Universidad Michoacana de San Nicolás de Hidalgo Instituto de Investigaciones sobre los Recursos Naturales

Programa de Maestría en Ciencias en Ecología Integrativa del Instituto de Investigaciones sobre Recursos Naturales, UMSNH.

Análisis de la asimetría fluctuante y cambios morfológicos en poblaciones de *Ambystoma dumerilii* en vida libre y cautiverio

TESIS

QUE PRESENTA:

Berenice Ramírez López

Como requisito para obtener el título de:

Maestra en Ciencias en Ecología Integrativa.

DIRECTORA DE TESIS:

Dra. Yurixhi Maldonado López

CO-DIRECTOR DE TESIS:

Dr. Luis H. Escalera Vázquez

REVISORES:

Dra. Ireri Suazo Ortuño

Dra. Ana María Pierro González

Dr. Omar Domínguez Domínguez

Morelia, Michoacán Febrero del 2022.

Índice:

1. Resumen

*Ambystoma dumerilii (*Achoque) es una especie microendémica en peligro crítico según la Unión Internacional para la Conservación de la Naturaleza (UICN), cuyo hábitat está altamente perturbado, por lo que una estrategia de conservación ha sido implementar la crianza en cautiverio. Sin embargo, en ambas condiciones, se presentan diferentes factores de estrés que afectan el desarrollo de los anfibios, induciendo cambios morfológicos y anormalidades. Por ello nuestro estudio se centró en analizar y comparar la morfología de Achoques criados en cautiverio y de vida libre, utilizando la asimetría fluctuante, morfometría y alometría. Nuestros resultados muestran que la forma del cuerpo es más larga y delgada en organismos de cautiverio en relación en los individuos del lago, y los caracteres morfológicos de los individuos del lago son más grandes y asimétricos. Todos los individuos muestran relaciones hipoalométricas entre el tamaño corporal y los caracteres morfológicos. La asimetría fluctuante es mayor en los individuos de cautiverio. Por lo tanto, los organismos del lago pueden tener tamaños más grandes debido a factores como la dieta, que a diferencia de los organismos en cautiverio se alimentan de acociles, peces pequeños, entre otros organismos que pueden favorecer un mejor desarrollo así como la disponibilidad que es diferente en ambas condiciones, así como la disponibilidad de espacio.

Palabras clave: asimetría fluctuante, patrones alométricos, Ambystoma, Achoque, cautiverio, vida silvestre.

2. Abstract

Ambystoma dumerilii (Achoque) is a critically endangered microendemic species according to the IUCN, whose habitat is highly disturbed. A conservation strategy has been to implement captive breeding. However, in both conditions, there are different stress factors that affect amphibian development, inducing morphological changes and abnormalities. Therefore, our study focused on comparing the morphology of Achoques in captivity and free life, using fluctuating asymmetry, morphometry and allometry as tools. Our results show that the body shape is elongated in lake individuals, and morphological characters show that they are larger and asymmetric in the lake. Allometry patterns for all individuals show hypoalometric relationships between body size and morphological characters. Therefore, lake organisms may have larger sizes due to various factors such as diet, space availability or other conditions that may be present in their environment. They also presented higher levels of fluctuating asymmetry, which coincides with other studies since they can be subjected to different pressures on the organisms that may be promoting lower rates of development of these traits on growth.

***Keywords*:** fluctuating asymmetry, allometric patterns, *Ambystoma*, Achoque, captivity, wild life.

Agradecimientos.

El presente trabajo forma parte de los proyectos: “Conservation and long-term management of the Achoque (*Ambystoma dumerilii*) and its habitat”. Zoológico de Chester, Reino Unido.

“Evaluating populations of the michoacan axolot (*A. dumerilii*) in Lake Pátzcuaro for the recovery of local management and fisheries”. Financiado por The Mohamed bin Zayed Species Conservation Fund, no. de proyecto **182518929.**

A la Dra. Yurixhi Maldonado López por todo su apoyo y enseñanzas durante el desarrollo de este trabajo tanto en campo, en los laboratorios y durante toda la redacción, pero particularmente por su paciencia y comprensión durante todo este tiempo.

Dr. Luis H. Escalera Vázquez por siempre estar disponible para aclararme dudas y apoyarme en la resolución de problemas, así como su apoyo en campo.

A la Dra. Ireri Suazo Ortuño, Dra. Ana María Pierro González y al Dr. Omar Domínguez Domínguez que a través de su conocimiento aportaron valiosos comentarios para mejorar la calidad de este proyecto.

A todos mis compañeros que durante estos dos años vivimos grandes momentos y se formo una bonita amistad.

A todos aquellos, quienes me acompañaron en campo Viridiana, Francisco Martínez, Indira Figueroa, Ricardo, así como a Joan S., gracias por todo el tiempo y esfuerzo que aportaron y por esos momentos tan divertidos.

Gracias a mis padres por sus palabras de aliento, su apoyo y amor, gracias a ustedes me eh logrado superar. A mis hermanos por siempre ayudarme y darme tantos momentos de alegría, son la luz en mis ojos.

A Luis por toda su paciencia y apoyo, gracias por siempre estar ahí.

A toda mi familia todos ustedes son las personas que me han ayudado a crecer y por los cuales soy la persona que está aquí en este momento, gracias por su apoyo en los malos momentos son los que llenan de alegría mi vida, cada uno siempre me enseña algo nuevo. Le gradezco a la vida haber nacido rodeada de personas maravillosas.

A todos mis amigos por siempre escucharme, son la familia que pude elegir y sin importar el tiempo que pasé para mí siempre podrán contar conmigo.

Dedicado a todos mis seres queridos que a lo largo de este tiempo se han ido, sigo pensando en ustedes y espero hacer que se sientan orgullosos donde quiera que estén. Un abrazo hasta el cielo.

3. Introducción

A nivel mundial la tasa de extinción de especies ha aumentado, por lo que se considera que el planeta se encuentra en la sexta extinción a consecuencia de las actividades antropogénicas (McCallum, 2015). Entre los taxones más vulnerables se encuentran los anfibios, ya que cerca del 43.2% de sus especies está en vías de extinción o presenta alguna disminución en sus poblaciones (Grenyer *et al*. 2006; Wake y Vredenburg, 2008; Bower, Lips, y Georges, 2017). Son múltiples las causas que interactúan de manera sinérgica o individual, ocasionando el declive de las poblaciones en este grupo, como es la pérdida y fragmentación del hábitat, la exposición a radiación UV, urbanización, cambio climático, la introducción de especies invasoras, así como la contaminación y las enfermedades emergentes (Stuart *et al*. 2004; Cohen *et al.* 2019). Aunque todos estos factores son relevantes, existe el consenso de que el principal promotor de la disminución de las poblaciones de anfibios es la destrucción y la alteración del hábitat debido al cambio de uso del suelo (Mattoon, 2000; Stuart *et al*. 2004; Cohen *et al*. 2019). Estos factores representan una fuente de estrés fisiológico muy importante que compromete el estado general de salud de los anfibios (Blaustein *et al.* 2011).

3.1. *Efecto del estrés ambiental en la morfología de los anfibios*

El estrés ambiental induce respuestas fisiológicas que son reguladas por el eje hipotalámico-pituitario-interrenal que activa la producción de glucocorticoides, principalmente corticosterona en los anfibios, provocando múltiples efectos en cascada en los procesos fisiológicos (Brodeur, 2020). Esta respuesta fisiológica tiene un efecto negativo sobre el sistema inmune, sobre el crecimiento y reproducción, afectando la salud del organismo (Hill *et al.* 2016; Waye, 2019; Ramírez-Hernández *et al.* 2019). El efecto del estrés ambiental en anfibios es frecuentemente evaluado a través del análisis de células blancas de la sangre, ya que los glucocorticoides alteran la producción de leucocitos, aumentando la cantidad de neutrófilos (células que proliferan en respuesta al estrés) y disminuyen la cantidad de linfocitos (moduladores del sistema inmunológico) (Davis y Maerz, 2008). Por ejemplo, en *Ambystoma ordinarium* se encontró una relación positiva entre el grado de perturbación del hábitat y el índice de neutrófilo/linfocitos, en particular en las proporciones de neutrófilos y monocitos que fueron más altas que en otras especies de *Ambystoma* (Ramírez-Hernández *et al*. 2019).

Sin embargo, cuando el estrés ambiental es crónico, puede tener efectos negativos sobre el desarrollo de los anfibios, induciendo cambios y anormalidades morfológicas (Davis *et al.* 2018; Waye, 2019; Cayuela *et al*. 2020; Drew R *et al*. 2020; Gangenova *et al.* 2020) como deformación gonadal (Papoulias *et al.* 2013), cambios en el largo corporal (Delgado-Acevedo, 2008; Santini *et al.* 2018), presencia de malformaciones en las extremidades y branquias (Johnson y Lunde, 2001; Johnson y Lunde, 2005; Soto-Rojas *et al*. 2017), y cambios en el tamaño de distintos caracteres morfológicos (Guo *et al*. 2017; Zhelev *et al.* 2019). Entre los factores de estrés relacionados a estos cambios morfológicos en anfibios, está la contaminación por pesticidas, plaguicidas, o metales pesados (Ruiz *et al.* 2010; Papoulias *et al.* 2013; Zhang *et al*. 2018), como es el caso de *Fejervarya limnocharis*, que presentó una disminución en el tamaño y el peso de los organismos en relación con la contaminación por herbicidas (Thammachoti *et al.* 2012). Se ha estudiado la infección por parásitos y sus efectos sobre los anfibios, como los tremátodos del género *Ribeiroia* que inducen malformaciones en las extremidades, como es el caso de la *Rana boyli* donde las anormalidades que presenta están asociadas a *Ribeiroia ondatrae* (Kupferberg *et al.* 2009) (Figura 1).

Figura 1. *Pseudacris regilla* con polimelia por quistes de *Ribeiroia ondatrae*. Tomada de Henle K, Dubois A. y Vershinin V. 2017

Los caracteres morfológicos de los anfibios pueden variar en tamaño y forma debido al estrés ambiental como la pérdida del hábitat, cambio de uso de suelo, y perturbación del hábitat (Guo *et al*. 2017; Zhelev *et al.* 2019). Por ejemplo, en *Ambystoma ordinarium* existe una relación positiva entre altos niveles de perturbación del hábitat, y el aumenta en la proporción de anormalidades en branquias (poli-branquia, braqui-branquia) y extremidades (micromelia, ectrodactilia, braquidactilia, polidactilia, sindactilia) (Soto-Rojas *et al*. 2017) (Figura 2, 3). Las especies *Elachistocleis bicolor* (Sapito panza amarilla), *Physalaemus cuvieri* (Ranita Ladradora) y *Odontophrynus americanus* (Escuercito común), presentan cambios morfológicos como disminución de la condición corporal, de la masa corporal y tamaño corporal, asociado al cambio de uso de suelo a plantaciones de pinos (Gangenova *et al*. 2020). En otro estudio, las especies de anfibios, *Lissotriton helveticus* (Tritón palmeado), *Pelophylax perezi* (Rana común), *Salamandra salamandra* (Salamandra común), *Triturus marmoratus* (tritón jaspeado) presentaron un mayor tamaño corporal y mayor condición corporal en sitios urbanizados en comparación con los sitios conservados, asociado a la alimentación y menor depredación (Iglesias-Carrasco *et al*. 2017). Las especies *Elachistocleis bicolor* (Sapito panza amarilla), *Physalaemus cuvieri* (Ranita Ladradora) y *Odontophrynus americanus* (Escuercito común), presentan cambios morfológicos como disminución de la condición corporal, de la masa corporal y tamaño corporal, asociado al cambio de uso de suelo a plantaciones de pinos (Gangenova *et al.* 2020).

