011; Funk and Dunlap, 1999; Grant et al., 2014; Price et al., 2012; Wilk et al., 2014; Zambrano et al., 2007).

We registered evidence of human disturbance at different levels of intensity in all sampled streams, including those ranked at the optimum level of habitat condition by RBPs scores. The most obvious signals of disturbance were deforestation, agriculture, cattle grazing, vehicular traffic and introduced predators (trouts) (Alvarado-Díaz et al., 2003; Ruiz-Martínez et al., 2014; Shaffer et al., 2004; Soto-Rojas, 2012; Soto-Rojas et al., 2017). Identifying factors that negatively impact size of wild populations is a necessary step before implementing mitigation actions. Although the association between habitat quality and population size makes intuitive sense, in our study of *A. ordinarium* we provide quantitative evidence of this relation. We accomplished this by gauging population size through a gradient of poor to optimum level of habitat condition. To identify the level of habitat quality of surveyed streams we used an efficient and cost-effective methodology (RBPs) that allowed us to visually establish habitat condition. RBPs rank habitat condition of streams by gauging habitat structural features that bear on water quality and health of animal communities (Boonsoong et al., 2009; Manentli et al., 2009; Petty et al., 2013; Soto-Rojas et al., 2017; Sweeten and Ford, 2016). Although in our study design we evaluated habitat quality of streams incorporating the joint effect of the variables considered by RBPS, we can hypothesize on the impact that condition of individual variables may have on population size of *A. ordinarium*. For instance, the high level of substrate embeddedness in lower habitat quality streams was probably the result of the top soil being eroded and transported by runoff from low-vegetation covered hillsides to adjacent streams. The higher level of substrate embeddedness in streams of lower habitat quality likely results in a reduction of the surface area available for macroinvertebrates, which constitute the main component of *A. ordinarium*'s diet (Ruiz-Martínez et al., 2014). This high level of substrate embeddedness might also result in a reduction of sites for egg deposition, since this species generally lay its eggs under rocks and roots (Anderson and Worthington, 1971).

The lower frequency of riffles and bends in streams of lower habitat quality frequently results in a lower diversity of biological communities and makes these streams more vulnerable to surges due to storms. Additionally, bends migth provide refuge, protecting salamanders from storms, mostly occurring during the summer in our study area. The decline of *A. ordinarium* population sizes can be further explained by the lack of vegetation cover that protects the streams from floods and maintains the system stable (Del Signore et al., 2014).

It has been reported that reproduction and survival of amphibians can be negatively affected by environmental disturbance (Barrett et al., 2017; Todd and Rothermel, 2006). Therefore, the alteration of the physicochemical variables of the water (i. e. temperature, conductivity, oxygen dissolved and pH) could be lethal for aquatic organisms as their vital functions are markedly sensitive to fluctuations in physicochemical variables (Ficetola et al., 2011). An increase on the temperature due to low forest cover migth decrease the amount of oxygen dissolved or increase the conductivity in the water. Furthermore, conductivity can also increase because of runoff, resulting in a negative alteration of physiology and behavior of aquatic salamanders by limiting cutaneous gas exchange, movement and feeding behavior (Chambers, 2011).

As it has been reported that reproduction and survival of amphibians can be negatively affected by environmental disturbance (Barrett et al., 2017; Todd and Rothermel, 2006), it will be important that future studies investigate how the reduction in population size of *A. ordinarium* as a result of habitat quality disminution is affecting individuals' performance.

In six (TUR, PR, LNZ, GOL, VAQ, REJ) of the 14 sampled streams we registered small population sizes (range = 10 – 26 individuals) and recaptures were not obtained. The percentage of recaptures was 22.60%, similar to studies in which population size of other salamanders'species was estimated using population models (Burgmeier et al., 2011; O'Donnell and Semlitsch, 2015; Unger et al., 2012; Welsh Jr et al., 2015). Two (RBP and CMP) of the total of 16 sampled streams were not included in the population estimation analysis, as despite presenting a low habitat quality score, salamander population size was relatively high. Causes of the apparent paradox shown by these two streams are not easy to explain. The reason behind the atypical behavior observed on these populations was beyond the scope of our experimental design.

One possible explanation is that food availability was favorably affected, countering the negative effects of level of habitat quality (Hache et al., 2013). Piñon et al. (2014) reported that CMP is a site where the input of organic matter has favored a higher diversity and abundance of macro invertebrates. Similar conditions might be present in RBP as the urban expansion has markedly increased in this area.

Although the abundance in these two sites did not show negative signs, these sites adjusted significantly with the rest of the sites in a model that evaluated the probability of a character in an individual to be abnormal in response to habitat quality (Soto-Rojas et al., 2017). This suggests that even when the number of individuals did not show signs of affectation, there are already other alarming signs that migth affect species survival (Johnson et al., 2006; Soto-Rojas et al., 2017).

Our model of population estimation adjusted in almost 90%, which seems reasonable to evaluate the effect of the habitat quality on population size of wild riverine species. Synergistically, introduced predators and the loss and modification of habitats may exacerbate and accelerate the negative effects on populations of wild vertebrates. Monitoring the species and generating actions for their conservation, such as the eradication of trout, are crucial for the viability and persistence of this species as its conservation status is already critical.

5. Literature Cited

Alvarado-Díaz, J., García-Garrido, P., Suazo-Ortuño, I. 2003. Food habits of a paedomorphic population of the Mexican salamander *Ambystoma ordinarium* (Caudata: Ambystomatidae). *The Southwestern Nautralist*. 48 (1), 100–102.

Anciães, M., Marini, M.Ã. 2000. The efects of fragmentation on fluctuating asymmetry in passerine birds of brazilian tropical forests. *Journal of Applied Ecology*. 37, 1013-1028.

Anderson, J. D., Worthington, R. D. 1971. The life history of the mexican salamander *Ambystoma ordinarium* Taylor. *Herpetological*. 27 (2), 165-176.

Armstrong, D.P., 2005. Integrating the metapopulation and habitat paradigms for understanding broad-scale declines of species. *Conservation Biology*. 19 (5), 1402–1420.

Baillie, J. E. M., Griffiths, J., Turvey, S. T., Loh, J., Collen, B. 2010. Evolution los status and trends of the world`s vetrebrates. Zoological Society of London, United Kindom.

Barbour, M.T., Gerritsen, J., Snyder, B.D., Stribling, J.B. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates and fish, Second Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water; Washington, D.C.

Barrett, K., Helms, B.S., Guyer, C., Schoonover, J.E.2010. Linking process to pattern: causes of stream-breeding amphibian decline in urbanized watersheds. *Biological Conservation.* 143 (9), 1998–2005.

Barret, K., Crawford, J.A., Reinstein, Milanovich, J.R. 2017. detritus quality produces species-specific tadpole growth and survivorship responses in experimental wetlands. *Journal of Herpetology*. 51 (2), 227-231.

Bendik, N. F., Sissel, B.N., Fields, J.R., O'Donnell, L.J., Sanders, M.S. 2014. Effects of urbanization on abundance of jollyville plateau salamanders (*Eurycea tonkawe*). *Hepetological Conservation and Biology*. 9 (1), 206-222.

Boonsoong, B., Sangpradub, N., Barbour, M.T., Simachaya, W. 2010. An implementation plan for using biological indicators to improve assessment of water quality in Thailand. *Environ Monit Assess*. 165, 205-215.

Boonsoong, B., Sangpradub, N., Barbour, M.T. 2009. Development of rapid bioassessment approaches using benthic macroinvertebrates for Thai streams. *Environ Monit Assess*. 155, 129-147.

Burgmeier, N.G., Unger, S.D., Sutton, T.M.,Williams, R.N. 2011. Population status of the Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*) in Indiana. *Journal of Herpetology*. 45 (2), 195-201.

Caughley, G. 1994. Direction in conservation biology. *Journal of Animal Ecology*. 63, 215-144.

Ceballos, G., Ehrlich, R. P., Barnosky, D. A., García, A., Pringle, M. R., Palmer, M.T. 2015. Accelerated modern human–induced species losses: Entering the sixth mass extinction. *Science Advances*. 1-5.

Chambers, D. L. 2011. Increased conductivity affects corticosterone levels and prey consumption in larval amphibians. *Journal of Herpetology*. 45 (2), 219–223.

Cushman, S. A. 2006. Effects of habitat loss and fragmentation on amphibians: a review and prospectus. *Biological Conservation*. 128, 231–240.

Davies, E. P., Nelson, M. 1994. Relationships between riparian buffer widths and the effects of logging on stream habitat, invertebrate community composition and fish abundance. *Marine and Freshwater Research*. 45 (7), 1289-1305.

Del Signore, A., Lenders, H. J. R., Hendriks, A. J., Vonk, J. A., Mulder, C., Leuven, R. S. E. W. 2014. Size-mediated effects of water-flow velocity on riverine fish species. *River Research and Applications*. 32 (3), 390-398.

Denoël, M., Lehmann, A. 2006. Multi-scale effect of landscape processes and habitat quality on newt abundance: Implications for conservation. *Biological Conservation*. 130 (4), 495- 504.

Ethier, K., Fahrig, L. 2011. Positive effects of forest fragmentation, independent of forest amount, on bat abundance in eastern Ontario, Canada. *Landscape Ecology*. 26, 865–876.

Feeley, K. J., Terborgh, J. W. 2008. Direct versus indirect effects of habitat reduction on the loss of avian species from tropical forest fragments. *Animal Conservation*. 11, 353–360.

Ferraz, G., Nichols, D. J., Hines, E. L., Stouffer, C. P., Bierregaard jr. O.R., Lovejoy, E. T. 2007. A large-scale deforestation experiment: Effects of patch area and isolation on Amazon birds. *Science*. 315, 238–241.

Ficetola, F.G., Marziali, L., Rossaro, B., De Bernardi, F., Padoa-Schioppa, E. 2011. Landscape-stream interactions and habitat conservation for amphibians. *Ecological Applications*. 21 (4), 1272–1282.

Funk, C.W., Dunlap, W.W.1999. Colonization of high-elevation lakes by long-toed salamanders (Ambystoma macrodactylum) after the extinction of introduced trout populations. *Canadian Journal of Zoology*. 77, 1759–1767.

Gall, B. G., Mathis, A. 2010. Innate predator recognition and the problem of introduced trout. *Ethology*. 116 (1), 47-58.

García-Garrido, P., Alvarado-Díaz, J. Suazo-Ortuño, I. 2016. *Ambystoma ordinarium*. predation by a black-necked gartersnake (*Thamnophis cyrtopsis*). *Mesoamerican Herpetology*. 3 (2), 461-462.

Grant, C.H.E., Wiewell A. N.M., Rice, C.K. 2014. Stream-water temperature limits occupancy of salamanders in mid-atlantic protected areas. *Journal of Herpetology*. 48 (1), 1-5.

Hache, S., Marc-André, V., Bayne, M.E. 2013. Experimental evidence for an ideal free distribution in a breeding population of territorial songbird. *Ecology*. 94 (4), 861-869.

Hamer, J.A., McDonnell, J. M. 2008. Amphibian ecology and conservation in the urbanising world: A review. *Biological Conservation*. 141 (10), 2432–2449.

Hannah,M. D., Malcolm, A. I., Soulsby, C., Youngson, F. A. 2008. A comparison of forest and moorland stream microclimate, heat exchanges and thermal dynamics. *Hydrological Processes*. 22 (7), 919–940.

Hartel, T., Nemes, S., Cogălniceanu, D., Ollere, K., Schweiger, O., Cosmin-Ioan Moga., Demeter, L. 2007. The effect of fish and aquatic habitat complexity on amphibians. *Hydrobiologica*. 583, 173–182.

Hocking, J.D., Babbitt, J.K., Yamasaki, M. 2013. Comparasion of silvicultura and natural disturbance effects on terrestrial salamanders in northern hardwood forest. *Biological Conservation.* 167, 194-2002.

Huang, J., Zhan, J., Yan, H., Wu, F., Deng, X. 2013. Evaluation of the impacts of land use on water quality: a case study in the Chaohu Lake Basin. *The Scientific World Journal*. 2013, 1-7.

Johnson, J.T.P., Preu, R.E., Sutherland, R.D., Romansic, M.J., Han, B., Blaustein, R.A. 2006. Adding infection to injury: synergistic effects of predation and parasitism on amphibian malformations. *Ecology*. 87 (9), 2227-2235.

Kiesecker, M.J., Blaustein, R.A., Belden, K.L. 2001. Complex causes of amphibian population declines. *Nature*. 410 (6820), 681- 684.