Figura 2. Subadulto de *Salamandrella keyserlingii*, especie con alta variabilidad en el número de falanges: oligodactilia (extremidad anterior derecha), braquidactilia (extremidad anterior izquierda) y cola acortada; según la ubicación y el autor, la variabilidad digital puede considerarse parte de la variación normal o anormal. Tenga en cuenta que cuatro dedos son el fenotipo normal. Tomada de Henle K, Dubois A. y Vershinin V. 2017.

Figura 3. Tipos de anomalías morfológicas identificadas en *A. ordinarium*: (a) micromelia, (b) ectrodactilia, (c) braquidactilia, (d) sindactilia, (e) polimelia, (f) polidactilia, (g) ectromelia, (h) cola incompleta, (i) cola regenerada, (j) polibranquia, (k) ectrobranquia, (l) braquibranquia, (m) sinbranquia. Tomada de Soto-Rojas *et al.* 2017.

Dado que los anfibios son un grupo susceptible a los cambios ambientales es importante conocer la variación morfológica derivada de las condiciones ambientales (Iglesias-Carrasco et al. 2017). Por ejemplo, en *Rhinella marina* encontraron un cambio en diversos rasgos morfológicos que estaban asociados a un mejor rendimiento locomotor como una pelvis más larga, una columna vertebral presacra más corta y una cabeza más estrecha en sitios con una mayor abundancia de arañas y serpientes (Figura 4). Diversas actividades antropogénicas como la urbanización pueden generar cambios en los organismos. En la especie *Feirana quadranus* evaluaron los efectos en el largo de hocico a cloaca, y el tamaño de las extremidades en función de los cambios de temperatura, la variación de la precipitación, disponibilidad y calidad del alimento, además de la radiación UV, encontrando una correlación significativa entre el estrés ambiental y la longitud de las extremidades traseras (Wang *et al.* 2018).

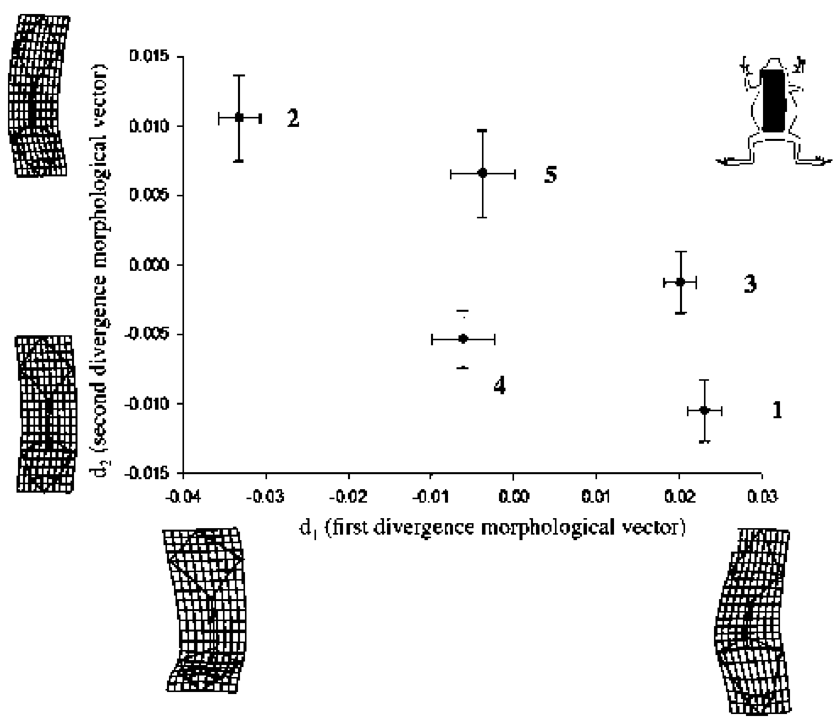


Figura 4. Divergencia morfológica entre poblaciones de *R. marina*. Tomado de Langerhans *et al.* 2014.

3.2. Asimetría fluctuante en anfibios

La asimetría fluctuante (AF) en anfibios es una medida del efecto del estrés ambiental durante el desarrollo de los organismos (Palmer y Strobeck, 2003; Benítez y Parra, 2011; Zhelev *et al.* 2015). La AF es el resultado de pequeñas irregularidades aleatorias en los procesos de desarrollo que ocurren incluso bajo condiciones genéticas y ambientales constantes (Tucić, Budečević *et al.* 2018). La AF es la varianza en las diferencias entre el lado izquierdo y derecho en organismos simétricamente bilaterales y proporciona una medida de la capacidad de un individuo para amortiguar el estrés interno (genético) y el externo (ambiental) durante su ontogenia (Møller y Shykoff, 1999). La asimetría fluctuante es considerada como una expresión de inestabilidad en el desarrollo de los órganos y estructuras biológicas, resultado de perturbaciones externas (Cuevas-Reyes *et al.*, 2013). Los organismos que a lo largo de su desarrollo toleran niveles bajos de estrés, logran amortiguar los efectos y disminuyen los errores aleatorios en el desarrollo (Freeman *et al.* 2005; Maldonado-López *et al.* 2019). Sin embargo, cuando los niveles de estrés ambiental son altos, los organismos se ven rebasados en su capacidad para amortiguar esos errores, presentando AF (Leamy y Klingenberg, 2005; Cuevas-Reyes *et al.* 2013; Maldonado-López *et al.* 2019).

La AF es un indicador del estado de salud de los organismos (Zakharov *et al*. 1991; Zhelev *et al.* 2014) que detecta problemas en la población antes de que muestren evidencia de declive (Niemeier *et al.* 2019). Los anfibios presentan AF en condiciones de estrés ambiental como contaminación (Zhelev *et al*. 2014), cambios en la temperatura, pH, electroconductividad (Zhelev *et al*. 2014), acidificación (Söderman *et al.* 2007) y pérdida del hábitat (Delgado-Acevedo y Restrepo, 2008). Por ejemplo, la Rana Europea común, *Pelophylax ridibundus*, presenta altos niveles de AF de diez rasgos morfológicos, como el número de manchas en las extremidades y tiras en el dorso, asociados a la alta contaminación del hábitat por aguas residuales domésticas y metales pesados (Zhelev *et al.* 2019). La urbanización también induce AF en anfibios, *Physalaemus cuvieri* (Ranita ladradora) presenta mayores niveles de AF de los dígitos, en una población en una zona urbanizada, en comparación con un sitio conservado (Eisemberg y Bertoluci, 2016). Guo *et al*. (2017) encontró que *Bufo raddei* presentó mayores niveles de AF asociados a contaminación por metales pesados. En otro estudio, *Pelophylax ridibundus* (rana europea común) y *Pseudepidalea viridis* (sapo verde) mostraron mayores niveles de AF en rayas y manchas de la piel de las patas, dorso y la longitud corporal asociados a los altos niveles de perturbación antropogénica (Zhelev *et al.* 2014). *Eleutherodactylus coqui* presentó un aumento en la AF de la longitud radio-cúbito relacionada con la pérdida del hábitat (Delgado-Acevedo y Restrepo, 2008). *Pelophylax perezi* (rana común) muestra mayores niveles de AF en el húmero, el radio-cúbito, el metatarsiano y el tibio-peroné, asociados a la perturbación del hábitat (Niemeier, 2019).

3.3. *Alometría en anfibios*

Los anfibios pueden mostrar diferentes patrones de alometría, entendido como variaciones intraespecíficas en las relaciones morfométricas entre caracteres morfológicos y proporción de crecimiento corporal (Delgado-Acevedo y Restrepo, 2008; Gangenova *et al.* 2020). Las diferencias en los patrones alométricos se pueden detectar cuando se compara el tamaño de un rasgo con otro individuo de igual tamaño. La alometría positiva ocurre cuando los caracteres morfológicos tienen un mayor crecimiento que el tamaño corporal, mientras que la alometría negativa se asocia con un menor crecimiento de los caracteres morfológicos que el tamaño corporal (Fairbairn, 1994; Fairbairn, 1997; Fox *et al.* 2015).

Factores ambientales como una mayor radiación solar y diferencias en temperaturas pueden promover la variación en los patrones alométricos en los anfibios (Buckley *et al.* 2005; Delgado-Acevedo y Restrepo, 2008; Gangenova *et al.* 2020). En un metaanálisis, Tejedo *et al.* (2010) examinaron los patrones alométricos de la longitud de las patas traseras y la forma de la cabeza de los anfibios anuros post-metamórficos en relación con las señales ambientales. Sus resultados mostraron que los anuros aumentaron el desarrollo más que la tasa de crecimiento y mostraron cabezas más pequeñas en relación al tamaño general asociado a temperaturas más altas. De la misma manera, un incremento en la disponibilidad de recursos incrementó el crecimiento más que el desarrollo, con un aumento paralelo en la longitud de las patas traseras, pero sin cambios en la forma de la cabeza. Márquez-García *et al*. (2009) encontraron que los individuos de *Rhinella spinulosa* desarrollaron miembros posteriores más pequeños en estanques con altos niveles de desecación, con un efecto en los patrones alométricos sobre el diámetro del ojo, la longitud de la nariz a la boca y la longitud de las patas traseras.

4. Antecedentes

México representa uno de los puntos principales de biodiversidad o *hotspots* en el mundo (Myers *et al.* 2000; Ceballos *et al.* 2009; Alvarado-Díaz *et al*. 2013), con alrededor de 376 especies de anfibios, que incluyen 237 anuros, 140 salamandras y dos cecilidos, representando así a tres órdenes de anfibios, lo que posiciona a México en el quinto lugar mundial de biodiversidad de anfibios (Alvarado-Díaz *et al.* 2013; Parra-Olea *et al.* 2014).

Michoacán presenta una importante biodiversidad de anfibios, con 59 especies, debido a la gran diversidad climática, altitudinal y de vegetación, a consecuencia de la situación geográfica, dentro del área de confluencia de dos grandes regiones biogeográficas, Neártica y Neotropical (Huacuz -Elías, 2005; Alvarado-Díaz *et al.* 2013). Estas condiciones han permitido que nueve especies de salamandras se distribuyen a lo largo del estado de Michoacán, de los cuales seis son miembros de la Familia Ambystomatidae: *Ambystoma amblycephalum, Ambystoma andersoni, Ambystoma dumerilii, Ambystoma ordinarium, Ambystoma rivulare* y

Ambystoma velasci (Alvarado-Díaz *et al.* 2013). Los miembros de esta familia son considerados depredadores ápices en los sitios en los que habitan (Alvarado-Díaz *et al*. 2013; Soto-Rojas *et al.* 2017).