Lowe, H. W., Bolger, T. D. 2002. Local and landscape-scale predictors of salamander abundance in New Hampshire headwater streams. *Conservation Biology*. 16 (1), 183–1.

Manentli, R., Ficetola, F.G., De Bernandi, F. 2009. Water stream morphology and land scape: complex habitat determinants for the fire salamander *Salamandra salamandra. Amphibia-Reptilia*. 30, 7-15.

Montes-Calderon, M.A., Alvarado-Díaz, J., Suazo-Ortuño, I. 2011. Abundancia, actividad espacial y crecimiento de *Ambystoma ordinarium* Taylor 1940 (Caudata: Ambystomatidae) en Michoacán, México. *Biológicas*. 13(1), 50-53.

Morris, W. F., Doak, D. F. 2002. Quantitative conservation biology: theory and practice of population viability analysis. Sinauer Associates, Sunderland, Massachusetts, USA.

Morris, F.W., Bloch, L.P., Hudgens, R.B., Moyle, C.L., Stinchcombe, R.J. 2002. Populationn viability analysis in endangered species recovery plans: Past use and future improvements. *Ecological Applications*. 12 (3), 708-712.

Nadaï- Monoury, E., Gilbert, F., Lecerf, A. 2014. Forest canopy cover determines invertebrate diversity and ecosystem process rates in depositional zones of headwater streams. *Freshwater Biology*. 59 (7), 1532-1545.

O'Donnell, M.K., Semlitsch, D.R. 2015. Advancing terrestrial salamander population ecology: the central role of imperfect detection. *Journal of Herpetology*. 49 (4), 533-540.

Petranka, W.J., Smith, K.C., Scott, F.A. 2004. Identifying the minimal demographic unit for monitoring pond-breeding amphibians. *Ecological Applications*. 14 (4), 1065-1078.

Pike, A.D., Webb, K. J., Shine, R. 2011. Removing forest canopy cover restores a reptile assemblage. *Ecological Applications*. 21 (1), 274–280.

Piñon, F.M.A., Pérez, M.R.M., Torres, G. U., Pineda, L.R. 2014. Integridad biótica de la microcuenca del Río Chiquito, Morelia, Michoacán, México, basada en la comunidad de macroinvertebrados acuáticos. *Revista de Biología Tropical*. 64 (2), 221-231.

Price, J. S., Browne, A. R., Dorcas, E. M. 2012. Evaluating the effects of urbanisation on salamander abundances using a before-after control-impact design. *Fresh water Biology*. 57 (1), 193–203.

Rouse, D.J., Bishop, A.C., Struger, J. 1999. Nitrogen pollution: An assessment of its threat to amphibian survival. *Environmental Health Perspectives*. 107 (10), 799-803.

Ruiz-Martínez, L., Alvarado-Díaz, J., Suazo-Ortuño, I., Pérez-Munguía, R. 2014. Diet of *Ambystoma ordinarium* (Caudata : Ambystomatidae) in undisturbed and disturbed segments of a mountain stream in the trans-mexican volcanic belt. *Salamandra*. 50 (2), 63–70.

Shaffer, H.B., Wake, D., Parra-Olea, G., Flores-Villela, O., Arias Caballero, P. Aguilar, X. *Ambystoma ordinairum.* 2004. IUCN Red List of Threatened Species. http://dx.doi.org/10.2305/IUCN.UK.2015-4.RLTS.T59066A53974247.en. Downloaded on september 2017.

Smith, M.A., Green, D.M., 2005. Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations?. *Ecography*. 28 (1), 110– 128.

Soto-Rojas, C. 2012. Uso y seleccion del microhábitat de la salamandra de montaña *Ambystoma ordinarium* (Amphibia: Caudata). Tesis de Maestría. Programa Institucional de Maestría en Ciencias Biologicas, UMSNH. 1-57.

Soto-Rojas C., Suazo-Ortuño, I., Laos, J.A.M., Alvarado-Díaz, J. 2017. Habitat quality affects the incidence of morphological abnormalities in the endangered salamander *Ambystoma ordinarium*. *PLOSE ONE.* 12(8), 1-15.

Strojny, A.C., Hunter Jr, L.M. 2010. Relative abundance of amphibians in forest canopy gaps of natural origin vs. timber harvest origin. *Animal Biodiversity and Conservation*. 33 (1), 1-13.

Sunny, A., Monroy-Vilchis, O., Fajardo, V., Aguilera-Reyes, U. 2014. Genetic diversity and structure of an endemic and critically endangered stream river salamander (Caudata. *Ambystoma leorae*) in México. *Conservation Genetics*. 15, 49-59.

Sweeten, E.S., Ford, M.W. 2016. Validation of a stream and riparian habitat assessment protocol using stream salamanders in the southwest Virginia coalfields. *Journal of the American Society of Mining and Reclamation*. 5(1), 45-66.

Todd, D.B., Rothermel, B.B. 2006. Assessing quality of clearcut habitats for amphibians: Effects on abundances versus vital rates in the southern toad (*Bufo terrestris*). *Biological Conservation*. 133, 178-185.

Unger, D.S., Burgmeier, G.D., Williams, N.R. 2012. Genetic markers reveal high PIT tag retention rates in giant salamanders (*Criptobranchus alleganiensis*). *Amphibia-Reptilia*. 33, 313-317.

Vallan, D. 2000. Influence of forest fragmentation on amphibian diversity in the nature reserve of Ambohitantely, Highland Madagascar. *Biological Conservation*. 96, 31-43.

Walsh, J.C., Roy, H.A., Feminella. W.J., Cottingham, D.P., Groffman, M.P., Morgan II, P.R. 2005. The urban stream syndrome: current knowledge and the search for a cure. *Journal of the North American Benthological Society*. 24 (3), 706–723.

Weisrock, W. D., Shaffer, B. H., Storz, L. B., Voss, R. S. 2006. Multiple nuclear gene sequences identify phylogenetic species boundaries in the rapidly radiating clade of mexican ambystomatid salamanders. *Molecular Ecology*. 15 (9), 2489–2503.

Welsh Jr. H.H., Waters, R.J., Hodgson, R.G., Weller, J.T., Zabel, J.C. 2015. Responses of the woodland salamander *Ensatina eschscholtzii* to commercial thinning by helicopter in late-seral Douglas-fir forest in northwest California. *Forest Ecology and Management.* 335, 156-165.

Wilk, J.R., Ricklefs, D.J., Raphael, G.M. 2014. Abundance of western red-backed salamanders (*Plethodon vehiculum*) in the Washington coast range after headwater stream-buffer manipulation. *Northwestern Naturalist*. 95, 92–102.

Willson, D.J., Dorcas, E.M. 2003. Effects of habitat disturbance on stream salamanders: implications for buffer zones and watershed management. *Conservation Biology*. 17 (3), 763–771.

Winandy, L., Denoël, M. 2011. The use of visual and automatized behavioral markers to assess methodologies: a study case on PIT-tagging in the Alpine newt. *Behavior Research Methods*. 43 (2), 568-576.

Zambrano, L., Vega, E., Herrera, M., Prado, E., Reynoso, V. H. 2007. A population matrix model and population viability analysis to predict the fate of endangered species in highly managed water systems. *Animal Conservation*. 10 (3), 297-303.

CAPÍTULO II. EFECTO DE LA CALIDAD DEL HÁBITAT SOBRE LA INCIDENCIA DE ANORMALIDADES MORFOLÓGICAS EN LA SALAMANDRA DE MONTAÑA *Ambystoma ordinarium*

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RESEARCH ARTICLE

Habitat quality affects the incidence of morphological abnormalities in the endangered salamander *Ambystoma ordinarium*

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Abstract

Identification of early warning signals previous to the occurrence of population decline or extinction is a major challenge for the conservation of animal species. Prevalence of morphological abnormalities in a population can be one of these signals. We registered morphological abnormalities in the salamander *Ambystoma ordinarium*. We also evaluated the relation between habitat quality and the prevalence of abnormalities in this species. We used scores from rapid bioassessment protocols (RBPs) to assess the habitat quality of streams inhabited by *A. ordinarium*. A preliminary survey indicated that of 29 streams where this species has been historically registered, 13 might have few or no *A. ordinarium*. The association between habitat quality and the incidence of morphological abnormalities was evaluated in these 16 streams. Of 502 sampled individuals, 224 (44.62%) had at least one body abnormality. Of the 224 individuals with body abnormalities, 84 (37.5%) presented more than one abnormality. Of a total of 5,522 evaluated morphological characters, 344 (6.74%) were abnormal. Partial loss of gills and missing digits were the most frequent abnormalities. Results of a binomial logistic regression indicated that the probability of a character of an individual to be abnormal was significantly associated with habitat quality; as the levels of the quality of the habitat increased, the prevalence of morphological abnormalities decreased. These results suggest that RBPs are a quick and useful method for assessing the habitat quality of streams inhabited by *A. ordinarium*. Given that RBPs provide rapid and cost-effective assessments of the ecological health of aquatic ecosystems, it will be important to test if the RBPs protocols can be used to rapidly assess habitat quality for other species of stream amphibians. The negative association between habitat quality and the prevalence of morpohological abnormalities that we found indicates that habitat condition plays an important role in the high number of abnormalities registered in *A. ordinarium*. Therefore, our results suggest that one of the several negative effects of habitat degradation on amphibians is an increase in the frequency of morphological abnormalities with marked consequences for the survival and general fitness of aquatic amphibians.

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Introduction

The loss of amphibian populations is a well-known example of the biodiversity crisis presently occurring on a global scale [1]. A number of factors, such as habitat loss and degradation, the introduction of invasive species, environmental contamination, diseases and parasites, unsustainable use and global climate change, have been recognized as causes of amphibian declines [2]. In association with this amphibian decline, an apparent increase in morphologic abnormalities has also been registered [3]. In most vertebrates, the presence of morphological abnormalities among individuals of a population is frequently associated with mutations, developmental flaws and traumas. In addition, the background incidence of abnormailities in wild vertebrate populations ranges between 0 and 5% [4], although higher incidences of abnormalities have been reported in some species of amphibians (10–50%) [5, 6]. Johnson et al. [5] define abnormalities as any gross deviation from the normal range of morphological variation, including malformations (permanent structural defects resulting from abnormal development) and deformities (alterations to an otherwise correctly formed structure). In amphibians, these types of abnormalities frequently involve loss of digits or portions of limbs [1]. These abnormalities affect an individual's fitness since their occurrence increases susceptibility to predation and lowers the individual's efficiency in prey capture and mate acquisition [7]. Moreover, the energy assigned for repairing of abnormalities limits the availability of energy needed for other vital functions, such as feeding and reproduction [8]. An increase in the frequency of morphological abnormalities in amphibians has been associated with a number of environmental stressors, including ultraviolet radiation (UVB), pesticides, endoparasitosis, chemical contamination, damage caused by intraspecific interactions as well as by predation attempts [9, 10]. Even though various studies report a marked increment in the incidence of morphological abnormalities in amphibians during the last decades [1, 11], there are relatively few studies (e.g. [12, 13]) that evaluate the relationship between habitat disturbance and the incidence of abnormalities.

The Michoacan stream salamander (*Ambystoma ordinarium*) is a facultatively paedomorphic ambystomatid species inhabiting mountain streams in coniferous forests in the central Trans-Mexican Volcanic Belt. Localities where this species has been registered are situated between the vicinity of Patzcuaro Lake in the state of Michoacan and Tianguistenco in the western portion of the state of México [14]. The total occupation range of this salamander is less of 500 km^2, and it is designated as an endangered species by the IUCN (www.iucnredlist.org accessed February 9, 2017). *A*. *ordinarium* is considered as a species in the category of Special Protection by the Mexican government (AmphibiaWeb; accessed February 12, 2017).

Considering that identifying environmental factors associated with the reported increment in morphological abnormalities is important for amphibian conservation [7, 15] and that the streams inhabited by *A*. *ordinarium* present different levels of habitat disturbance, our main aim in this study was to evaluate the role of habitat quality in the incidence of morphological abnormalities (sensu [5]) in this salamander. We addressed the following questions: 1) what types of morphological abnormalities are present in wild populations of *A*. *ordinarium*?, 2) is the method of rapid biossessment protocols (RBPs) of Barbour et al. [16] a reliable predictor of habitat quality for *A*. *ordinarium*?, and 3) what is the association between morphological abnormalities of *A*. *ordinarium* and habitat quality? We hypothesized that in streams with high disturbance levels, incidence of morphological abnormalities will be greater than in streams of low disturbance levels. Although in some species of amphibians morphological abnormalities have been linked to bite injuries inflicted among conspecifics during development (e.g. [6]), and therefore more abnormalities may be expected in productive high quality habitats, we hypothesized that incidence of morphological abnormalities will be greater in streams with high disturbance levels. This predicition was formulated by considering that the greater levels of habitat structural complexity in higher quality streams occupied by *A*. *ordinarium* provide numerous and diverse refuges [17], protecting individuals from bites potentially inflicted by conspecifics and aquatic predators.