Específicamente, *Ambystoma dumerilii*, conocido comúnmente como Achoque, es una salamandra microendémica del lago de Pátzcuaro (Aguilar-Miguel, 2005). *Ambystoma dumerilii* es una especie de uso comercial y de gran importancia ecológica y cultural, que ha sido muy explotada y al igual que otras especies de la familia Ambystomatidae, es susceptible a las actividades antropogénicas (Huacuz, 2002; Cano-Martínez *et al*. 2007; Mendoza, 2015). Las condiciones del lago de Pátzcuaro se han deteriorado debido a la alta perturbación derivada de factores antropogénicos como cambio de uso de suelo, contaminación por aguas residuales, herbicidas y pesticidas, entre otros (Zambrano *et al.* 2011; Ramírez-Herrejón *et al.* 2014). Además, el lago de Pátzcuaro es un sistema lacustre cálido, poco profundo y de baja transparencia que se encuentra actualmente en un estado eutrófico por su alto contenido de material nitrogenado y fosforado (Mendoza, 2015; Tomasini *et al.* 2016). Ha disminuido su profundidad en 6 metros desde 1939 y presenta tasas de sedimentación de alrededor de 100,000 m^3 cada año (Ramírez-Herrejón *et al.* 2014). De acuerdo con Aguilar-Miguel (2005), *Ambystoma dumerilii* ha restringido su área de ocupación a menos de 10 km^2. A consecuencia de estos cambios, la población en vida silvestre ha disminuido de manera significativa, e incluso algunos autores proponen que el Achoque está cerca de la extinción (Zambrano *et al.*, 2011). *Ambystoma dumerilii* es considerada una especie en peligro crítico según la UICN (2020) y protegida bajo la categoría de Protección Especial por SEMARNAT (NOM-ECOL059-2010).

4.1. Estrategias de conservación de la especie

Como medida para la conservación de especies en peligro de extinción, se ha implementado la reproducción *ex situ* en zoológicos, acuarios, laboratorios de investigación, así como colecciones privadas (Zippel *et al.* 2011; Michaels, *et al*. 2014), con la intención de poder mantener poblaciones de estas especies que está en alto riesgo de extinción en el medio silvestre (Stuart *et al*. 2008; Amphibian Conservation Action Plan 2007; IUCN 2014). En 2006 se lanzó el Arca de los Anfibios (AArk), como parte del Plan de Acción para la Conservación de Anfibios (ACAP), como una estrategia de rescate, crianza y manejo de diversas especies (Zippel *et al*. 2011). En México, la reproducción en cautiverio de la familia Ambystomatidae ha sido empleada como una estrategia para su conservación por diversas instituciones, como el plan de Acción para la Conservación de las Especies de *Ambystoma* (PACE: *Ambystoma*). Este programa forma parte del eje de Conservación y Manejo de la Biodiversidad, de la Estrategia de la CONANP 2040, que expresa la importancia de "Desarrollar e implementar programas de acción para la recuperación de especies en riesgo, vinculados con los Programas de Manejo de ANP" (Semarnat, 2018). Existen diversos centros de crianza de especies del género *Ambystoma*, como el Instituto de Biología de la UNAM, el cual trabaja con *Ambystoma mexicanum* (Servín, 2011), el Centro de Investigaciones Biológicas y Acuícolas de Cuemanco dedicado a la reproducción masiva de distintas especies de *Ambystomas* para su conservación (Arana, 2016). *Ambystoma granulosum* y *Ambystoma lermaense* son reproducidas *ex situ* dentro del Centro de Investigación en Recursos Bióticos, UNAM (Aguilar-Miguel et. al. 2009). Para *Ambystoma dumerilii* se han desarrollado diferentes unidades de manejo de esta especie, como el Huacuz- Pátzcuaro, el cual actualmente cuenta con organismos reproductores, colección científica del laboratorio de biología de la UMSNH. Además, existen colecciones privadas, y se cuenta con la UMA Jimbani Erandi que pertenece a la comunidad de las monjas Dominicas, las cuales elaboran un jarabe de Achoque a través de un aprovechamiento sustentable (Velarde -Mendoza, 2012), y el PIMVS Jimbani Tzipekua en el municipio de Pátzcuaro, dedicado a la educación ambiental y el aprovechamiento comercial (Velarde -Mendoza, 2018).

4.2. *Problemática en cautiverio*

La crianza en cautiverio de anfibios es una buena alternativa para la conservación de las especies. Idealmente, las poblaciones en cautiverio deberían de ser mantenidas en condiciones controladas, para disminuir la exposición al estrés ambiental a niveles muy bajos o nulos (García-Feria *et al*. 2017). Sin embargo, son frecuentes las problemáticas asociadas al control de diversos parámetros en cautiverio, que pueden inducir estrés en los anfibios, dado que, al estar expuestos a condiciones no óptimas dentro del cautiverio, pueden estar bajo estrés crónico (O'Donnell *et al.* 2017). Uno de los principales problemas es la adaptabilidad de los organismos a las condiciones de cautiverio, dado que hay especies de salamandras que tienen requerimientos de microclima y microhábitat altamente especializadas, por lo que replicar estas condiciones en cautiverio es complicado, como es el caso de *Ambystoma cingulatum* (O'Donnell *et al.* 2017). Otro factor de gran relevancia es el mantenimiento de la temperatura apropiada para los organismos, evitando así enfermedades infecciosas por patógenos, como bacterias, parásitos y hongos como *Batrachochytrium dendrobatidis* (O'Donnell *et al.* 2017; García-Feria *et al*. 2017).

La calidad del agua es otro factor de estrés ya que, si no cuenta con un filtrado adecuado y recambio de manera continua, puede contener sustancias disueltas como amoniaco, urea o toxinas que son absorbidas por la superficie ventral de la piel y es una causa común de muerte en anfibios (Vosjoli 1999). El espacio físico es un factor importante, ya que de este depende que los organismos puedan realizar determinados procesos biológicos. Los espacios limitados y un mal manejo en el filtrado del agua implican estrés en los organismos, ya que aumentan la susceptibilidad a enfermedades. Un ejemplo es el síndrome de la salida de toxinas que provoca la absorción por la piel de sustancias disueltas en el medio acuático, como el amoniaco y la urea, entre otras toxinas que causan la muerte en anfibios de cautiverio (Vosjoli 1999). La alimentación deficiente, principalmente deficiencia de vitamina A, genera metaplasia epitelial en especies como *Gastrotheca cornuta, Hemiphractus fasciatus*, y *Hylomantis lemur* (Pessier *et al.* 2014), además de asociarse a la inmunosupresión dejándolos susceptibles a patógenos (Michaels, *et al.* 2014). Una alimentación inadecuada con bajo aporte nutricional tiene efectos negativos sobre el desarrollo. En un estudio en *Ambystoma mexicanum,* la dieta con un solo tipo de alimento rico en nutrientes, como lo es el alimento con gusanos de sangre, ocasionó un mayor crecimiento de los individuos, esto en comparación con las otras dietas con las cuales los organismos crecieron más lento y alcanzaron tasas de crecimiento medias como con *Daphnia* y con alimento mixto. En otras especies se ha reportado que el uso de alimentos mixtos o variados favorece el desarrollo de los organismos a través de tasas medias de crecimiento, también reportaron que la dieta con base en gusanos de sangre tiene un desarrollo acelerado con una tasa de crecimiento alta, sin embargo, no encontraron relación significativa en la influencia de su comportamiento (Slight *et al*. 2015).

Por lo tanto, nuestro objetivo fue analizar y comparar la morfología, asimetría fluctuante y patrones alométricos de *Ambystoma dumerilii* en individuos capturados del lago y criados en cautiverio. Nuestras preguntas fueron: 1) ¿Existe diferencia en la asimetría fluctuante de cabeza y cuerpo entre individuos de vida libre y de cautiverio?; 2) ¿Hay alguna variación en el tamaño corporal y de los caracteres morfológicos entre individuos de vida libre y de cautiverio?; 3) ¿Los patrones alométricos son distintos entre individuos de vida libre y de cautiverio?

5. Objetivo General

Analizar y comparar la morfología de *Ambystoma dumerilii* de una población del Lago de Pátzcuaro y una población en cautiverio.

6. Objetivos Particulares

1. Comparar las características morfológicas de los individuos de *Ambystoma dumerilii* de una población del Lago de Pátzcuaro y otra población en cautiverio.

2. Determinar la asimetría fluctuante de cabeza y cuerpo de individuos de *Ambystoma dumerilii* del Lago de Pátzcuaro y de cautiverio.

3. Determinar y comparar los patrones alométricos de los caracteres morfológicos de individuos de *Ambystoma dumerilii* del Lago de Pátzcuaro y de cautiverio.

5. Hipótesis:

Las características o condiciones en las que podemos encontrar a los organismos de *Ambystoma dumerilii* puede modificar su morfología, debido a que influyen en el óptimo desarrollo de los individuos. Y dado que en ambas condiciones estudiadas los organismos están sometidos a factores no óptimos, esperamos encontrar diferencias morfológicas representadas en el tamaño de diversos caracteres como en las extremidades y cabeza de los organismos, además esperamos encontrar niveles de asimetría fluctuante y patrones alométricos.

7. Capítulo 1

Morphological differentiation of *Ambystoma dumerilii* in captivity and wildlife conditions

Berenice Ramírez-López[1], Ireri Suazo-Ortuño[1], Luis H. Escalera-Vázquez[2] Omar Domínguez Domínguez[2], Yurixhi Maldonado-López[3].

[1]Instituto de Investigaciones sobre los Recursos Naturales, Universidad Michoacana de San Nicolás de Hidalgo, Avenida Juanito Itzícuaro SN, Nueva Esperanza, 58330 Morelia, Michoacán, México.

[2]Facultad de Biología, Universidad Michoacana de San Nicolás de Hidalgo, calle Gral. Francisco J. Múgica s/n A-1, Felícitas del Río, 58030 Morelia, Michoacán, México.