Materials and methods

Ethics statement

This study was carried out in strict accordance with the guidelines for use of live amphibians and reptiles in field research compiled by the American Society of Ichthyologists and Herpetologists (ASIH). All procedures with animals were approved by the Committee on Biosafety and Bioethics (CBB) of the Institute of Chemical Biology of the Universidad Michoacana de San Nicolás de Hidalgo. In the research protocol, adult salamanders were visually detected and captured, anesthetized (by immersion in a solution of tricaine methanesulfonate, 0.25 g/l diluted in distilled water for an average of 15 minutes) and evaluated; they then recovered from anesthesia and were released the same day at the site of capture. The biological study, the protocol and the final report were overseen by the CBB.

Field surveys

We evaluated the presence of *A*. *ordinarium* in all 29 streams where it has been historically recorded [18, 19]. In each of these streams, a transect 800 m long was laid at the middle section, and salamanders were visually searched at the bottom of the stream by two persons for three hours. These surveys were carried out during February 2014. Sites correspond to mountain streams (altitude range = 1,950–3,400 m) in the central portion of the Trans-Mexican Volcanic Belt in Mexico [19, 20], immersed originally in pine, pine-oak, and fir forests. Anthropogenic disturbance inflicted on streams and adjacent areas includes bank erosion from trampling by vehicle traffic, cattle and people, selective logging of riparian vegetation, and conversion of upslope vegetation to suit agricultural activities [14]. In 16 of the 29 surveyed streams, we found populations of *A*. *ordinarium* (Fig 1). To collect field data on morphological abnormalities, surveys were performed during the dry season (March–July) of 2014. A 100 m long transect was laid at the middle section of each of the 16 streams where *A*. *ordinarium* populations existed. Each transect was sampled upstream three times (one time per day for three consecutive days). The sampling effort was standardized to 18 person/hours per transect (2 persons x 3 hours x 3 sampling days). Within each transect, salamanders were visually detected and captured with handheld nets capture (scientific collecting permit number SGPA/DGVS/04187/13). Once captured, salamanders were anesthetized by immersion in a solution of MS-222 (tricaine methanesulfonate, 0.25 g/l diluted in distilled water) for an average of 15 minutes [21, 22]. Only adult individuals (> 60 mm snout-vent length [18]) were considered in this study. Before the effect of anesthesia ended, salamanders were weighed (gr) (Primo Digital Scale), marked with a PIT (passive integrated transponder) tag (11.5 mm long and 2.1 mm in diameter by Trovan ID100A) following the technique of Winandy and Denoël [23] and photographed. PIT tagging was part of a complementary study to estimate population size of this species. In the present study, tags were used to avoid counting individuals more than once in the consecutive surveys. Photographs were used to identify morphological abnormalities. Photographs were taken of the venter, sides and front of the body (Fig 2). The same person took all photographs. Each image was referenced with an ID number. Once the procedure was completed and salamanders recovered from anesthesia, they were released the same day at the site of capture.

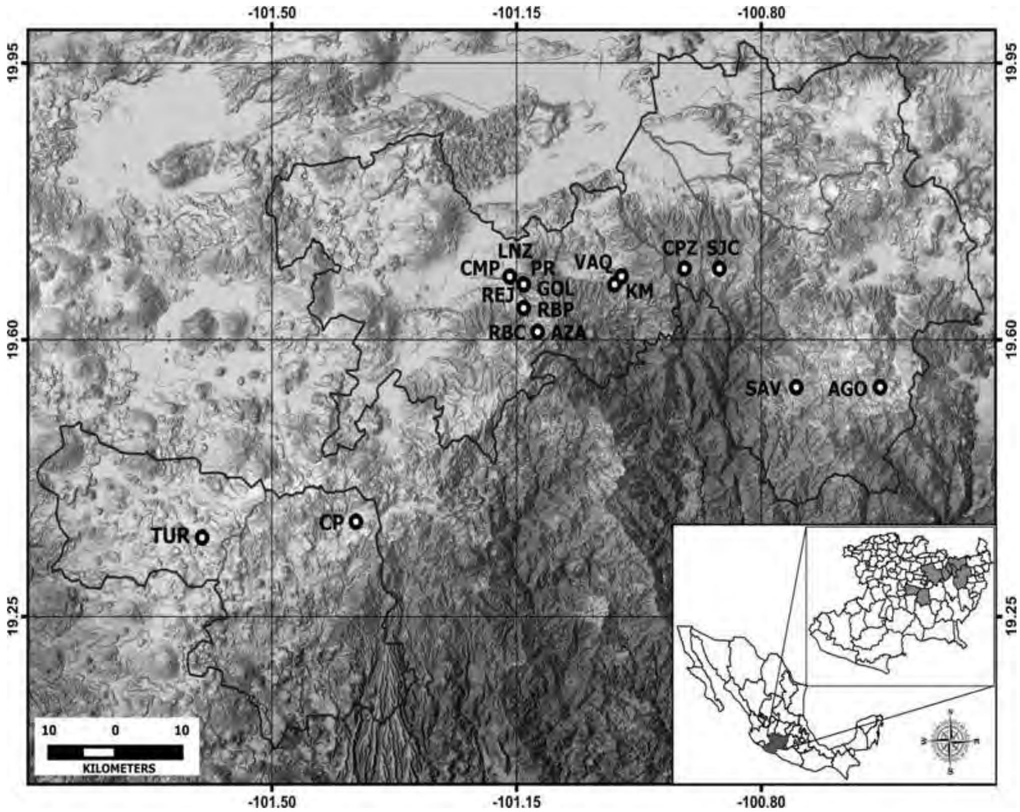

Fig 1. Distribution of streams sampled for morphological abnormalities of *A. ordinarium*. See Table 1 for acronyms.

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Identifying morphological abnormalities

Images were used to visually search for morphological abnormalities in the laboratory by the same person. Each abnormality of forelimbs, hindlimbs and tail was defined as follows: micromelia (short limbs), ectrodactyly (missing digits), brachydactyly (missing part of a limb), syndactyly (fusion of fingers), polymelia (extra number of limbs and extra number of hands or feet), polydactyly (extra number of digits), ectromelia (missing a limb), incomplete tail (missing part of tail), and regenerated tail [6, 15, 24, 25] (Fig 3). We used the following terms in referring to gill abnormalities: polygills (extra number of gills), ectrogills (missing gills), braquigills (missing part of a gill) and syngills (fusion of gills) (Fig 3). Images of the ventral body part were used to determine snout-vent length (SVL), length of tail and total body length by using the software ImageJ [22].

Habitat quality

We used the rapid bioassessment protocols (RBPs) of Barbour et al. [16] to assess the habitat quality of all of the 29 surveyed streams where historically *A. ordinarium* has been registered. This protocol is a visually based method that evaluates habitat condition by using a categorical

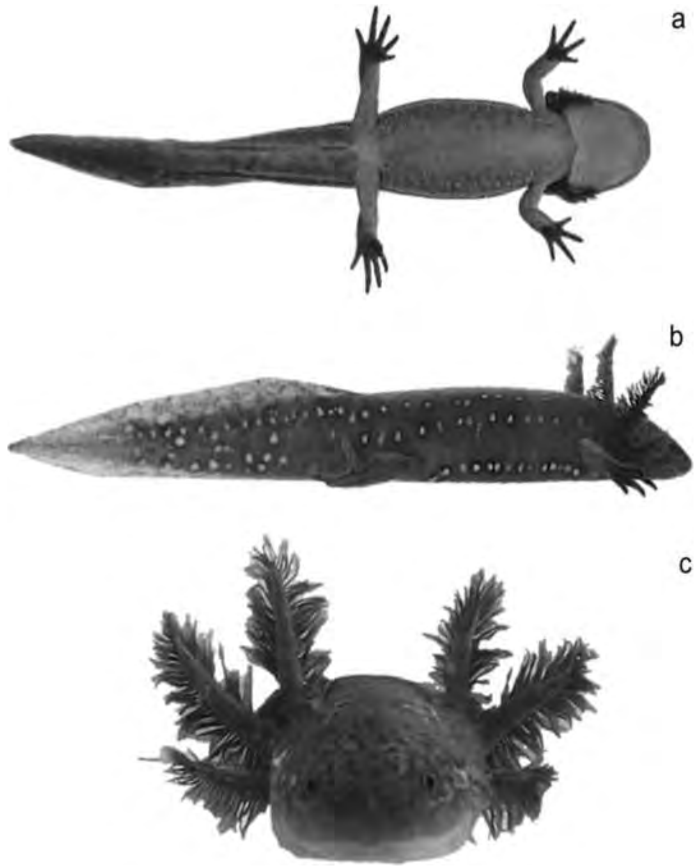

Fig 2. (a) ventral view, (b) lateral view, and (c) frontal view of the body of *A. ordinarium*.

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scoring system to appraise habitat condition [16]. Following the RBPs, the variables measured to assess habitat quality were the vegetative protection of streambank, type and embeddedness of substrate, channel flow status, patterns of velocity and depth, sediment deposition, riffle frequency, and human alterations. These variables were recorded along a 100 m long transect laid along the middle section of each stream. Scored values range from 0 (indicating a markedly disturbed condition) to 200 (indicating a habitat in pristine condition) [16]. The evaluated streams were assigned to four habitat condition categories according to RBPs scores: poor (0–59), marginal (60–112), suboptimal (113–165) and optimal (166–200).

Physichochemical variables

To test if RBPs are a good predictor of habitat quality for *A. ordinarium*, we evaluated if the range of values of physicochemical and structural variables that are relevant for this species in its habitat [17] and that are not considered by the RBPs matched the categories of habitat condition considered by RBPs. This set of variables was found to be significant for the selection of aquatic microhabitats by *A. ordinarium* [17]. A 100 m long transect was laid in the middle of each of the 29 streams where the presence of *A. ordinarium* was historically reported. Every 10 m along the 100 m transect at sampled streams a gauging point was established. At each of these points, the following measurements were made: water temperature, conductivity and dissolved oxygen (by using a YSI 85,Yellow Spring, Ohio, USA), pH (with a conductronic PC18), water velocity and flow (with a flowmeter model FP211, Global Water), air temperature and humidity (measured at 1 m above water surface with a microstation Kestrel 4500), width and depth of the stream (with a tape measure) and canopy cover (with a densiometer).

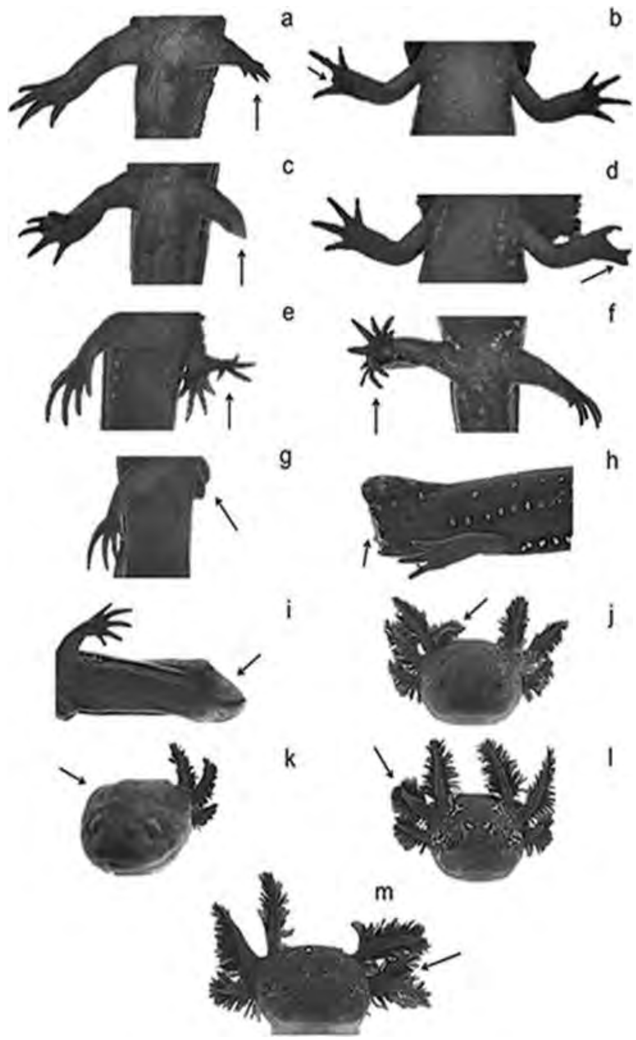

Fig 3. Types of morphological abnormalities identified in *A. ordinarium*: (a) micromelia, (b) ectrodactyly, (c) brachydactyly, (d) syndactyly, (e) polimely, (f) polydactyly, (g) ectromelia, (h) incomplete tail, (i) regenerated tail, (j) polygills, (k) ectrogills,(l) braquigills, (m) syngills.