[3]CONACyT-Instituto de Investigaciones sobre los Recursos Naturales, Universidad Michoacana de San Nicolás de Hidalgo, Avenida Juanito Itzícuaro SN, Nueva Esperanza, 58330 Morelia, Michoacán, México

Corresponding author: yurixhimaldonado@gmail.com

Abstract

Ambystoma dumerilii, known as Achoque, is a microendemic salamander from Lake Pátzcuaro, considered as a critically endangered species according to the IUCN (2020) and it is under Special Protection by SEMARNAT (NOM-ECOL 059-2010), since the population in the wild has decreased significantly and is close to extinction. The main threats are high levels of water contamination, high levels of eutrophication in addition to the fact that invasive species can be found within the achoque habitat. For these reasons, an important conservation effort has been the maintenance of achoques in captivity. However, captivity is known to be a stressor derived from non-optimal conditions that can have important physiological consequences that are reflected in body conditions. Therefore, our objective was to analyze and compare the morphology of *A. dumerilii*, using different parameters such as morphological character sizes, geometric morphology, fluctuating asymmetry and allometry, in individuals from Lake Patzcuaro and from captivity. We found that individuals from the lake presented greater sizes in morphological characters and the SVL of captive individuals, while we find in almost all the traits that have a negative allometric relationship with the body size in individuals from the lake and captivity, so that the environmental conditions are favoring the growth of the SVL while the extremities develop more slowly, as well as differences in FA between lake and captive salamanders, being the lake organisms the ones that presented higher levels of FA, so this could be an indicator of the anthropogenic disturbance conditions to which they are subjected.

Keywords: fluctuating asymmetry, allometric patterns, *Ambystoma*, Achoque, captivity, wild life.

Introduction

The Ambystomatidae family is composed of the genus *Ambystoma*, with 35 species distributed from southern Canada and Alaska to the southern limit of the Mexican highlands (Casas-Andreu *et al.* 2003). In Mexico, there are 18 species of which 16 are classified in some category risk according to the NOM-059-Semarnat-2010 (Parra-Olea, *et al.* 2014). Specifically, *Ambystoma dumerilii,* known as Achoque, is a micro-endemic salamander from the Lake Pátzcuaro, which population is close to extinction (Zambrano *et al*. 2011), even is considered a critically endangered species according to the IUCN (2020) and under special protection by SEMARNAT (NOM-ECOL 059-2010). This species, like others of the Ambystomatidae family, is a very sensitive species to anthropogenic activities (Soto-Rojas *et al*. 2017), and its habitat has suffered a great deterioration due to land use change and water contamination (Zambrano *et al*. 2011). The land use of the lake basin has changed for agriculture, livestock production, forestry, as well as urban activities such as the discharge of untreated wastewater (Delgadillo *et al*. 2011; Ramírez-Herrejón *et al*. 2014).

The endemic nature of *A. dumerilii* and the critical status of the habitat, have led to bred this species in captivity as a conservation strategy (Huacuz, 2002; IUCN, 2020). Therefore, the maintenance of achoques in captive colonies have result of vital importance to enhance conservation efforts. However, captive populations can present morphological and physiological problems associated to long-term stress derived from non-optimal conditions (Michaels *et al*. 2014 Assis *et al.* 2015; Titon *et al.* 2017). As has been documented in other amphibian species, a wide range of stressors in captivity, such as a poor diet, inadequate habitat, and restricted movement (Morgan and Tromborg 2007, Burghardt, 2013) have (Titon *et al*. 2017).

Regardless of the stress source, amphibians’ response underlies phenotypic plasticity, shaping an organism's phenotype (Denver, 2009). Permanent stress source induces an overproduction of glucocorticoids, the hormones of stress, that affect negatively amphibians' growth and induce changes in their morphology (Davis and Maerz, 2010; Davis *et al.* 2018; Davis *et al.* 2019; Drew R *et al.* 2020; Gangenova *et al*. 2020), leading to permanent alterations in morphology (Brunson *et al.* 2001; Matthews, 2002; Hu *et al.* 2008). Morphological changes in amphibians occur during their ontogenetic development, from the larvae phase to adult stage (Shi *et al*. 1996; Steinicke *et al.* 2015), hence amphibians of the same species show different morphological forms, depending on the stress degree suffered during their development (Tejedo *et al*. 2010). Morphological changes derived from environmental stress involve different morphological parameters such as: 1) size and shape of morphological traits; 2) allometric patterns and; 3) fluctuating asymmetry.

Environmental stress decreased growth with smaller size at metamorphosis (Hayes,1997; Denver *et al.* 2002; Delgado-Acevedo and Restrepo, 2008; Henríquez *et al.* 2009; Cayuela *et al.* 2017; Iglesias-Carrasco *et al.* 2017). Changes in morphology (Goater, 1994; Denver *et al.* 1998; Relyea, 2003) and shape and size of morphological traits also varies in predictable ways in response to environmental stress (Morrison *et al*. 2004; Phillips *et al.* 2006), for example, amphibians can develop smaller hindlimbs in individuals presented in fragmented habitats (Buckley *et al.* 2005; Delgado-Acevedo and Restrepo, 2008; Steinicke *et al.* 2015).

Variation in morphological traits often scales with overall body size, defined as morphological allometry (Shaffer, 1984; Fairbairn, 1997). However, the degree of such correspondence can range from nearly perfect covariance of a trait with body size (i.e., isometry) to highly uncorrelated, where specific morphological traits change more or less rapidly with increasing body size (i.e., allometry). Positive allometry occurs when morphological characters have greater growth than body size, while negative allometry is associated with lower growth of morphological characters than body size (Fairbairn, 1997; Fox *et al*. 2015). Changes in allometric patterns can influence amphibians' fitness (Delgado-Acevedo and Restrepo 2008; Tejedo *et al.* 2010; Schulte-Hostedde *et al.* 2011). For example, in salamanders, the scaling relationships of head shape with body size have been related to larval diet and predation risk (Van Buskirk, 2011; Shaffery and Relye, 2015; Anderson *et al*. 2016). Positive allometric relationships between head and body size enhance frogs singing in sexual selection (Riva *et al.* 2020).

Stressful conditions induce changes during development that result in morphological asymmetry (Lens *et al.* 2002; Wright and Zamudio, 2002), such as fluctuating asymmetry (FA) (Zhelev *et al.* 2014) that measure of small, random deviations of bilateral symmetrical traits, reflecting developmental instability of the organisms. In disturbed or high stress environments, metamorphosis and growth can be accelerated (Lowe et al., 2006; Crump, 1989a), leading to higher FA (Møller and Manning, 2003). For example, *Pelophylax ridibundus* and *Pseudepidalea viridis* showed higher FA in sites with high levels of anthropogenic disturbance (Zhelev *et al*. 2014). In a similar study, *Pelophylax ridibundus* in highly contaminated sites, presented high levels of FA where they evaluated the levels of FA in ten morphological traits while individuals in uncontaminated sites presented FA in three morphological characters (Zhelev *et al*. 2015; Zhelev *et al*. 2019).

Understanding how the environment may shape morphological responses in amphibians is an important challenge for conservation strategies, providing important insights into the response capacity of this sensitive group (Lomolino *et al*. 2001). Due to the deterioration of the lake Patzcuaro and the stress induced in captivity, we expected to find physiological stress in individuals of *A. dumerilii* that can be reflected in their morphological traits. Our objective was to analyze and compare the *Ambystoma dumerilii* morphology with different parameters such as FA, geometric morphometry and allometric patterns, in individuals from the lake and captivity. We hypothesized that the anthropogenic disturbance present in the lake and the maintenance of optimal conditions in captivity would cause stress in the organisms, therefore, we expect to detect it using FA, with an increase in the condition where they are most stressed. Our results will allow us to know the conditions of the organisms in both populations, in addition to provide useful information for future plans for the reintroduction of this species to its habitat, and the improvement of captivity conditions.

Materials and methods

Sampling

We analyzed 107 individuals, 60 individuals from the lake and 47 from captivity. The captive organisms were sampled at the Regional Center for Fisheries Research in Pátzcuaro, which is a management unit for the conservation and sustainable use of wildlife. At this center, *Ambystoma dumerilii* is bred in captivity. The organisms are kept in a covered area that protects them from direct sunlight. Between 6 to 8 individuals are kept in oval recycled plastic tubs of approximately 180 liters, with artificial shelters, natural aquatic plants and hiding places made of PVC. Temperatures are maintained around 17°C ± 1°C. The achoques are fed with mixed diet with brine shrimp, fish fillet and tubifex (Tubificidae) and acociles (*Cambarellus patzcuarensis, Cambarellus montezumae*) (Huacuz, 2002; Valverde-Mendoza, 2018). The pH oscilate between 7.5 and 7.8. The salamanders sampled were adults between 10 and 15 1.5-year-old so they were adult organisms with a SVL greater than 60 mm.

The wild specimens were sampled in Lake Pátzcuaro that is located at the west of the volcanic plateau of central Mexico, at coordinates 19 ° 32 ′ N -19 ° 42 ′ N, 101 ° 32 ′ W -101 ° 42 ′ W. The samplings were carried out monthly from March 2019 to December 2020, using 180 traps of 50 by 40 cm in the form of cylinders whose entrance is conical and whose structure is metallic, each of the lines covers an area of 500 linear meters (Delgadillo *et al*. 2014; Ramírez-Herrejón *et al*. 2014). The traps are fixed at the bottom of the lake by the weight of the trap. To identify the organism collected in the lake, we use a microchip marking system, to avoid resampling. Adult individuals were classified with a minimum snout-vent length (SVL) of 60 mm (Anderson and Worthington, 1971).

Measurements

For all salamanders, we registered snout-vent length (SVL, mm) and total length (mm), using a digital caliper (Metaltex de 5 kg). The sex of each individual was determined based on the cloacal bulge. We used the snout-vent length (SVL) as a standard measure of body size (Gangenova *et al.* 2020). To analyze all morphological traits, we obtained a digital image from the dorsal part of each organisms with a high-resolution camera, making sure that the objective was always parallel and at the same distance. We also used a base to which we added millimeter paper to record the scale. These images were used for the morphometric measurements, FA and allometry (Alarcón-Ríos *et al.* 2017; Soto-Rojas *et al*. 2017).

Morphological traits

To determine differences in body shape between individuals from captivity and lake conditions, we used the photography to measured the following morphometric traits: Eye to Eye Distance (EED), Head Width (HW), Head length (HL), Body Width (BW), Total length (TL), Tail Width (TW), Tail Length (TLe), Femur length right and left (FLR, FLL), Tibia-Fibula length right and left (TFLR, TFLL), Radius-Ulna Length right and left (RULR, RULL), Humerus length right and left (HLR, HLL) (Figure 1A). The sizes were measured with the software ImagenJ program 1.44.

Geometry Morphometric

In order to detect the differences in body morphology and size between individuals that occurs in the lake and captivity conditions, we used geometric morphometric techniques (Vega-Trejo et al., 2014). In each digital image, we put 22 landmarks along corporal shape and two additional landmarks over the centimeter as size reference (Figure 1A). Each set of landmarks indicates the body shape and corresponds to homologous loci, which are repeatable and unambiguous in all individuals (Bookstein, 1991; 1997). To digitalize the coordinates of 22 landmarks of each individual, we employed the TpsDig program (Rohlf,and Slice 1991; Rohlf, 2015), and then, a Procrustes superimposition analysis was performed using the Integrated Morphometrics Package (IMP series: http://www.canisius.edu/~sheets/morphsoft.html) to align the landmark coordinates and eliminate size effect (Vega-Trejo et al. 2014). Later, the mean configuration of all individuals for condition was considered as reference of shape variables (Procrustes distances) and calculated by a superimposition coordinates analysis (Cuevas-Reyes et al. 2018). Finally, we applied a canonical variate analysis (CVA) to evaluate the differences between from lake and captivity conditions, considering the configuration of all individuals (Cuevas-Reyes et al. 2011).

Allometry

To evaluate allometric relationships between body size and all morphological traits, we used the SLV as standard measure of body size and compared between captivity and lake conditions (Delgado-Acevedo and Restrepo 2008; Zhelev *et al*. 2014). Morphological measurements were analyzed using a normalization technique to scale data and remove allometric effects (Lleonart *et al.* 2000). This method, based on theoretical equations of allometric growth, adjusts the shape considering allometry and scales all individuals to the same size. The absolute values of morphometric characters were standardized as proposed by Lleonart *et al*. (2000). The absolute measurements were converted by using the equation: $Yi^* = Yi\ (SVL_0/SVLi)^b$, Yi* is the size-adjusted proportion character of specimen i; Yi is the body character; SVL_0 is the mean value of SVL at which all characters are adjusted; SVLi is the SVL of specimen i; b is the within-habitat treatment regression slope of log (Y) against log (SVL). All variables were log transformed. We evaluated the effect of lake and captivity on SVL and its allometric relationship with morphological characters, applying an ordinary least squares regression between snout-vent length (x-axis: mm) and size of morphological characters (y-axis: mm) for individuals from the lake and captivity. We calculated the allometric slope for each regression. For those variables in which a correlation was identified, we applied an analysis of covariance (ANCOVA) to test the difference in regression slopes between individuals from the lake and captivity.

We used a scaled morphological database to analyze differences in morphological characters between both conditions, using a one-way ANOVA test to determine the lake and captivity effects on each morphological character. Individuals from the lake and captivity were considered as an independent variable and morphological characters as dependent variables. We used an analysis of variance (ANOVA) to determine the differences in FA levels between individuals from the lake and captivity. In all cases, the normality was tested after suitable transformations.

Fluctuating asymmetry

With the digital image obtained for each individual, we measured FA of abdomen and head (Figure 1B). Fluctuating asymmetry was calculated as the absolute value of the difference among the distances from the middle to the left and right margins of the body part (|Ai – Bi|), divided by the average distance (*Ai* + *Bi* / 2), to correct for the fact that asymmetry may be size-dependent. Additionally, 10 individuals were blindly re-measured, without reference to previous measurements to control the measurement error in FA. We then evaluate the degree of significance of FA relative to measurement error using two-way mixed-model ANOVA. The significance of the interaction (individual × body part × side) indicated that variation in FA was greater than expected by measurement error: ($F_{9,25} = 22.4$; $P < 0.002$).

Fluctuating asymmetry is found when the Right-minus-Left differences are normally distributed with a mean value of zero, unlike directional asymmetry that is found when the R-minus-L differences are also normally distributed, but with a mean significantly different from zero, and antisymmetry, characterized by a platykurtic or bimodal distribution of R-minus-L differences about a mean of zero (Palmer and Strobeck, 1986). To determine whether our data fitted only FA and no other types of asymmetry, we performed a Student's t test and Lilliefors' normality test to evaluate whether mean values of signed right-minus-left values differed significantly from zero. We found that R-minus-L measurements did not differ from zero ($t = 1.1$; $P > 0.05$), and therefore, we discarded the presence of directional asymmetry in our data. In the same way, we also reject the presence of antisymmetry because our data (R-minus-L) exhibited a normal distribution ($P > 0.05$).

Once determined that our data fitted only in FA criterium, we used an analysis of variance (ANOVA) to determine the differences in FA levels between individuals that occur in the lake and captivity condition. In all cases, the normality was tested after suitable transformations.

Results

Morphometric differences

We found differences in morphological characters between both populations, with individuals from the lake with greater head width, head length, total length, tail width, tail length, femur length of left side, tibia-fibula length of left side, radius-ulna length of right and left sides, humerus length of right and left sides, radius-ulna length of left and right side and SVL than individuals from captivity. In the case of body width, femur length right, tibia-fibula length right we didn`t find differences (Table 1).

Based on a coordinate superimposition analysis, we found differences in body shape between individuals of the lake and captivity (Figure 2A). Two well-segregated groups were formed with lake and captive individuals in our PCA analysis (Figure 2A). The wireframe graph based on Procrustes coordinates shows that body shape of individuals from the lake were thinner than individuals from captivity (Figure 2B). This difference in body shape between individuals from captivity and lake is supported by discriminant analysis, where both distances (values) of Mahalanobis (3.95) and Procrustes (0.046) were significant (P = 0.0001).

Allometry

We observed significant allometric relationships in individuals of *A. dumerilii* from the lake and captivity. We found that almost all traits showed a negative allometric relationship with body size. In the case of tail width, radius-ulna length of left side, fibula length of left size showed an isometric relationship with body size. Femur length of left size showed a positive relationship (Table 2).

ANCOVA analyses showed significant differences between slopes in some allometric relationships of both habitat conditions. In individuals from the lake, the slopes of the allometric equations between body size and humerus length of right size and radius-ulna length of right side were higher than in individuals from captivity (HLR: F = 77.6; P = 0.0001. RULR: F = 105.9; P = 0.0001). In the rest of traits, the slopes of the allometric equations were higher in individuals from captivity (HW: F = 10.68; P = 0.0001. HL: F = 4.1; P = 0.0001. BW: F = 112.7: P = 0.0001. TL: F = 47.6; P = 0.0001. TW: F = 62.23; P = 0.0001. TLe: F = 115.5: P = 0.0001. FLR: F =41.02: P = 0.0001. TFLR: F = 28.7: P = 0.0001. FLL: F = 53.29 P = 0.0001. TFLL: F = 40.89: P = 0.0001).

Fluctuating asymmetry

We found differences in FA between salamanders from the lake and salamanders from captivity conditions. Our results show higher FA in the body (F=27.9, d.f.=1, P = 0.0001) and head (F= 47.1, d.f.= 1, P= 0.067) of individuals sampled in the lake.

Discussion

Although captivity is a necessary strategy to face imminent extinctions of endangered species such (Michaels *et al.* 2014a, 2014b). Morphological changes in amphibians occur during their ontogenetic development, from the larvae phase to adult stage (Shi *et al.* 1996; Steinicke *et al.*

2015), hence amphibians of the same species can show different morphological forms, depending on the stress degree suffered during their development (Tejedo *et al.* 2010). Our results showed that individuals from the lake are bigger, have higher FA and the slopes of allometric correlations of almost all traits were lower in the lake, suggesting that SVL increases faster according to morphological characters than in captivity. Individuals from captivity present all morphological characters shorter and narrower than individuals from the lake.

In our study, individuals from the lake are bigger than individuals from captivity. This result is the opposite of what would be expected if we hypothesize that the conditions in the lake were more stressful than captivity, and amphibians exposure to environmental stressors decreased their growth rate of main morphological traits such as body size and body traits size (Delgado-Acevedo and Restrepo, 2008; Tejedo *et al.* 2010). However, *A. dumerilii* do not show a decrease in size, since the bigger individuals and higher FA occurs in individuals from the lake. To explain the relationship between growth and FA, it has been hypothesized that a favorable environment, such as greater availability of foods with higher nutrients (Milligan *et al*. 2008), allow a faster growth of organisms, prompting higher developmental instability and FA levels (Hochwender and Fritz, 1999, Lempa *et al.* 2000, Martel *et al.* 1999). The main reason is that there are trade-offs between growth rate and life-history traits such as developmental stability (Sibly and Calow, 1984), and within species, different genotypes in certain environments need to change from optimizing growth rate to optimizing developmental quality. Therefore, we can expect higher FA in individuals of *A. dumerilii* that reach an optimal growth rate for their development in lake conditions, showing that fluctuating asymmetry might not be an unequivocal indicator of environmentally induced stress, since other factors can be involved, such as genetic stress or growth rate (Milligan *et al.* 2008, Velickovic and Perisic, 2006).

An important factor may be the resource availability that can lead to larger body size (Jessop *et al*. 2006, Wu *et al.* 2006). *Ambystoma dumerilii* has a high degree of trophic specialization, and consumes mainly *Cambarellus* species, an abundant resource in lake Patzcuaro (Huacuz, 2008), but also crustaceans, insects, worms, small fish and tadpoles (Aguilar-Miguel, 2005; Velarde-Mendoza, 2012; SEMARNAT, 2018). In captivity, a diet composed of mosquito larvae, Daphnia, rotifers, earthworms, brine shrimp, tubifex, pieces of fish fillet and mixed feed has been reported (Slight *et al*. 2015; Valverde-Mendoza, 2018), but specifically in captivity, *A. dumerilii* organisms feed on fish fillets, earthworms, tubifex, and occasionally feed on acociles. This is an important point to consider since it has been reported that the type of feeding has an influence on the growth and development of organisms, so it could be one of the factors that influence the differences found in both conditions, since have compared types of diets such as mixed resulting in a slower growth rate than a bloodworm-only diet, and it appears that for captive aquatic species, an unvarying but good-quality diet is a better choice , since mixed diets are better is not a universal truth (Sligth *et al.* 2015). Although there is a lack of knowledge about the nutritional requirements, available information shows that diet supplementation has a positive impact on amphibian health (Livingston *et al*. 2014; Berner *et al.* 2013). Food restriction induces whole body corticosterone production (Crespi and Denver, 2005; Denver *et al.* 2009) and, consequently, amphibians develop small body size (Peacor and Pfister 2006; Krause *et al.* 2011). On the other hand, when food is abundant, organisms suffer low stress and common phenotypic traits are shown, such as growth rates (Metcalfe and Monaghan 2001; Hector *et al.* 2011).

Morphology of amphibians is directly related to movement, locomotion ability and individual's performance (Aubret and Shine, 2008; Irschick and Garland, 2001; Ijspeert and Cabelguen, 2006). In our study, some traits are correlated with the fast-swimming performance in salamanders, necessary to escape from predation but also to capture prey (Van Buskirk and Schmidt, 2000; Urban, 2010). For example, tail morphology is known to have an impact on the locomotor performance while swimming (Buskirk and Schmidt, 2000; Vorndran *et al.* 2002), large tails are adaptations for rapid acceleration (Duellman and Trueb, 1986), and are associated to the response to chemical predator cues (Van Buskirk and McCollum, 2000). A greater tail surface area can also generate greater thrust, so a greater stride can be achieved (Aubret and Shine, 2008; Hawkins and Quinn, 1996). The morphology of *Ambystoma* species is adapted to have larger tails but also larger heads to achieve greater acceleration ability, high-acceleration bursts and high velocity swimming (Hoff *et al*., 1989). Head width is positively related to propulsive performance (Fitzpatrick *et al.* 2003) and may serve as an important stabilizing function. We found larger and wider tails in individuals from the lake, suggesting that these individuals have developed greater capacity of movement.