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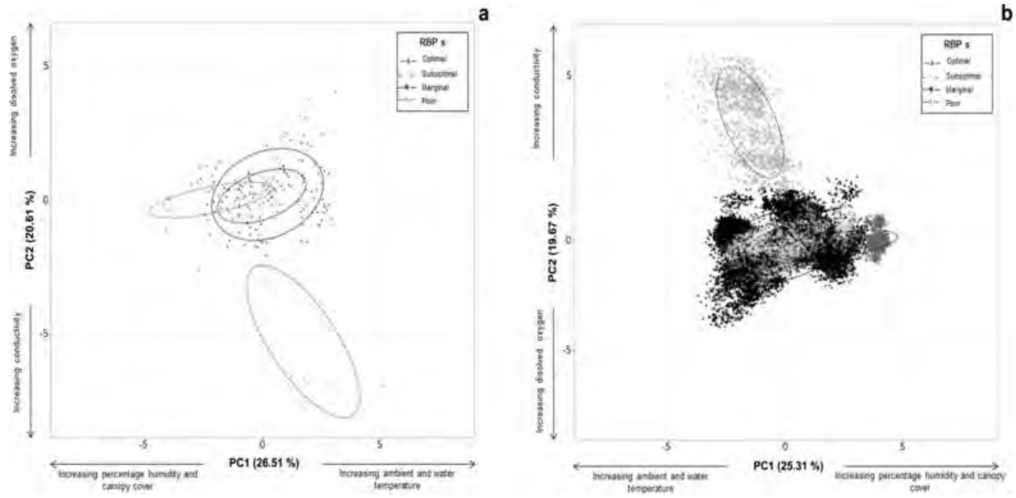

Fig 4. (a) Analysis of principal component (PCA) of physichochemical and structural variables of the streams where *A. ordinarium* has been historically recorded. Categories of habitat condition according to RBPs scores: optimal, suboptimal, marginal and poor and (b) bootstrap simulation of PCA results.

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Analysis

To assess if the the range of values of the physichochemical and structural variables that were measured, in addition to those considered by RBPs, corresponded to the categories of habitat condition established by RBPs (optimal, suboptimal, marginal, poor), we used a principal component analysis with R Core Team [26]. Previous to PCA analysis, we performed autoscaling of sampled variables. To estimate the distribution of categories of the habitat condition established by RBPs, we employed a non-parametric bootstrap approach. We resampled times the physichochemical and structural variables one thousand times at each sampled stream. The PCA showed that values of evaluated physichochemical and structural variables were separated, with sampling points in the inferior right quadrant corresponding to the most disturbed streams, and sampling points in the upper left quadrant corresponding to the most conserved streams (Fig 4A and 4B). A less defined cluster of points in the middle part of the figure corresponds to streams with intermediate conditions of disturbance (Fig 4A and 4B). The PCA ordination corresponded to labels of RBPs categories of habitat condition (Fig 4A and 4B). Therefore, to explore the relationship between morphological abnormalities and habitat quality we employed the scores of RBPs.

To assess the relationship between habitat quality and the probability of abnormalities for each body character, we considered eleven visible characters by individual: six gills (three on each side), four limbs (two forelimbs, two hindlimbs) and a tail. Therefore, a total of 5,522 body characters were evaluated (11 characters x 502 individuals). The evaluation of each character was independent, and each was registered as either abnormal (success) or normal (failure) (binary variable). Assuming that habitat quality is a factor that can directly or indirectly influence the health status of an organism, we used a binomial logistic regression model [27] to assess the relationship between habitat quality and the probability of abnormality of a body character. In this regression model, we used the values of RBPs as a variation factor. We assumed that the probability of a body character of an individual of the population to be abnormal was determined by habitat quality. This model is given in terms of the following:

$$Y_i \sim \text{Bin}\,(\text{N}, P_{i,})$$

where

Yi = the number of body abnormalities (successes) in N = 11 body characters evaluated to the i-th individual, i = 1,. . .,502.

P_i = the probability that a character of an individual of the population is abnormal is modeled based on

$$P_i = \frac{e^{\alpha+\beta X_i}}{1+e^{\alpha+\beta X_i}},$$

where X_i is the RBPs score corresponding to habitat of the i-th individual and (α,β) is the vector of regression coefficients. The value of α yields probability when the RBPs score is zero. The coefficient β is the amount the logit (log-odds) changes with a one-unit change in RBPs score:

$$ln\left(\frac{P_i}{1-P_i}\right) = \alpha + \beta X_i.$$

Results

Habitat quality

The scores of RBPs indicated that two of the 29 sampled streams were in the optimal category of habitat condition (172–178), 10 in suboptimal condition (123–157), 14 in marginal condition (63–110) and three in poor condition (0.0–59) (Table 1, S1 Fig). PCA analysis showed that the physichochemical and structural variables (those not considered by RBPs) presented a continuous distribution (Fig 4A), with no single site segregating from the rest based on physichochemical and structural features of the habitat. The first two PCA components explained 47.7% of the variability in the data. In the first PCA component, streams with an optimal habitat condition presented a higher percentage of tree cover (-0.24) and higher values of air humidity (-0.50) compared to streams in the suboptimal, marginal and poor habitat categories. These streams in the lower categories of habitat condition presented higher water (0.44) and air temperatures (0.55). In general, in the second PCA component streams with a lower habitat condition presented higher conductivity (-0.47) and a lower concentration of dissolved oxygen (0.60) in the water. Bootstrap analysis showed an affinity of the distribution of physichochemical and structural data in relation to RBPs' scores of habitat quality (Fig 4B).

Morphological abnormalities

Of the 502 individuals collected, 224 (44.62%) presented at least one morphological abnormality. Of these, 140 presented one abnormality, 60 two abnormalities, 22 three and 2 individuals presented four abnormalities. Of the total of 5,522 analyzed characters, 334 were abnormal (6.04%). Of the 334 abnormal characters, braquigills and ectrodactyly with an ocurrence of 26% and 23%, respectively, were the most frequent abnormalities, followed in descending order by micromelia (8.98%), incomplete tail (8.38%), poligills (8.08%), syndactyly (6.89%), ectrogills (5.39%), brachydactyly (4.19%), ectromelia (3.89%), polydactyly (1.80%), regenerated tail (1.50%), polymelia (0.90%), microdactyly (0.60) and syngills (0.30%).

Probability of abnormalities

The results of the binomial logistic regression showed that the probability of a character of an individual of the population being abnormal was significantly associated with the scores of

Table 1. Scores of RBPs, habitat condition and the abundance of *A. ordinarium* in the 29 sampled streams where this species has been historically recorded.

				Coordinates		
Locality	**Acronyms**	**RBPs scores**	**Habitat condition**	**Longitude**	**Latitude**	**Abundance**
Agostitlán	AGO	156	Suboptimal	100˚37'40.3"	19˚32'9.8"	45
San josé de la Cumbre	SJC	172	Optimal	100˚51'31.4"	19˚41'11."	78
San Antonio Villalongín	SAV	134	Suboptimal	100˚44'54.9"	19˚32'5."	5
Carindapaz	CPZ	154	Suboptimal	100˚54'25.7"	19˚41'15.8."	21
Kilómetro 23	KM	81	Marginal	101˚0'5.1"	19˚39'34."	9
Campestre	CMP	63	Marginal	101˚9'27.4"	19˚40'51.4."	50
Linezo	LNZ	75	Marginal	101˚9'22.8"	19˚40'45.9"	10
Piedra redonda	PR	125	Suboptimal	101˚8'41"	19˚40'3"	13
Cruz del Plato	CP	154	Suboptimal	101˚16'58.7"	19˚24'53.5"	22
Río Bello C	RBC	178	Optimal	101˚7'25.7"	19˚36'39.6"	32
Río Bello P	RBP	105	Marginal	101˚8'17.2"	19˚38'28.3"	80
Turirán	TUR	157	Suboptimal	101˚35'54.1"	19˚21'15.4"	26
Agua Zarca	AZA	153	Suboptimal	101˚7'29.1"	19˚36'49.8"	64
Golondrinas	GOL	123	Suboptimal	101˚8'35"	19˚39'58"	22
Vaquerito	VAQ	145	Suboptimal	101˚0'7.6"	19˚40'54.5"	12
Reja	REJ	110	Marginal	101˚8'42.3"	19˚40'14.04"	13
Puerto Garnica	PGA	85	Marginal	101˚49'19.4"	19˚40'38"	0
Pino Gordo	PGO	134	Suboptimal	100˚47'56.7"	19˚39'33.9"	0
Mil Cumbres	MCU	60	Marginal	100˚46'27.8"	19˚37'15.4"	0
Piedra redonda	PRE	16	Poor	101˚0'48.6"	19˚38'56.3"	0
Zurumbeneo	ZUR	87	Marginal	101˚1'3.3"	19˚41'57.7"	0
San Gregorio	SGR	66	Marginal	101˚32'20.6"	19˚24'3.6"	0
Paso del Muerto	PMU	101	Marginal	101˚36'34.8"	19˚19'29.2"	0
Tepetate	TEP	17	Poor	101˚34'54.8"	19˚22'22.9"	0
Cieneguillas	CIE	91	Marginal	100˚44'58.5"	19˚42'43"	0
Ojo de Agua	OAG	111	Marginal	100˚45'13.4"	19˚44'39.3"	0
Querendaro	QUE	83	Marginal	100˚53'8"	19˚48'28.7.3"	0
Los Sauces	SAU	91	Marginal	100˚42'5.9"	19˚40'3.8"	0
Iratzio	IRT	26	Poor	101˚24'37.23"	19˚38'31.74"	0

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habitat condition of RBPs (P-value < 0.001, $\beta = -0.01$; $SD = 0.001$)). The higher the RBPs scores the lower the probability of individuals presenting morphological abnormalities (Fig 5).

Discussion

The results of our survey of the 29 streams where historically *A. ordinarium* has been recorded must be taken with a certain level of caution. Failing to detect *A. ordinarium* in 13 of the 29 surveyed streams does not necessarily imply that the species has become extinct in these streams. This species is more sensitive to thyroid hormone-induced metamorphosis than other ambystomatids [6]. Considering that surveys were carried out to register larvae/and or paedomorphic individuals, it is possible that the detection of individuals was negatively affected if there was a higher proportion of metamorphic forms in streams where this species was not detected. An additional consideration in interpreting both the presence/absence of *A. ordinarium* and the incidence of morphological abnormalities is the short window of time (February for presence/absence and March-July for abnormalities) in which field surveys were performed. This period of time corresponds to the dry season in the study area and is when

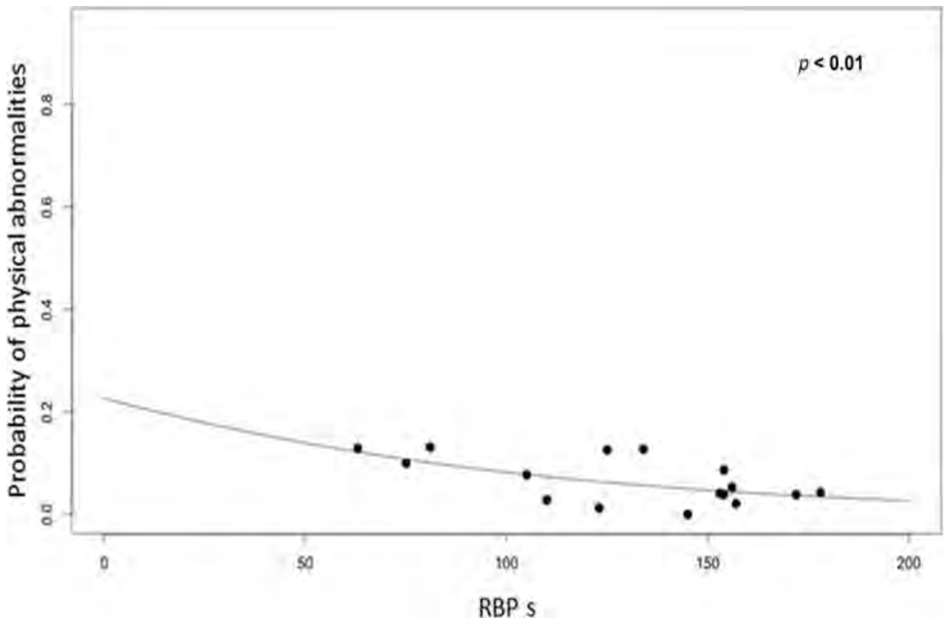

Fig 5. Results of the model of binomial logistic regression of the probability of the character of an individual of *A. ordinarium* being abnormal in relation to habitat quality (RBPs scores). The estimated parameters are α = -1.22, SD = 0.18, P-value < 0.01; β = - 0.01, SD = 0.001, P-value < 0.01 (S1 Table).

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the mountain streams present lower depth and flow rate, as well as clearer water—conditions that allow a better detectability of salamanders in the streams. Therefore, possible seasonal variations in the incidence of abnormalities were not detected with our sampling design.

Understanding how animals in nature respond to the various anthropogenic environmental stressors that threaten their survival is critical for the planning of conservation strategies and actions [28]. Animal species, especially inhabitants of streams, such as the salamander *Ambystoma oridnarium*, are frequently threatened by human activities like urbanization, deforestation and agriculture that negatively affect habitat conditions [13, 29, 30]. Considering the fast pace at which habitats are being transformed, it is important to identify methods that provide rapid and confident assessments of the effects of modifications of habitat quality on the health of populations. In this study we found that the range of values of physicochemical and structural variables that are relevant for *A. ordinarium* in its natural habitat [17] is positively associated with the habitat condition categories of RBPs. Streams that according to scores of RBPs were of optimal habitat condition (high habitat quality) presented a higher percentage of canopy cover, lower values of water conductivity, lower water temperature and higher levels of dissolved oxygen than streams with lower scores of habitat condition. These results suggest that the RBPs of Barbour et al. [16] are a quick and useful method for assessing the habitat quality of streams inhabited by *A. ordinarium.* Given that RBPs provide rapid and cost-effective assessments of water quality and ecological health of aquatic ecosystems [31], it will be important to test if the RBPs protocols can be used to rapidly assess habitat quality for other species of stream amphibians.

Habitat quality is frequently studied in relation to abundance, density, richnness and diversity of species. Some of these measures, however, such as abundance and density may not be adequate predictors of the well-being and physiological performance of individuals [27, 32, 33]. Considering that morphological abnormalities in amphibians have been associated with various types of environmental stressors (e.g., chemical contamination, UBV radiation, infection of parasites, predation attempts), it is reasonable to expect a higher rate of abnormalities in habitats with low levels of environmental quality. Thererfore, our main hypothesis in this study was that we would find an association between habitat quality and prevalence of morphological abnormalities in *A. ordinarium.* Results supported this hypothesis since binomial regression analysis showed a negative association between habitat quality (as indicated by RBPs scores) and the probablility of morphological abnormalities. As the scores of habitat condition were higher, the probability of abnormalities became lower.

The negative association between habitat quality and the prevalence of morphological abnormalities that we found suggests that habitat condition plays an important role in the high number of abnormalities registered in *A. ordinarium.* Only two of the 29 surveyed streams were assigned to the optimal category of habitat condition according to RBPs scores. Ten streams were assigned to the suboptimal, 14 streams to the marginal and three streams to the poor categories of habitat condition. Therefore, 93% of the surveyed streams presented lower than optimal habitat conditions, resulting apparently in a high number of salamanders with morphological abnormalities.

Morphological abnormalities in amphibians have been frequently associated with a reduction in survival and fitness [34, 35]. The 44.6% of individuals of *A. ordinarium* with morphological abnormalities recorded in this study is markedly higher than the background incidence of 0 to 5.0% reported in some wild vertebrate populations in natural habitats [4] and near the higher end of the range of abnormalities reported in some amphibian species (10–50%) [6, 7]. Additionally, of the 224 individuals evaluated for abnormalities, 84 (37.5%) presented more than one abnormality. It is pertinent to acknowledge that in contrast to other studies we included gill abnormalities in the calculation of the 44.6% of sampled individuals with at least one type of abnormality. Even though the gill abnormalities recorded might have been in part related to different proportions of metamorphic forms in dissimilar habitats, if we substract from the estimation those individuals with gill abnormalities, the resultant figure (29.4%) is still markedly higher than the background reported incidence (0–5%).

Loss of digits has been reported as the most frequent morphological abnormality in amphibians (e.g. [5, 36]). To our knowledge, ours is the first study about wild amphibians that reports morphological abnormalities in gills. Braquigills, the partial loss of gills was the most frequent abnormality (26%), whereas ectrodactyly, the loss of digits presented an incidence of 23.0%. Gills in urodeles are responsible for up to 60–70% of oxygen uptake and CO2 removal [37]. Therefore, a reduction of gill surface as a result of injures likely affects the fitness of those individuals with this type of morphological abnormality.

Reported causes of morphological abnormalities in amphibians include road proximity [38], trematode parasites [39, 40], predation attacks [41], radiation [42] and chemical contaminants [43]. Our experimental design did not allow us to identify the relationship between the type of abnormalities and specific causative agents. The negative association between habitat quality and prevalence of abnormalities offered us the possibility of exploring the role of some possible causal factors.

The proximity of roads has been associated with the incidence of morphological abnormailities in frogs [38]. The most obvious effects are direct injury or death from contact with vehicles [44]. Of the streams that we surveyed, those with lower scores of habitat quality had paved or gravel roads parallel to or crossing the streams. Traffic on these roads included trucks, motorcycles, cars and bicycles. Cattle also trampled portions of the banks of these lower habitat quality streams. Both traffic crossing the bed of streams and trampling of banks by cattle are potential causes of some of the morphological abnormalities related to body injures, such as partial or total loss of gills, digits, extremities and tail. Roads also crossed some of the streams with better habitat quality scores, but elevated bridges were used for traffic passage.

Predation attempts have also been considered as one cause of body injures in aquatic amphibians [8]. Therefore, another possible source of abnormalities related to body injures is attacks by predators such as Odonata nymphs [14] and garter snakes (*Thamnophis cyrtopsis* [45]). Predatory fish are also one of the major predators of salamanders in aquatic escosystems [46] and can inflict injures such as partial or total loss of tail, digits, gills and extremities [47]. In three of the 16 streams sampled for prevalence of abnormalities, introduced rainbow trout (*Oncorhynchu mykiss*), a known predator of aquatic salamanders [46] was registered in this study.

Parasite infection is another possible cause of the morphological abnormalities recorded in this study. It has been reported that morphological abnormalities such as polymelia (extra number of limbs, hands or feet), polydactyly (extra number of digits), micromelia (short limbs) and syndactyly (fusion of digits) are associated with the presence of parasites such as trematodes (i.e. *Robeiroia sp*.) [7, 24, 36]. It has been suggested that the process of regeneration of limbs and digits in salamanders is sensitive to the effects of parasites, resulting in abnormal structures [48]. In our study, although of low prevalence, polymelia, polydactyly, micromelia and syndactyly were registered. Snails of the family Planorbidae are intermediate hosts in the life cycle of *Robeiroia* [49], and the presence of these snails in the diet of *A*. *ordinarium* [14] indicates that these snails are present in at least some of the streams inhabitad by this salamander. This finding suggests the possible implication of parasites in the incidence of some of the abnormalities registered. Additionally, changes in water quality caused by human activities favor a proliferation of planorbid snails [11].

The increasing prevalence of morphological abnormalities has recently been suspected as a potential factor implicated in the decline of amphibian populations and species [1]. To our knowledge, this is the first field study that shows a direct association between the incidence of morphological abnormalities and the quality of the habitat in an endangered salamander throughout its entire distribution range. Therefore, our results suggest that one of the several negative effects of habitat degradation on amphibians is an increase in morphological abnormalities with marked consequences for the survival and general fitness of aquatic amphibians. Studies evaluating body abnormalities can provide important information for determining the causal elements and can be a sign of the health status of the ecosystem, since the proliferation of body abnormalities can not only cause death to individuals but may be a warning sign of environmental degradation [50, 51, 52].

Supporting information

S1 Fig. Pictures showing habitat condition a) optimal, b) suboptimal, c) marginal, and d) poor.
(TIF)

S1 Table. Data set underlying findings in this study.
(DOCX)

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References

1. Blaustein AR, Johnson PT. The complexity of deformed amphibians. Fron. Ecol. Environ. 2003; 1: 87–94.
2. Gibbons JW, Scott DE, Ryan TJ, Buhlmann KA, Tuberville TD, Metts BS, et al. The Global Decline of Reptiles, Déjà Vu Amphibians: Reptile species are declining on a global scale. Six significant threats to reptile populations are habitat loss and degradation, introduced invasive species, environmental pollution, disease, unsustainable use, and global climate change. BioScience 2000; 50: 653–666.
3. Hoppe DM. History of Minnesota frog abnormalities: do recent findings represent a new phenomenon?. J. Iowa Acad. Sci. 2000; 107: 86–89.
4. Reeves MK, Medley KA, Pinkney AE, Holyoak M, Johnson PT, Lannoo MJ. Localized hotspots drive continental geography of abnormal amphibians on US Wildlife Refuges. PlOS ONE 2013; 8: e77467. https://doi.org/10.1371/journal.pone.0077467 PMID: 24260103
5. Johnson PTJ, Lunde KB, Ritchie EG, Reaser JK, Launer AE. Morphological abnormalities patterns in a California amphibians community. Herpetologica 2001; 57: 336–352.
6. Thomson S, Muzinic L, Muzinic C, Niemiller ML, Voss SR. Probability of regenerating a normal limb after bite injury in the Mexican axolotl (Ambystoma mexicanum). Regeneration 2014; 1:27–32.
7. Johnson PT, Preu ER, Sutherland DR, Romansic JM, Han B, Blaustein AR. Adding infection to injury: synergistic effects of predation and parasitism on amphibian malformations. Ecology 2006; 87: 2227–2235. PMID: 16995623
8. Semlitsch RD, Reichling SB. Density-dependent injury in larval salamanders. Oecologia 1989; 81: 100–103. https://doi.org/10.1007/BF00377017 PMID: 28312164
9. Ankley GT, Diamond SA, Tietge JE, Holcombe GW, Jensen KM, DeFoe DL, et al. Assessment of the risk of solar ultraviolet radiation to amphibians. I. Dose-dependent induction of hindlimb malformations in the northern leopard frog (Rana pipiens). Environ. Sci. Tech. 2002; 36: 2853–2858.
10. Johnson PT, Lunde KB. Parasite infection and limb malformations: a growing problem in amphibian conservation. In Lannoo M, editor. Amphibian declines: the conservation status of United States species. University of California Press, Berkeley. 2005. Pp. 124–138.