Individuals of *A. dumerilii* found in captivity may not develop traits associated with movement, as individuals from the lake due to the effect of the size of the enclosure. Aquatic species have elongated bodies because this species may use their whole trunk for swimming using a posterior traveling wave along their bodies (Deban and Schilling 2009). Aquatic salamanders have an undulatory swimming gait with limbs tucked against the body (Frolich and Biewener, 1992). However, this species also uses their limbs for aquatic walking on the substrata (Azizi and Horton 2004). In the case of *A. dumerilii* it can move towards the water column, however, by being considered epibentonic, it can move swimming or with its limbs (Aguilar-Miguel, 2005 en:

Natiralista, 2021; Montes-Calderón, Alvarado-Días and Suazo-Ortuño, 2011). Our results show that lacustrine organisms have larger limbs so, as in other species of the *Ambystoma* gender, it has been reported that some species like *A. ordinarium* have a displacement between 4 and 20 meters (Aguilar-Miguel, 2005 in Natiralista, 2021; Montes-Calderón, Alvarado-Días and Suazo-Ortuño, 2011; Escalera-Vázquez *et al.* 2018), while *A. maculatum* has a range of 3.3 to 29.4 m (Duellman and Trueb 1994), that match with the displacements of another salamander that have an average distance of 10m2 (Duellman and Trueb, 1994). So that *A. dumerilii* could show similar ranges of species. On the other hand, in captivity there are limitations of artificial conditions (Alvarez and Nicieza, 2002; Altwegg and Reyer, 2003; Relyea and Hoverman, 2003), where they have limited spaces, they are generally simple, without competitors or predators of other species. Therefore, it is very difficult to replicate natural abiotic conditions (Essner and Suffian, 2010). In this way, Ambystoma salamanders perceive captivity as "mildly stressful" (Davis et al. 2011), however they show smaller sizes due to suboptimal environments and present less locomotive capacity due to the limited space available to purchase from Lake organisms that may have greater displacement, greater susceptibility to hunger, and higher mortality when raised in conditions where resources are limited (Alvarez and Nicieza, 2002; Altwegg and Reyer, 2003; Relyea and Hoverman, 2003).

Variation in morphological traits scales with body size, ranging from the perfect covariance of a trait with body size (isometry) to highly uncorrelated, where morphological traits grow more slowly than body size (negative allometry), and when morphological traits grow faster is called positive allometry (Fairbairn 1994; Fairbairn 1997). The ontogenetic allometry is the source of morphological variation during the growth process (Murta-Fonseca *et al*. 2020). In our study, allometric relationship between SVL and morphological traits were highly consistent, with most of the morphological characters showed negative allometric patterns or hypoallometry in many traits and the slopes of allometric correlation of many traits were lower in salamanders from the lake than captivity. We suggest that these results are related to environmental conditions in the lake, that promotes lower development rates of these traits over the growth. Therefore, it is possible that such traits were affected in their growth when the development rates were more promoted than growth rates (Tejedo *et al*. 2010).

It is important to mention that although we not measure individual's fitness and performance, all variations in phenotypic traits such as body size and body proportions, affect performance. Amphibians with smaller body size are associated with reduced survival (Altwegg and Reyer 2003) and may be more dehydrate compared with bigger individuals (Beck and Congdon

2000; Gray and Smith 2005). It is important to ask if individuals in captivity are functionally similar to their wild counterparts (Calisi and Bentley, 2009; Fariaa *et al.* 2010).

The suboptimal environmental conditions can lead to stress that affects the health of the organisms and modifies their morphology as well as their behavioral and physiological (Denver, 1997b), the captivity centers are designed to replicate the wild environmental parameters, in order to maintain to animals in good long-term health and potentially improve the fitness of these organisms, on occasions in order to seek their reintroduction (Izquierdo et al. 2001; Davis et al. 2011), so our study could help to rethink the conditions in which the organisms are found, since negative allometric patterns were found in relation to the extremities, which plays an important role for their fitness, in addition to AF levels that despite being lower than those found in the lake are an indication of stress.

Since different populations of the same species are not in the same conditions, the inclusion of morphological diversity data in biodiversity conservation seems to be an important developing strategy for reducing biodiversity losses under global change (Des Roches *et al.* 2018). We highlight the importance of conducting studies of morphology in Ambystomas to know how novel environments influence their morphological traits and try to understand the role of the expression of different phenotypes in the ecological context.

Literature

A. C. M., and Gomes, F. R. (2017). Captivity effects on immune response and steroid plasma levels of a Brazilian toad (Rhinella schneideri). Journal of Experimental Zoology A, 327(2-3), 127–138

Aguilar-Miguel, X. y Casas-Andreu, G. (2005). Ambystoma dumerilii. Algunas especies de anfibios y reptiles contenidos en el Proyecto de Norma Oficial Mexicana PROY-NOM-059-ECOL-2000. Facultad de Ciencias, Centro de Investigación en Recursos Bióticos, Universidad Autónoma del Estado de México. Recuperado el 20 de agosto, 2020 de: Bases de datos SNIBCONABIO. Proyecto W035. México. D.F. Recuperado de: http://www.conabio.gob.mx/conocimiento/ise/fichasnom/Ambystomadumerilii00.pdf

Alarcón-Ríos, L., Velo-Antón, G., and Kaliontzopoulou, A. (2017). A non-invasive geometric morphometrics method for exploring variation in dorsal head shape in urodeles: sexual dimorphism and geographic variation in Salamandra salamandra. Journal of morphology, 278(4), 475-485.

Altwegg, R., Reyer, H. U. (2003). Patterns of natural selection on size at metamorphosis in water frogs. Evolution 57, 872–882.

Alvarez, D., Nicieza, A.G. (2002). Effects of induced variation in anuran larval development on postmetamorphic energy reserves and locomotion. Oecologia 131, 186–195.

AmphibiaWeb. (2019). *Ambystoma dumerilii*: Lake Pátzcuaro Salamander. University of California, Berkeley, CA, EE. UU. Recuperado el 13 de enero, 2021 en: http://amphibiaweb.org/species/3831.

Anderson, J. D., and Worthington, R. D. (1971). "The life history of the Mexican salamander *Ambystoma ordinarium* Taylor." Herpetologica, 27, 165-176.

Anderson, T. L., Ousterhout, B. H., Drake, D. L., Burkhart, J. J., Rowland, F. E., Peterman, W. E., and Semlitsch, R. D. (2016). Differences in larval allometry among three ambystomatid salamanders. Journal of Herpetology, 50(3), 464-470.

Assis, V. R., Titon, S. C. M., Barsotti, A. M. G., Titon, B., Jr., and Gomes, F. R. (2015). Effects of acute restraint stress, prolonged captivity stress and transdermal corticosterone application on immunocompetence and plasma levels of corticosterone on the cururu toad (*Rhinella icterica*). PLoS One, 10(4), e0121005.Recuperado el 12 de julio, 2020 de: https://doi.org/10.1371/journal.pone.0121005

Aubret, F., and Shine, R. (2008). The origin of evolutionary innovations: locomotor consequences of tail shape in aquatic snakes. Functional Ecology, 22(2), 317-322.

Azizi, E. and Hortpn, J. M. (2004). Pattern of axial and appendicular movements during aquatic walking in the salamander Siren lacertina. Zoology 107: 111–120.

Beck, C. W., & Congdon, J. D. (2000). Effects of age and size at metamorphosis on performance and metabolic rates of Southern Toad, *Bufo terrestris*, metamorphs. Functional Ecology, 14(1), 32-38.

Berner, Nancy J., Heil, Leslie A., and Romero, L. Michael Source. (2013). Seasonal Variation in Corticosterone in Free-Living and Captive Eastern Red-spotted Newts *Notophthalmus viridescens viridescens*. Journal of Herpetology, 47(3): 466-470

Blaustein, A. R., Han, B. A., Relyea, R. A., Johnson, P. T., Buck, J. C., Gervasi, S. S., and Kats, L. B. (2011). The complexity of amphibian population declines: understanding the role of cofactors in driving amphibian losses. Annals of the New york Academy of Sciences,1223 (1), 108-119.

Bookstein, F. L. (1991). Morphometric tools for landmark data: geometry and biology. Biometrical journal, New York, US. 35, 512-512.

Bookstein, F. L. (1997). Landmark methods for forms without landmarks: morphometrics of group differences in outline shape. Medical image analysis, 1(3), 225-243.

Bower, Deborah S, Karen R Lips, and Arthur Georges. (2017). “Devastating Fungal Diseases” Science, 357 (6350): 454–56.

Brodeur, J. C., Bahl, M. F., Natale, G. S., and Poliserpi, M. B. (2020). Biomarker and hematological fieldwork with amphibians: is it necessary to sample all night?. Environmental Science and Pollution Research, 27(14):17152-17161.

Brunson, K.L., Avishai-Eliner, S., Hatalski, C.G., Baram, T.Z. (2001). Neurobiology of the stress response early in life: evolution of a concept and the role of corticotropin releasing hormone. Molecular Psychiatry, 6, 647–656.

Buckley, C. R., Michael, S. F., & Irschick, D. J. 2005. Early hatching decreases jumping performance in a direct-developing frog, Eleutherodactylus coqui. Functional ecology, 67-72.

Burghardt, G. M. (2013). Environmental enrichment and cognitive complexity in reptiles and amphibians: Concepts, review, and implications for captive populations. Applied Animal Behaviour Science, 147(3-4), 286-298.

Calisi, R. M., and G. E. Bentley. (2009). Lab and field experiments: are they the same animal? Hormones and Behavior 56:1–10.

Casas-Andreu, G. Aviña, R. C., and Miguel, X. A. (2003). Un regalo poco conocido de México al mundo: el ajolote o axolotl (Ambystoma: Caudata: Amphibia). Con algunas notas sobre la crítica situación de sus poblaciones. CIENCIA ergo-sum, 10(3), 304-308.

Cayuela, H., Quay, L., Dumet, A., Léna, J. P., Miaud, C., and Rivière, V. (2017). Intensive vehicle traffic impacts morphology and endocrine stress response in a threatened amphibian. Oryx, 51(1), 182-188.

Cayuela, H., Valenzuela-Sánchez, A., Teulier, L., Martínez-Solano, Í., Léna, J. P., Merilä, J., ... and Schmidt, B. R. (2020). Determinants and consequences of dispersal in vertebrates with complex life cycles: a review of pond-breeding amphibians. The Quarterly Review of Biology, 95(1), 1-36.

Cohen, Jeremy M., Taegan A. McMahon, Chloe Ramsay, Elizabeth A. Roznik, Erin L. Sauer, Scott Bessler, David J. Civitello, et al. (2019). "Impacts of Thermal Mismatches on Chytrid Fungus *Batrachochytrium dendrobatidis* Prevalence Are Moderated by Life Stage, Body Size, Elevation and Latitude." Ecology Letters, 22 (5): 817–25.

Comisión Nacional de Áreas Naturales Protegidas. (2018). Programa de Acción para la Conservación de las Especies Ambystoma spp, SEMARNAT/CONANP, (pp. 26-27), México.