11. Beebee TJC, Griffiths RA. The amphibian decline crisis: a watershed for conservation biology? Biol. Conserv. 2005; 125: 271–285.
12. Johnson PT, Lunde KB, Thurman EM, Ritchie EG, Wray SN, Sutherland DR, et al. Parasite (Ribeiroia ondatrae) infection linked to amphibian malformations in the western United States. Ecol. Monogr. 2002; 72: 151–168.
13. Taylor B, Skelly D, Demarchis LK, Slade MD, Galusha D, Rabinowitz PM. Proximity to pollution sources and risk of amphibian limb malformation. Environ. Health. Perspect. 2005; 113:1497–1501. https://doi.org/10.1289/ehp.7585 PMID: 16263502
14. Ruiz-Martínez L, Alvarado-Díaz J, Suazo-Ortuño I, Pérez-Munguía R. Diet of Ambystoma ordinarium (Caudata: Ambystomatidae) in undisturbed and disturbed segments of a mountain stream in the trans-Mexican Volcanic Belt. Salamandra 2014; 50: 63–70.
15. Martínez-Silvestre A, Amat F, Carranza S. Natural incidence of body abnormalities in the montseny newt, *Calotriton arnoldi*. Herpetology Notes 2014; 7: 277–279.
16. Barbour MT, Gerritsen J, Snyder B, Stribling J. Rapid bioassessment protocols for use in streams and wadeable rivers: Periphyton, benthic macroinvertebrates and fish, Second Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water; Washington: USEPA. 1999.
17. Soto RC. Uso y selección del microhábitat de la salamandra de montaña Ambystoma ordinarium (Amphibia: Caudata). Tesis de Maestría. Programa Institucional de Maestría en Ciencias Biológicas, UMSNH. 2012.
18. Anderson JD, Worthington RD. The lifehistory of the Mexican salamander *Ambystoma ordinarium* Taylor. Herpetologica 1971; 27:165–176.
19. Weisrock DW, Shaffer HB, Storz BL, Storz SR, Voss SR. Multiple nuclear gene sequences identify phylogenetic species boundaries in the rapidly radiating clade of Mexican ambystomatid salamanders. Mol. Ecol. 2006; 15: 2489–2503. https://doi.org/10.1111/j.1365-294X.2006.02961.x PMID: 16842422
20. Shaffer HB. Phylogenetics of model organisms: the laboratory axolotl, Ambystoma mexicanum. Sys. Biol. 1993; 42: 508–522.
21. Bendik NF, Gluesenkamp AG. Body length shrinkage in an endangered amphibian is associated with drought. J. Zool. 2013; 290: 35–41.
22. Pokhrel LR, Karsai I, Hamed MK, Laughlin TF. Dorsal body pigmentation and sexual dimorphism in the marbled salamander (Ambystoma opacum). Ethol. Ecol. Evol. 2013; 25: 214–226.
23. Winandy L, Denoël M. The use of visual and automatized behavioral markers to assess methodologies: a study case on PIT-tagging in the Alpine newt. Behav. Res. Meth. 2011; 43: 568–576.
24. Ankley GT, Degitz SJ, Diamond SA, Tietge JE. Assessment of environmental stressors potentially responsable for malformations in North American anuran amphibians. Ecotoxicol. Environ. Saf. 2004; 58: 7–17. https://doi.org/10.1016/j.ecoenv.2004.01.004 PMID: 15087157
25. Bowerman J, Johnson PTJ, Bowerman T. Sublethal predators and their injured prey: linking aquatic predators and severe limb abnormalities in amphibians. Ecology 2010; 91: 242–251. PMID: 20380213
26. R Core Team. R: A Language and Environment for Statistical Computing (Version 3.1.0). C,Vienna, Austria: R Foundation for Statistical Computing. Retrieved from http://www.R-project.org/. 2014.
27. Johnstone CP, Reina RD, Lill A. Impact of anthropogenic habitat fragmentation on population health in a small, carnivorous marsupial. J. Mammal. 2010; 91:1332–1341.
28. Wu W, Xu Z, Yin X, Zuo D. Assessment of ecosystem health based on fish assemblages in the Wei River basin, China. Environ. Monit. Assess. 2014; 186: 3701–3716. https://doi.org/10.1007/s10661-014-3651-7 PMID: 24535402
29. Kaczmarski M, Kolenda K, Rozenblut-Kościsty B, Sośnicka W. Phalangeal bone anomalies in the European common toad Bufo bufo from polluted environments. Environ. Sci. Pollut. Res. 2016; 23: 21940–21946.
30. Kang C, Shin Y, Kim YE, Moon J, Kang JY, Jang Y. The incidence of abnormalities in the fire-bellied toad, Bombina orientalis, in relation to nearby human activity. J. Ecol. Environ. 2016; 39: 11–16.
31. Harding JS. Stream Habitat Assessment Protocols for wadeable rivers and streams in New Zealand. University of Canterbury, School of Biological Sciences. 2009.
32. Kie JG. Performance in wild ungulates: measuring population density and condition of individuals. General Technical Report PSW-106. Pacific Southwest Forest and Range Experiment Station, USDA Forest Service, Berkeley, California, USA. 1988.
33. Ousterhout BH, Anderson TL, Drake DL, Peterman WE, Semlitsch RD. Habitat traits and species interactions differentially affect abundance and body size in pond-breeding amphibians. J. Anim. Ecol. 2015; 84:914–924. https://doi.org/10.1111/1365-2656.12344 PMID: 25643605

34. Johnson PTJ, Kellermanns E, Bowerman J. Critical Windows of disease risk: amphibian pathology driven by developmental changes in host resistance and tolerance. Func. Ecol. 2011; 25: 726–734.
35. Johnson PTJ, Rohr JR, Hoverman JT, Kellermanns E, Bowerman J. Living fast and dying of infection: host life history drives interspecific variation in infection and disease risk. Ecol. Lett. 2012; 15: 235–242. https://doi.org/10.1111/j.1461-0248.2011.01730.x PMID: 22221837
36. Johnson PTJ, Lunde KB, Ritchie EG, Launer AE. The effect of trematode infection on amphobian limb development and survivorship. Science 1999; 284:802–804. PMID: 10221912
37. BurggrenW Just J. 1992. Developmental changes in physiological systems. In in Feder ME, Burggren WW, editors. Environmental physiology of the amphibians. University of Chicago Press. Chicago. Pp.467–530.
38. Reeves MK, Dolph CL, Zimmer H, Tjeerdeman RS, Trust A. Road proximity increases risk of skeletal abnormalities in Wood Frogs from national wildlife refuges in Alaska. Environ. Health Perspect. 2008; 116:1009–1040. https://doi.org/10.1289/ehp.10963 PMID: 18709167
39. Sessions SK, Ruth SB. Explanation for naturally occurring supernumerary limb in amphibians. J. Exp. Zool. 1990; 254: 38–47. https://doi.org/10.1002/jez.1402540107 PMID: 2348164
40. Johnson PTJ, Sutherland DR, Kinsella JM, Lunde KB. Review of the trematode genus *Ribeiroia* (Psilostomidae): ecology, life history and pathogenesis with special emphasis on the amphibian malformation problem. Adv. Parasitol. 2004; 57:191–253. https://doi.org/10.1016/S0065-308X(04)57003-3 PMID: 15504539
41. Eaton BR, Eaves S, Stevens C, Puchniak A, Paszkowski CA. Deformity levels in wild populations of the wood frog (*Rana sylvatica*) in three ecoregions of western Canada. J. Herpetol. 2004; 2: 283–287.
42. Ankley GT, Tietge JE, Holcombe GW, DeFoe DL, Diamond SA, Jensen KM, et al. Effects of laboratory ultraviolet radiation and natural sunlight on survival and development of *Rana pipiens*. Environ Sci Tech. 2000; 78: 1092–1100.
43. Gardiner DM, Hoppe DM. Environmentally induced limb malformations in mink frogs (*Rana septentrionalis*). J. Exp. Zool. 1999; 284: 207–216. PMID: 10404649
44. Andrews KM, JW, Jochimsen DM. Literature Synthesis of the Effects of Roads and Vehicles on Amphibians and Reptiles. Federal Highway Administration (FHWA), U.S. Department of Transportation, Report No. FHWA-HEP-08-005. Washington, D.C. 2006.
45. García-Garrido P, Alvarado-Diaz J, Suazo-Ortuño I.2016. *Ambystoma ordinarium*. Predation by a Black-necked Gartersnake (*Thamnophis cyrtopsis*). Mesoam. Herpetol; 3: 461–462.
46. Wells KD.The ecology and Behavior of Amphibians. Competition, Predation and the Hidroperiod Gradient. University of Chicago Press. 2007. Pp 776–781.
47. Shule CD, Semlitsch RD. Western mosquitofish (*Gambusia affinis*) bolster the prevalence and severity of tadpole tail injuries in experimental wetlands. Hydrobiologia 2014; 723:131–144.
48. Schottoefer AM, Cole R, Beasley VR. Relationship of tadpole stage to location of echinostome cercariae encystment and the consequences for tadpole survival. J. Parasitol. 2003; 89: 475–482. https://doi.org/10.1645/0022-3395(2003)089[0475:ROTSTL]2.0.CO;2 PMID: 12880244
49. Pinto HA, Jardin RC, Orlofske SA, Johnson PTJ, Melo AL. Biomphalaria straminea (Mollusca: Planorbidae) as an intermediate host of Ribeiroia sp. (Trematoda: Psilostomatidae) in Brazil. J. Parasitol. 2013; 99: 914–918. https://doi.org/10.1645/GE-3214.1 PMID: 23421393
50. Meyer-Rochow V, Asashima M. Naturally ocurring morphological abnormalities in wild population of the japanese newt *Cynops pyrrhogaster* (Salamandridae; Urodela; Amphibia). Zool. Anz. 1988; 221: 70–80.
51. Harris RN. Nonlethal injury to organisms as a mechanism of population regulation. Am. Nat. 1989; 134: 835–847.
52. Burkhart JG, Ankley G, Bell H, Carpenter H, Fort D, Gardiner D, et al. Strategies for Assessing the Implications of Malformed Frogs for Environmental Health. Environ. Health Persp. 2000; 108: 83–90.

DISCUSIÓN GENERAL

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La tasa actual de extinción de especies y la disminución de poblaciones de vertebrados silvestres reflejan los efectos de las actividades realizadas por el hombre, que en su mayoría repercuten negativamente y se agudizan con la demanda de recursos naturales para el sustento humano y el crecimiento poblacional a nivel mundial (Baillie et al. 2010, Pimm et al. 2014, Stuart et al. 2008). Si bien las interacciones entre especies y hábitats a largo plazo pueden resultar en la evolución de adaptaciones que promueven la adecuación de las especies, en la actualidad la alta tasa de transformación de hábitats y modificación de interacciones, limita la respuesta de adaptación de las especies (Ceballos et al. 2015).

Sin embargo, hay especies que responden positivamente a la transformación del hábitat asociado a actividades humanas. Esta respuesta positiva frecuentemente se refleja en el incremento en abundancia de estas especies. En las especies afectadas negativamente por estos cambios las abundancias de sus poblaciones disminuyen súbita o paulatinamente y en algunos casos se extinguen (Bennett y Saunders 2010).

Una de las causas principales asociada a actividades humanas que afectan negativamente a las especies silvestres es la pérdida y modificación de los hábitats (Barrett et al. 2010). Esta amenaza puede afectar la adecuación de las especies directa o indirectamente. Por ejemplo, a medida que se modifica la estructura de los hábitats, la disponibilidad de refugios y sitios para termorregulación, descanso, alimentación y reproducción puede disminuir, limitando la adecuación de las especies (Davies y Nelson 1994, Hamer y McDonnell 2008, Kiesecker et al. 2001, Mitchell et al. 2012, Price et al. 2010, Stuart et al. 2004).

Adicionalmente, los cambios del hábitat por actividades antrópicas frecuentemente modifican variables ambientales microclimáticas y fisicoquímicas (e.g. temperatura, humedad, oxígeno disuelto, pH, conductividad) que a su vez pueden afectar la fisiología y conducta de las especies (Chambers 2011, Chambers et al. 2013, Kiesecker 1996). Las variables ambientales que dependiendo de su disponibilidad e intensidad desencadenan una serie de reacciones fisiológicas, conductuales y fenotípicas en los organismos y pueden suscitar fluctuaciones o cambios en las tendencias poblacionales, anomalías genéticas y morfológicas que son consideradas estresores ambientales (Angulo 2002).

La combinación de un marcado vacío de información sobre biología y ecología de especies de anfibios y los altos niveles de extinción de especies y poblaciones, ha resultado en prioridades de atención especial para este grupo de tetrápodos (Pimm et al. 2014, Hamer y McDonnell 2008, Urbina-Cardona 2008). Adicionalmente, la presión negativa por la pérdida y modificación de sus hábitats, demanda la generación de información que permita entender cómo responden los anfibios a estos cambios, de lo cual dependerá en gran medida la eficiencia de las acciones de conservación.

El presente estudio permitió identificar la respuesta de una especie de anfibio a la pérdida y modificación de su hábitat y a los efectos de la introducción de un depredador no nativo (la trucha arcoíris *Oncorhynchus mykiss*). Se utilizó como modelo de estudio la salamandra de montaña en peligro de extinción *Ambystoma ordinarium*. Los resultados indicaron que las poblaciones de *A. ordinarium* disminuyeron significativamente a medida que disminuyó la calidad del hábitat y hubo presencia del depredador (trucha); mientras que significativamente las anormalidades corporales en los individuos aumentaron. No se registró la presencia de esta salamandra en los sitios que presentaron el mayor grado de perturbación (menor nivel de calidad de hábitat). El diseño experimental de este estudio no permitió identificar las causas directas de estas afectaciones, sin embargo, permitió proveer evidencia cuantitativa de estas relaciones.