Cuevas-Reyes, P., Canché-Delgado, A., Maldonado-López, Y., Fernandes, G. W., Oyama, K., and González-Rodríguez, A. (2018). Patterns of herbivory and leaf morphology in two Mexican hybrid oak complexes: Importance of fluctuating asymmetry as indicator of environmental stress in hybrid plants. Ecological Indicators, 90, 164–170.

Cuevas-Reyes, P., Oyama, K., González-Rodríguez, A., Fernandes, G. W., and Mendoza-Cuenca, L. (2011). Contrasting herbivory patterns and leaf fluctuating asymmetry in *Heliocarpus pallidus* between different habitat types within a Mexican tropical dry forest. Journal of Tropical Ecology, 27(4), 383-391.

Cuevas-Reyes P, Gilberti L, González-Rodríguez A, Fernandes G. W. (2013). Patterns of herbivory and fluctuating asymmetry in *Solanum lycocarpum* St. Hill (Solanaceae) along an urban gradient in Brazil. Ecol Indic 24:557–561.

Crespi E, Denver R. (2005). Roles of stress hormones in food intake regulation in anuran amphibians throughout the life cycle. Comparative Biochemistry and Physiology, Part A: Molecular and Integrative Physiology. 141, 381–390.

Crump, M. L. (1989a). Effect of habitat drying on developmental time and size at metamorphosis in *Hyla pseudopuma*. Copeia, 3, 794-797.

Davis, D. R., Ferguson, K. J., Schwarz, M. S., and Kerby, J. L. (2019). Effects of agricultural pollutants on stress hormones and viral infection in larval salamanders. Wetlands, 1-10.

Davis, A. K., and Maerz, J. C. (2008). Comparison of hematological stress indicators in recently captured and captive paedomorphic mole salamanders, *Ambystoma talpoideum*. Copeia 3, 613-617.

Davis, A. K., and Maerz, J. C. (2010). Effects of exogenous corticosterone on circulating leukocytes of a salamander (*Ambystoma talpoideum*) with unusually abundant eosinophils. International Journal of Zoology, 2010, 1-8.

Davis A. K. and Maney D. L. (2018). The use of glucocorticoid hormones or leucocyte profiles to measure stress in vertebrates: What's the difference?. Methods in Ecology and Evolution, 9. 1556-1568. DOI: 10.1111/2041-210X.13020

Davis, A. K., D. L. Maney, and J. C. Maerz. (2008). The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. Functional Ecology 22:760–772.

Davis, Andrew K., and Maerz, John C. (2011). Assessing Stress Levels of Captive-Reared Amphibians with Hematological Data: Implications for Conservation Initiatives Journal of Herpetology, 45(1): 40-44.

Delgado-Acevedo J., Restrepo C. (2008). The contribution of habitat loss to changes in body size, allometry, and bilateral asymmetry in two Eleutherodactylus frogs from Puerto Rico. Conservation Biology, 22: 773-782, DOI: 10.1111/j.1523-1739.2008.00930.

Deban, S. M. and Schilling, N. (2009). Activity of trunk muscles during aquatic and terrestrial locomotion in *Ambystoma maculatum*. J. Exp. Biol. 212: 2949–2959.

Delgadillo H, R., and Vargas Velázquez, S. (2014). Estudio ecosistémico del lago de Pátzcuaro: aportes en gestión ambiental para el fomento del desarrollo sustentable. Instituto Mexicano de Tecnología del Agua, Comisión Nacional del Agua, Secretaría de Medio Ambiente y Recursos Naturales. Jiutepec, Mor. 2, 1-204.

Demori, I., El Rashed, Z., Corradino, V., Catalano, A., Rovegno, L., Queirolo, L.,Salvidio S, Biggi E, Zanotti-Russo M, Canesi L, Catenazzi A and Grasselli, E. (2019). Peptides for skin protection and healing in amphibians. Molecules, 24(2), 347.

Denver, R.J. 1997b. Environmental stress as a developmental cue: corticotropin-releasing hormone is a proximate mediator of adaptive phenotypic plasticity in amphibian metamorphosis. Hormones and behavior 31, 169–179.

Denver RJ. (1998). Hormonal correlates of environmentally induced metamorphosis in the Western spadefoot toad, *Scaphiopus hammondii*. Gen Comp Endocrinol 110:326–36.

Denver RJ. (2009). Stress hormones mediate environment-genotype interactions during amphibian development. Gen. Comp. Endocrinol. 164, 20 – 31. doi:10.1016/j.ygcen.2009.04.016

Des Roches S, Post DM, Turley NE, Bailey JK, Hendry AP, Kinnison MT, Schweitzer JA, Palkovacs EP. (2018). The ecological importance of intraspecific variation. Nature Ecology and Evolution 2:57–64. DOI: 10.1038/s41559-017-0402-5.

Didde, R. D., and Rivera, G. (2019). Patterns of fluctuating asymmetry in the limbs of anurans. Journal of morphology, 280(4), 587-592.

Drew R. Davis, D. R. Davis, Katie J. Ferguson, K. J. Ferguson, Matthew S. Schwarz, M. S. Schwarz, and Jacob L. Kerby, J. L. Kerby. (2020). Effects of Agricultural Pollutants on Stress Hormones and Viral Infection in Larval Salamanders. Wetlands, 40, 577-586.

Duellman, W. E., and Trueb, L. (1994). Biology of amphibians. JHU press.

Faira J.M, Breshears D. D. (2005). Drought stress and fluctuating asymmetry in Quercus undulata leaves: confounding effects of absolute and relative amounts of stress?. Journal of Arid Environments 62, 235–249.

Fairbairn, D. J., and R. F. Preziosi. (1994). Sexual selection and the evolution of allometry for sexual size dimorphism in the water strider, Aquarius remigis. American Naturalist 144,101–118

Fairbairn, D. J. (1997). Allometry for sexual size dimorphism: pattern and process in the coevolution of body size in males and females. Annual Review of Ecology and Systematics 28, 659–687.

Fitzpatrick Benjamin M., Michael F. Benard, and James A. (2003). Fordyce Morphology and Escape Performance of Tiger Salamander Larvae (*Ambystoma tigrinum mavortium*) Journal of Experimental Zoology 297A, 147–159.

Frolich, L. M., and Biewener, A. A. (1992). Kinematic and electromyographic analysis of the functional role of the body axis during terrestrial and aquatic locomotion in the salamander *Ambystoma tigrinum*. Journal of Experimental Biology, 162(1), 107-130.

Fox, G. A., Cooper, A. M., and Hayes, W. K. (2015). The dilemma of choosing a reference character for measuring sexual size dimorphism, sexual body component dimorphism, and character scaling: cryptic dimorphism and allometry in the scorpion *Hadrurus arizonensis*. PLoS One, 10(3), 1-23.

Escalera-Vázquez, L. H., Hernández-Guzmán, R., Soto-Rojas, C., and Suazo-Ortuño, I. (2018). Predicting *Ambystoma ordinarium* habitat in central Mexico using species distribution models. Herpetologica, 74(2), 117-126.

Essner Jr, R. L., and Suffian, D. J. (2010). Herpetological Husbandry Captive Husbandry in the Rocky Mountain Tailed Frog, *Ascaphus montanus*. Herpetological Review, 41(2), 181.

Gangenova, E., Giombini, M. I., Zurita, G. A., and Marangoni, F. (2020). Morphological responses of three persistent native anuran species after forest conversion into monoculture pine plantations: tolerance or prosperity?. Integrative zoology, 15(5), 428-440.

García-Feria, L. M., Hernández-Jauregui, D. M. B., Bravo, D. V., and Olivares, R. A. C. (2017). El comercio de anfibios y la presencia de *Batrachochytrium dendrobatidis* en vida libre:¿ dispersión en círculo vicioso?. Neotropical Biology and Conservation, 12(1), 30-36.

Griffin A. S., Blumstein D. T. and Evans C. S. (2000). Training Captive-Bred or Translocated Animals to Avoid Predators. Conservation Biology. 14(5), 1317-1326.

Gilligan D. M. and Frankham R. (2003). Dynamics of genetic adaptation to captivity. Conservation Genetics, 4, 189-197.

Gondim, P. D. M., Rodrigues, J. F. M., and Cascon, P. (2020). Fluctuating asymmetry and organosomatic indices in anuran populations in agricultural environments in semi-arid brazil. Herpetological Conservation and Biology, 15(2), 354-366.

Gordon M. Burghardt. (2013). Environmental enrichment and cognitive complexity inreptiles and amphibians: Concepts, review, and implicationsfor captive populations. Applied Animal Behaviour Science 147: 286– 298

Gastón, M. S., Pereyra, L. C., and Vaira, M. 2019. Artificial light at night and captivity induces differential effects on leukocyte profile, body condition, and erythrocyte size of a diurnal toad. Journal of Experimental Zoology Part A: Ecological and Integrative Physiology, 331(2), 93-102.

Grenyer R., Orme C. D., Jackson S. F., Thomas H., Davies, R. G., Davies T J., Jones K. E., Olson V. A., Ridgely R. S., Rasmussen P. C., Ding Tzung-su., Bennett P. M., Blackburn Tim M., Gaston, K. J., Gittleman, J. L., Owens, Ian P F. (2006). Global distribution and conservation of rare and threatened vertebrates. Nature. 444(7115), 93-96.

Gray, M. J., and Smith, L. M. (2005). Influence of land use on postmetamorphic body size of playa lake amphibians. The Journal of wildlife management, 69(2), 515-524.

Guo, Rui, Wenya Zhang, Shiwei Ai, and Liang Ren. (2017). "Fluctuating Asymmetry Rather than Oxidative Stress in *Bufo Raddei* Can Be an Accurate Indicator of Environmental Pollution Induced by Heavy Metals." 189(6), 1-10. https://doi.org/10.1007/s10661-017-5991-6

Hallgren P., Martensson L. and Mathiasson L. (2009). Improved spectrophotometric vitellogenin determination via alkalilabile phosphate in fish plasma-a cost effective approach for assessment of endocrine disruption. International Journal of environmental analytical chemistry. 89(14): Pp. 1023-1042.

Hayes TB. (1997). Steroids as potential modulators of thyroid hormone activity in anuran metamorphosis. Am Zool 37: 185–194.

Hector KL, Bishop PJ, Nakagawa S. (2011). Consequences of compensatory growth in an amphibian. J Zool 286:93–101.

Hernández-Guzmán, R., Escalera-Vazquez, L. H., and Suazo-Ortuno, I. (2019). Predicting *Ambystoma ordinarium* distribution under different climate scenarios in central Mexico.Herpetological Journal, 29(2), 71-81.

Henríquez, P., Donoso, D. S., and Grez, A. A. (2009). Population density, sex ratio, body size and fluctuating asymmetry of *Ceroglossus chilensis* (Carabidae) in the fragmented Maulino forest and surrounding pine plantations. Acta Oecologica, 35, 811-818. https://doi.org/10.1016/j.actao.2009.08.012

Hoff, K. V., Huq, N., King, V. A., and Wassersug, R. J. (1989). The kinematics of larval salamander swimming (Ambystomatidae: Caudata). Canadian journal of zoology, 67(11), 2756-2761.