Los sitios donde históricamente se había reportado esta especie y no se registró su presencia fueron los que presentaron mayores niveles de afectación antrópica (Soto-Rojas et al. 2017). Si bien la respuesta de los anfibios depende del tipo de afectación y de la magnitud de ésta (Hamer y McDonnell 2008, Pimm et al. 2014), la extinción local de poblaciones de anfibios y en particular de las especies estrechamente ligadas a cuerpos acuáticos, se ha asociado con la urbanización, la pérdida de cobertura forestal, la conversión de bosque a cultivos, la contaminación, la transformación y la pérdida de cuerpos de agua (Adams et al. 2013, Cole et al. 2014, Currinder et al. 2014, Otto et al. 2014, Price et al. 2012, Urban et al. 2006).

Este tipo de estresores ambientales fueron identificados en el presente estudio. Los niveles mayores de estos estresores se presentaron en los sitios donde la especie no se registró. Sin embargo, en los arroyos en que su presencia fue registrada, la degradación de la calidad del hábitat fue aparente. En la actualidad existen pocos lugares donde las actividades del hombre no han tenido efecto (Wu et al. 2014) y es preocupante que este tipo de actividades se agudicen, ya que no solo los anfibios pueden responder negativamente a la antropización de los hábitats, sino también otros grupos de organismos.

En el caso de *A. ordinarium* si las presiones antrópicas persisten y se agudizan, su permanencia y viabilidad estará comprometida, ya que en los sitios donde aún está presente, el tamaño de las poblaciones disminuyeron a medida que disminuyó la calidad del hábitat. Estos resultados apoyan la hipótesis de que la adecuación y viabilidad de las especies se ven afectadas cuando se incrementa la modificación de sus hábitats, además de que presiones adicionales como la presencia de especies invasoras pueden acelerar los efectos negativos sobre las poblaciones, puesto que el efecto de los depredadores introducidos, en comparación con los depredadores locales, suele ser significativo pudiendo llegar a la extirpación de las especies locales (Funk y Dunlap 1999). Aunado a este tipo de estresores que operan negativamente sobre *A. ordinarium,* la vulnerabilidad de la especie aumenta debido a qué al disminuir las abundancias, las poblaciones son más vulnerables a eventos estocásticos, ambientales y genéticos (Armstrong 2005, Caughley 1994, Ferraz et al. 2007, Morris et al. 2002, Vallan 2000).

Otro signo de afectación de la especie fue la proliferación de anormalidades corporales a medida que la calidad del hábitat disminuyó. Es probable que la presencia de anormalidades corporales pueda disminuir la capacidad de los individuos para evadir depredadores, así como para alimentarse y reproducirse adecuadamente, y en consecuencia afectar la viabilidad de la especie (Johnson et al. 2006, Soto-Rojas et al. 2017). En base a los resultados obtenidos, es importante la identificación de factores adicionales que pueden afectar negativamente a la especie, como patógenos y contaminantes, que no fueron evaluados en este estudio.

Debido a la precaria situación en que se encuentra la especie en todo su rango de distribución (menor tamaño poblacional y mayor número de anormalidades corporales en sitios con menor calidad de hábitat) es urgente el monitoreo permanente de esta para identificar las causas directas que pueden estar afectando los patrones de ocurrencia, colonización y extinción local de las poblaciones de *A. ordinarium*. Así mismo es necesario realizar acciones encaminadas a la conservación in situ de la especie y la implementación de estudios encaminados a la reproducción ex situ, que permitan garantizar la viabilidad de *A. ordinarium*.

BIBLIOGRAFÍA

Angulo, A. 2002. Anfibios y paradojas: perspectivas sobre la diversidad y las poblaciones de anfibios. *Ecología Aplicada*. 1 (1), 87-91.

Adams, M.J., D.A.W. Miller., E. Muths., P.S. Corn., E.H.C Grant., L.L Bailey., G.M. Fellers., R.N. Fisher., W.J. Sadinski., H. Waddle., y S.C. Walls. 2013. Trends in amphibian occupancy in the United States. *PLOS ONE*. 8 (5), 1-5.

Armstrong, D.P. 2005. Integrating the metapopulation and habitat paradigms for understanding broad-scale declines of species. *Conservation Biology*. 19 (5), 1402–1420.

Baillie, J. E. M., J. Griffiths, S. T. Turvey, J. Loh, and B. Collen. 2010. Evolution Lost. Secretary 1-72.

Barrett, K., B. S. Helms., C. Guyer, and J. E. Schoonover. 2010. Linking process to pattern: causes of stream-breeding amphibian decline in urbanized watersheds. *Biological Conservation*, 143 (9), 1998-2005.

Bennett, A.F. y D.A. Saunders. 2010. Hábitat fragmentation and landscape. *Oxford University Press*. 88-106.

Caughley, G.1994. Directions in conservation biology. *Journal of Animal Ecology*. 63 (2), 215-244.

Ceballos, G., P. R. Ehrlich., A. D. Barnosky., A. García., R. M. Pringle., y T. M. Palmer. 2015. Accelerated modern human–induced species losses: Entering the sixth mass extinction. *Science advances*. 1-5.

Chambers, D. L. 2011. Increased conductivity affects corticosterone levels and prey consumption in larval amphibians. *Journal of Herpetology*. 45 (2), 219-223.

Chambers, D. L., J. M. Wojdak., P. Du, y L. K. Belden. 2013. Pond acidification may explain differences in corticosterone among salamander populations. *Physiological and Biochemical Zoology*. 86 (2), 224-232.

Cole, E. M., M. R. Bustamante., D. Almeida-Reinoso., y W. C. Funk. 2014. Spatial and temporal variation in population dynamics of Andean frogs: Effects of forest disturbance and evidence for declines. *Global Ecology and Conservation*. 1, 60-70.

Currinder, B., K. K. Cecala., R. M. Northington., y M. E. Dorcas. 2014. Response of stream salamanders to experimental drought in the southern appalachian mountains, USA. *Journal of freshwater ecology*. 29 (4), 579-587.

Davies, P., y M. Nelson. 1994. Relationships between riparian buffer widths and the effects of logging on stream habitat, invertebrate community composition and fish abundance. *Marine and Freshwater Research.* 45, 1289-305.

Ferraz, G., J.D. Nichols, J.E. Hines, P.C. Stouffer, R.O. Bierregaard Jr. y T.E Lovejoy. 2007.A large-scale deforestation experiment: Effects of patch area and isolation on Amazon birds. *Science.* 315, 238–241.

Funk, W. C., y W. W. Dunlap.1999. Colonization of high-elevation lakes by long-toed salamanders (*Ambystoma macrodactylum*) after the extinction of introduced trout populations. *Canadian Journal of Zoology*. 77 (11), 1759-1767.

Hamer, A. J., y M. J. McDonnell. 2008. Amphibian ecology and conservation in the urbanising world: A review. *Biological Conservation.* 141, 2432–2449.

Johnson, P.T., E.R. Preu., D.R. Sutherland., J.M. Romansic., B. Han., y A.R. Blaustein. 2006. Adding infection to injury: synergistic effects of predation and parasitism on amphibian malformations. *Ecology*. 87 (9), 2227-2235.

Kiesecker, J. 1996. Ph- mediated predator - prey interactions between *Ambystoma tigrinum* and *Pseudacris triseriata*. *Ecological Applications*. *6*(4), 1325-1331.

Kiesecker, J.M., A.R. Blaustein y L.K. Belden. 2001.Complex causes of amphibian population declines. *Nature* .410 (6820), 681- 684.

Mitchell,T., L.A. Alton, G.R. White y C.E. Franklin. 2012. Relations between conespecific density and effects of ultraviolet-b radiation on tadpole size in the striped marsh frog. *Conservation biology*. 26 (6), 547-555.

Morris, W., P. Bloch., B. Hudgens., L. Moyle, y J. Stinch-combe. 2002. Population viability analysis in endan-gered species recovery plans: past use and future improvements. *Ecological Applications*. 12 (3), 708–712.

Otto, C. R., G. J. Roloff., y R. E. Thames. 2014. Comparing population patterns to processes: abundance and survival of a forest salamander following habitat degradation. *PLOS ONE*. *9* (4), e93859.

Pimm, S.L., C.N. Jenkins., R. Abell., T.M. Brooks., J.L. Gittleman., L.N. Joppa., P.H. Raven., C.M. Roberts y J.O. Sexton. 2014. The biodiversity of species and their rates of extinction, distribution, and protection. Science. 344 (6178), 1246752, 1-10.

Price, J. S., K. K. Cecala, R. A. Browne y M. E. Dorcas. 2010. Effects of urbanization on occupancy of stream salamanders. *Conservation Biology*. 25 (3), 547-555.

Price, J.S., Browne, A.R., y Dorcas, E.M. 2012. Evaluating the effects of urbanisation on salamander abundances using a before-after control-impact design. *Freshwather Biology*. 57, 193 – 203.

Stuart, S.N., J.S. Chanson., N.A. Cox., B.E. Young, A.S.L. Rodrigues., D.L. Fischman., y R.W. Waller. 2004. Status and trends of amphibian declines and extinctions worldwide. Science. 306, 1783–1786.

Stuart, S.M., M. Hoffman., J.S. Chanson., N.A. Cox., R.J. Berridge, P. Ramani y B. Young. 2008. Threatened amphibians of the world. Lynx Edicions, Barcelona, Spain. IUCN, Gland, Switzerland and Conservación International, Arlington, Virginia, USA.

Urban, M. C., D. K. Skelly., D. Burchsted., W. Price., y S. Lowry .2006. Stream communities across a rural–urban landscape gradient. *Diversity and distributions*. 12 (4), 337-350.

Urbina-Cardona, J. N. 2008. Conservation of neotropical herpetofauna: research trends and challenges. *Tropical Conservation Science*. 1 (4), 359-375.

Vallan, D. 2000. Influence of forest fragmentation on amphibian diversity in the nature reserve of Ambohit- antely, highland Madagascar. *Biological Conservation*. 96, 31–43.

Wu, W., Z. Xu., X. Yin y D. Zuo. 2014. Assessment of ecosystem health based on fish assemblages in the Wei river basin, China. *Environmental monitoring and assessment*. 186 (6), 3701-3716.

ANEXO I

LOS AJOLOTES MICHOACANOS

Este artículo está en proceso de publicación:

Soto-Rojas, C., y I. Suazo-Ortuño. Los ajolotes michoacanos. Estudio de estado.CONABIO.

Los ajolotes michoacanos

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En la actualidad más de un tercio de las especies de anfibios se encuentran amenazadas o en peligro de extinción (Stuart *et al.* 2004). Los esfuerzos para entender este alarmante declive se han centrado principalmente en los anuros (sapos y ranas), mientras que se ha puesto poca atención sobre las especies de salamandras (Rovito *et al.* 2009), posiblemente debido a que estos organismos son más difíciles de observar.

Descripción del grupo

Los ajolotes también llamados Achoques, Achoqui o Achójki, por el pueblo purépecha (Velarde 2011), son conocidos por su morfología peculiar ya que tienen branquias externas y ramificadas que salen hacia atrás de la base de la cabeza en su estado larvario y pierden sus branquias cuando sufren metamorfosis (figura 1). Por lo general su piel es lisa en casi todo el cuerpo y presentan pliegues de cada lado, tienen una cabeza robusta, ancha y plana que contrasta con el resto del cuerpo que es más alargado, sus ojos son pequeños y no tienen parpados. El periodo de reproducción y puesta de huevos de esta familia es variable pero en general depositan sus huevecillos en la temporada invernal (Huacuz 2002, Soto Rojas 2012).

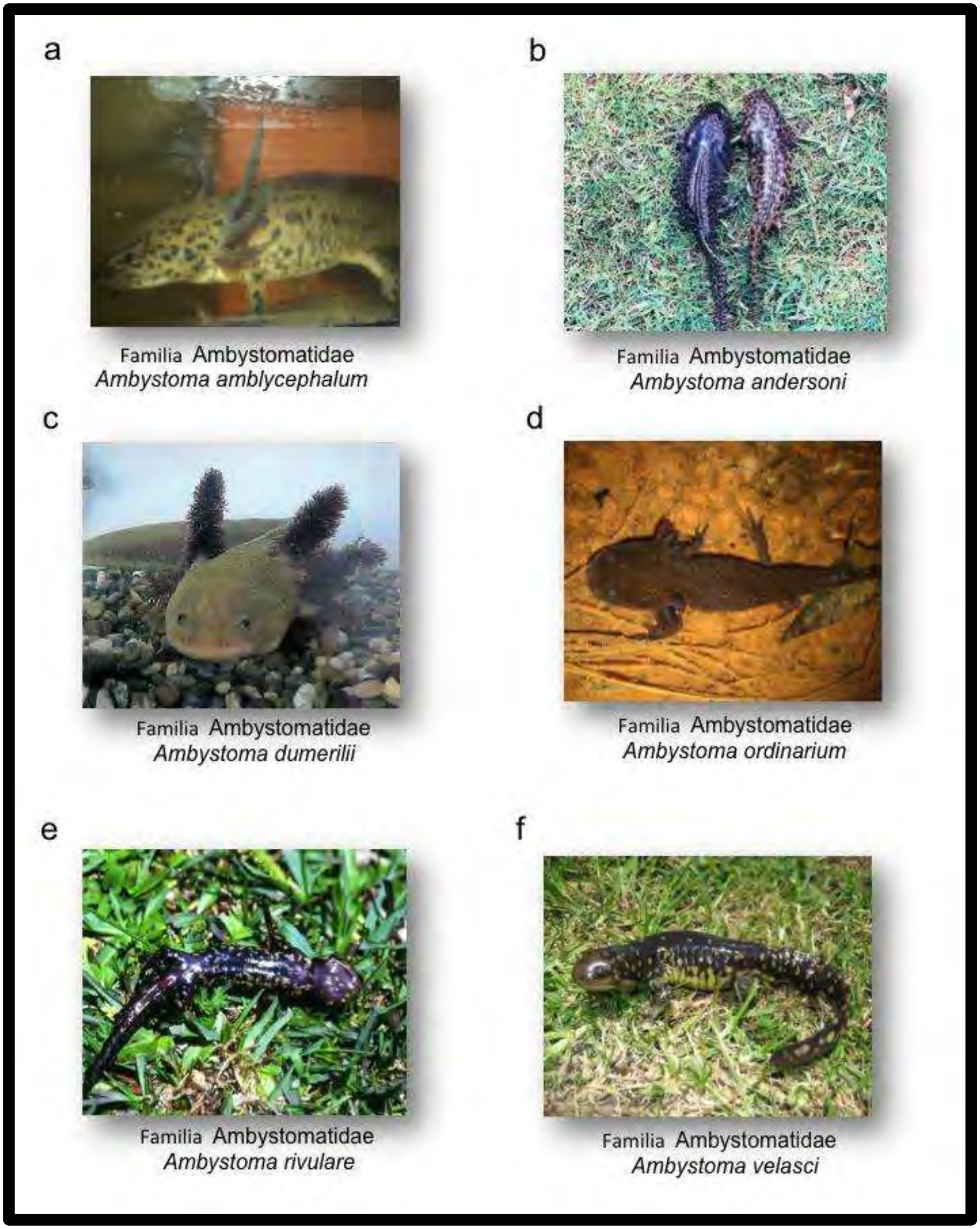

Figura 1. Ambystomidos de Michoacán: a) *Ambystoma amblycephalum*¸b) *A. andersoni*; c) *A. dumerilii*; d) *A. ordinarium*; e) *Ambystoma rivulare* y f) *Ambystoma velasci*. Fotos: Oscar Medina Aguilar.

Diversidad y Distribución

En México se han registrado 137 especies de salamandras pertenecientes a cuatro familias: Ambystomatidae (ajolotes), Plethodontidae (salamandras sin pulmones), Salamandridae (salamandras) y Sirenidae (sirenidos) de las cuales 87 especies se encuentran en alguna categoría de riesgo en la NOM-059-SEMARNAT-2010. De las cuatro familias de salamandras mexicanas, en Michoacán únicamente se encuentran representadas dos: Plethodontidae que presenta salamandras apulmonadas y está constituida por tres especies: *Pseudoeurycea belli*, *P. leprosa* y *P. longicauda*; y Ambystomatidae integrada por seis especies de ajolotes (cuadro 1, figura 1). Las seis especies de ambystomas presentes en Michoacán se encuentran incluidas en alguna de las categorías de conservación de la UICN y la NOM-059-SEMARNAT-2010 (cuadro 1).

Cuadro 1. Listado de las especies de ambystomas del estado de Michoacán de acuerdo al estado de conservación de la UICN (2014) y la Norma Oficial Mexicana Ecológica (NOM-059-SEMARNAT-2010).

Especie	Nombre común	Endemismo	Estado de Conservación UICN	NOM-059-SEMARNAT-2010
Ambystoma amblycephalum	Ajolote o salamandra	A Michoacán	CR	Pr
Ambystoma andersoni	Ajolote, Achoque de Zacapu	A Michoacán	CR	Pr
Ambystoma dumerilii	Ajolote, Achoque de Pátzcuaro	A Michoacán	CR	Pr
Ambystoma ordinarium	Ajolote de montaña, Ajolote o salamandra de arroyo	A México	EN	Pr
Ambystoma rivulare	Ajolote	A México	DD	A
Ambystoma velasci		A México	LC	Pr

A=Amenazada, Pr = Sujeta a protección especial, CR = En Peligro Crítico, EN = En Peligro, DD= Deficiencia de datos.

Habitan en lagos, lagunas, ríos perenes e intermitentes y zonas terrestres donde predomina la humedad. Todos los Ambystomatidos presentes en Michoacán se ubican en el Sistema Volcánico Transversal. Las especies que habitan estrictamente cuerpos de agua lénticos son *A. andersoni* (figura 1b) en el Lago de Zacapu y *A. dumerili* en el Lago de Pátzcuaro (figura 1c), y se consideran organismos paedomorficos estrictos, es decir han perdido su capacidad para transformarse y salir al medio terrestre. Mientras que *A. amplycephalum* (figura 1a) que habita en la región de Tacícuaro, Municipio de Morelia, *A. ordinarium* (figura 1d) que habita en los arroyos de montaña del Sistema Volcánico Transversal, *A. rivulare* (figura 1e) que habita en la Reserva de la Mariposa Monarca, y *A. velasci* que habita en el bajío Michoacano (figura 1f) viven en cuerpos de agua loticos perenes o intermitentes; además, pueden sufrir metamorfosis y perder las branquias (paedomórficos facultativos) lo que les permite salir del medio acuático e invadir el medio terrestre (Duellman y Trueb 1994).

En la entidad *A. ordinarium, A. rivulare* y *A. velasci* son endémicas a México mientras que los *Ambystoma dumerili, A. andersoni* y *A. amblycephalum*, son microendémicos al estado lo que significa, que solo están presentes en estos sitios y no se encuentran en ninguna otra parte del mundo (Shaffer 1989).

Importancia de los ajolotes

La importancia biológica de estos organismos endémicos se entrelaza con la riqueza cultural ya que son animales enigmáticos y sorprendentes que han formado parte de la cultura mexicana como deidades, alimento y medicina desde antes de la conquista hasta la actualidad. Hoy en día estos organismos forman partes de diversos mitos, de los cuales se puede mencionar que en la mitología Azteca se consideraba que el “axólotl” era la encarnación de un dios prehispánico que le tiene miedo a la muerte y quiere escapar de ella mediante sus poderes de transformación. En algunas leyendas nahuas, se cuenta que había un dios malvado que presumía y hacía cosas malas a los hombres y a los dioses. Entonces los dioses decidieron deshacerse de él y enviarlo al inframundo para que de ahí no pudiese salir, sin que se dieran cuenta el dios malvado los escucho y decidió esconderse en el Lago de Pátzcuaro para que no lo encontraran, entonces fue como surgió el achoque (Velarde 2011). A pesar de que no se han comprobado sus efectos, los ajolotes se utilizan para curar diversas afecciones, en particular las relacionadas con las enfermedades respiratorias y la tristeza (Velarde 2011).

Los ambystomas son considerados depredadores importantes y consumidores generalistas ya que pueden alimentarse de casi cualquier presa que puedan atrapar y engullir (Shaffer 1989); desde pequeños peces, artrópodos acuáticos y terrestres, hasta larvas de moscos, arañas, grillos, lombrices, huevos y larvas de otros anfibios (Duellman 1961, Alvarado-Díaz *et al.* 2003, Huacuz 2002). Incluso se ha reportado que estos organismos se comen entre sí, por lo que también son considerado caníbales (Lemos *et al.* 1999, Soto-Rojas 2012).

Acciones de conservación y recomendaciones

Actualmente estos organismos enfrentan diversas amenazas como la pérdida y contaminación de hábitat, depredación por animales introducidos, extracción para consumo, muerte por infecciones de hongos y los efectos colaterales del calentamiento global, entre otros (Blaustein *et al.* 1994, Huacuz 2002, Stuart *et al.* 2004,Velarde 2011, Soto-Rojas 2012). Estas amenazas sumadas a la falta de información sobre la biología y el estado de conservación actual de las especies que habitan en Michoacán, representan un panorama crítico para la supervivencia de los ambystomas. Se considera necesario desarrollar e implementar acciones de manejo y conservación que garanticen su persistencia, por ejemplo el saneamiento de los cuerpos de agua donde estas especies habitan, así como la prohibición total de su captura con fines comerciales y de autoconsumo, ya que en la región de la meseta purépecha aún se continúa con el saqueo desmedido de estas especies. Por otro lado, es urgente que las agencias gubernamentales financien proyectos de investigación que permitan evaluar el tamaño y estado actual de las poblaciones de cada una de estas especies, así como las condiciones en que se encuentran los cuerpos de agua y los hábitats en los que ocurren estas especies.

Referencias

Alvarado-Díaz, J., P. García e I. Suazo-Ortuño. 2003. Food habits of a paedomorphic population of the Mexican salamander, *Ambystoma ordinarium* (Caudata:Ambystomatidae). *The Southwestern Nautralist* 48: 100-102.

Blaustein, A.R., D.B. Wake y W.P. Sousa. 1994. Amphibian declines: judging stability, persistence, and susceptibility of populations to local and global extinctions. *Conservation Biology* 8:60-71.

Duellman, W.E. 1961. The amphibians and reptiles of Michoacán México. Univ. Kansas Pub. Musseum of Natural History 15:1-148.

Duellman, W.E. y L. Trueb. 1994. *Biology of Amphibians*. Johns Hopkins University

Huacuz E.D. 2002. Programa de Conservación y Manejo de *Ambystoma dumerilii*, el achoque del Lago de Pátzcuaro.Universidad Michoacana de San Nicolás de Hidalgo, FMCN A.C., Secretaría de Medio Ambiente y Recursos Naturales, México.

UICN. Unión Internacional para la Conservación de la Naturaleza Red List of Threatened Species. Version 2013. En: http.//www.iucnredlist.org, última consulta: 21 de Abril del 2013

Lemos, E.J.A., G.R. Smith, R.E. Ballinger y Ramírez B.A.1999. Status of protected endemic salamanders (Ambystoma: Ambystomatidae:Caudata) in the transvolcanic belt of México. *British Herpetological Society Bulletin* 68:1.4

Rovito, S. M., G. Parra-Oleo, C. R. Vázquez-Almazán, T. J. Papenfuss y D. B. Wake. 2009. Dramatic decline in neotropical salamander populations are an important part of the global amphibian crisis. *Proceedings of National Academic Sciences* 109:3231-3236

SEMARNAT. Secretaría de Medio Ambiente y Recursos Naturales. 2010. Norma Oficial Mexicana NOM-059-SEMARNAT-2010. Diario Oficial de la Federación (DOF), jueves 30 de diciembre de 2010.

Shaffer, H.B.1989. Natural History, Ecology, and Evolution of the Mexican "Axolotls". *Axolotl Newsletter* 18:5-11

Soto-Rojas C. 2012. Uso y selección del microhábitat de la salamandra de montaña *Ambystoma ordinarium*, Tesis de Maestria Institucional en Ciencias Biológicas, Universidad Michoacana de San Nicolás de Hidalgo. Morelia, Michoacán.

Stuart S. N., J. S. Chanson, N. A. Cox, B. E. Young, A.S.L. Rodrigues, D. L. Fischman y R. W. Waller. 2004. Status and trends of amphibian declines and extinctions worldwide. *Science* 306:1783-1786.

Velarde, M. T. 2011. Importancia ecológica y cultural de una especie endemic de ajolote (Ambystoma dumerilli) del Lago de Pátzcuaro, Michoacán. Tesis de Licenciatura. Facultad de Ciencias. Universidad Nacional Autónoma de México.