Huacuz-E. D. C. (2002). Programa de Conservación y Manejo de Ambystoma dumerilii: El Achoque del Lago de Pátzcuaro. UMSNH, Fondo Mexicano para la Conservación de la Naturaleza, A C. Secretaria de Medio Ambiente y Recursos Naturales. Morelia, Mich. Mex. Pp. 138.

Huacuz, E. D. (2008). Biología y conservación del género Ambystoma en Michoacán, México. Tesis de grado de doctoral, Universidad de Salamanca, Salamanca, España.

Hu, F., Crespi, E.J., Denver, R.J., (2008). Programming neuroendocrine stress axis activity by exposure to glucocorticoids during postembryonic development of the frog Xenopus laevis. Endocrinology 149, 5470–5481.

Iglesias-Carrasco, M., Martín, J., and Cabido, C. (2017). Urban habitats can affect body size and body condition but not immune response in amphibians. Urban Ecosystems, 20(6), 1331-1338.

Irschick D.J. and Garland T.J. (2001). Integrating function and ecology in studies of adaptation: Investigations of locomotor capacity as a model system. Annu Rev Ecol Syst 32:367–396.

Ijspeert, A. J., and Cabelguen, J.-M. (2006). Gait transition from swimming to walking: Investigation of salamander locomotion control using nonlinear oscillators. In H. Kimura, K. Tsuchiya, A. Ishiguro, and H. Witte (Eds.), Adaptive motion of animals and machines. Springer. 177–188. https://doi.org/10.1007/4-431-31381-8_16.

IUCN SSC Amphibian Specialist Group. (2020). *Ambystoma dumerilii*. The IUCN Red List of Threatened Species: e. T59055A53973725. https://dx.doi.org/10.2305/IUCN.UK.2020-1.RLTS.T59055A53973725.en. Downloaded on 28 November 2020.

Izquierdo, M.S., Fernandez-Palacios, H. and Tacon, A. G. J. (2001). Effect of broodstock nutrition on reproductive performance of fish. Aquaculture 197, 25–42.

James RS, Wilson RS. (2008). Explosive jumping: extreme morphological and physiological specializations of Australian rocket frogs (*Litoria nasuta*). Physiol Biochem Zool 8:176–185

Jessop, T. M., Madsen, T., Sumner, J., Rudiharto, H., et al. (2006). Maximum body Size among insular Komodo dragon populations covaries with large prey density. Oikos, 112, 422–429.

Krause ET, Steinfartz S, Caspers BA. (2011). Poor nutritional conditions during the early larval Stage reduce risk-taking activities of fire salamander larvae (*Salamandra salamandra*). Ethology 117:416–21.

Lens, L., von Dongen, S., Matthysen, E., (2002). Fluctuating asymmetry as an early warning system in the critically endangered Taita Thrush. Conserv. Biol. 16, 479–487

Lempa, K., Martel, J., Koricheva, J., Haukioja, E., Ossipov, V., Ossipova, S., and Pihlaja, K. (2000). Covariation of fluctuating asymmetry, herbivory and chemistry during birch leaf expansion. Oecologia, 122(3), 354-360.

Livingston S., Lavin S. R., Sullivan K., Attard L. and Valdes E. V. (2014). Challenges with effective nutrient supplementation for amphibians: A review of cricket studies. Zoo Biology, 33, 565-76.

Lleonart, J., Salat, J., and Torres, G. J. (2000). Removing allometric effects of body size in morphological analysis. Journal of theoretical Biology, 205(1), 85-93.

Lomolino MV, Channell R, Perault DR, Smith GA (2001). Down- sizing nature: anthropogenic dwarfing of species and ecosystems. In: Lockwood JL, McKinney ML, eds. Biotic Homogenization. Kluwer Academic/Plenum, New York

Lowe, W. H., Likens, G. E., and Cosentino, B. J. (2006). Self organization in streams: The relationship between movement behaviour and body condition in a headwater salamander. Freshwater Biology, 51, 2052–2062.

Martel, J., Lempa, K., and Haukioja, E. (1999). Effects of stress and rapid growth on fluctuating asymmetry and insect damage in birch leaves. Oikos, 208-216.

Matthews, S.G., (2002). Early programming of the hypothalamo–pituitary–adrenal axis. Trends in Endocrinology and Metabolism 13, 373–380.

Metcalfe NB, Monaghan P. (2001). Compensation for a bad start: grow now, pay later? Trends Ecol Evol 16:254–60.

Michaels, C. J., Gini, B. F., and Preziosi, R. F. (2014a). The importance of natural history and species-specific approaches in amphibian ex-situ conservation. The Herpetological Journal,24 (3), 135-145.

Michaels C.J., Downie J.R., Campbell-Palmer R. (2014b). The importance of enrichment for advancing amphibian welfare and conservation goals: a review of a neglected topic. Amphib Reptile Conser 8:7–23.

Michaels, C. J., Rendle, M., Gibault, C., Lopez, J., Garcia, G., Perkins, M. W., and Tapley, B. (2018). Batrachochytrium dendrobatidis infection and treatment in the salamanders Ambystoma andersoni, *A. dumerilii* and *A. mexicanum*. Herpetological Journal, 28(2).

Milligan, J. R., Krebs, R. A., and Mal, T. K. (2008). Separating developmental and environmental effects on fluctuating asymmetry in *Lythrum salicaria* and *Penthorum sedoides*. International Journal of Plant Sciences, 169(5), 625-630.

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

Morgan K. N. and Tromborg C. T. (2007). Sources of stress in captivity. Applied Animal Behaviour Science, 102, 262-302.

Morrison, C., J. M. Hero, and J. Browning. (2004). Altitudinal variation in the age at maturity, longevity, and reproductive lifespan of anuran in subtropical Queensland. Herptetologica 60, 34–44.

Møller and J. Manning. (2003). Growth and developmental instability. Veterinary Journal, 166:19–27.

Murta-Fonseca R. A., Manuella Folly, Luiz Fernando Carmo, Angele Martins. (2020). Growing towards disparity: geometric morphometrics reveals sexual and allometric differences in *Aparasphenodon brunoi* (Anura: Hylidae: Lophyohylinae) head shape Cuad. herpetol. 34 (1): 00-00

Novelo-Retana, A. (2018). Pátzcuaro y cuencas endorréicas cercanas. CONABIO. Recuperado el 29 de noviembre, 2019 en; http://www.conabio.gob.mx/conocimiento/regionalizacion/doctos/rhp_062.html

Palmer, A.R., Strobeck, C. (2003): Fluctuating asymmetry analyses revisited. In: Developmental Instability: Causes and Consequences. Polak, M., Ed., Oxford University Press, USA. 171, 279-319.

Palmer, A. R., and Strobeck, C. (1986). Fluctuating asymmetry: measurement, analysis, patterns. Annual review of Ecology and Systematics, 17(1), 391-421.

Peacor S, Pfister C. (2006). Experimental and model analyses of the effects of competition on individual size variation in wood frog (Rana sylvatica) tadpoles. J Anim Ecol 75:990–9.

Pessier, A. P., E. J. Baitchman, P. Crump, B. Wilson, E. Griffith, and H. Ross. (2014). Causes of mortality in anuran amphibians from an ex-situ survival assurance colony in Panama. Zoo Biol. 33(6):516–526.

Phillips, B. L., G. P. Brown, J. K. Webb, and R. Shine. 2006. Invasion and the evolution of speed in toads. Nature 439:803.

Relyea, R.A., Hoverman, J.T. (2003). The impact of larval predators and competitors on the morphology and fitness of juvenile treefrogs. Oecologia 134, 596–604.

Rodríguez, B. M. A. P. (2020). Caudata, O. Formato Adaptado de AARK “Ex Situ Management Guidelines” por el Grupo de Anfibios del Comité de Conservación de la ALPZA.

Rohlf, J.; Slice, D. (1990). Extensions of the Procrustes method for the optimal superimposition of landmarks. Systematic biology, 39(1), 40-59.

Rohlf, F. J. (2015). tpsDig, version 2.16: A program for digitizing landmarks and outlines for geometric morphometrics. Department of Ecology and Evolution, State University of New York, Stony Brook.

Riva-Tonini, J. F., Provete, D. B., Maciel, N. M., Morais, A. R., Goutte, S., Toledo, L. F., and Pyron, R. A. (2020). Allometric escape from acoustic constraints is rare for frog calls. Ecology and evolution, 10(8), 3686-3695.

Ramírez-Herrejón J. P., Zambrano L., Mercado-Silva N., Torres-Téllez A., Pineda-García F., Caraveo-Patiño J. and Balart E. F. (2014). Long term changes in the fish fauna of Lago de Pátzcuaro in Central México. Lat. Am. J. Aquat. Res., 42(1): 137-149, 137 DOI: 103856/vol42-issue1-fulltext-11.

Schulte-Hostedde, A. I., Bowman, J., and Middel, K. R. (2011). Allometry of the baculum and sexual size dimorphism in American martens and fishers (Mammalia: Mustelidae). Biological Journal of the Linnean Society, 104(4), 955-963.

Shaffer, H. B. (1984). Evolution in a paedomorphic lineage. II. Allometry and form in the Mexican ambystomatidae salamanders. Evolution, 38(6), 1207-1218.

Shaffer, B., O. Flores-Villela, G. Parra-Olea and D. Wake. (2004). *Ambystoma dumerilii*. En: IUCN 2009. IUCN Red List of Threatened Species. Recuperado el 28 de Agosto, 2021 de: http://www.iucnredlist.org/ details/

Shaffery, H. M., and R. A. Relyea. (2015). Predator-induced defenses in five species of larval Ambystoma. Copeia 103:552–562.

Shi, Y., Puzianowska-Kuznicka, M., and Stolow, M. A. (1996). Tadpole competence and tissue-specific temporal regulation of amphibian metamorphosis: roles of thryoid hormone and its receptors. BioEssays, 18, 391–399.

Sibly, R., and Calow, P. (1984). Direct and absorption costing in the evolution of life cycles. Journal of Theoretical Biology, 111(3), 463-473.

Slight, D. J., Nichols, H. J., and Arbuckle, K. (2015). Are mixed diets beneficial for the welfare of captive axolotls (*Ambystoma mexicanum*)? Effects of feeding regimes on growth and behavior. Journal of Veterinary Behavior, 10(2), 185-190.

Steinicke, H., Gruber, B., Grimm, A., Grosse, W. R, and Henle K. (2015). Morphological shifts in populations of generalist and specialist amphibians in response to fragmentation of the Brazilian Atlantic forest. Nature Conservation, 13, 47–59. doi: 10.3897/natureconservation.13.7428

Soto-Rojas C, Suazo-Ortuño I, Montoya Laos JA, Alvarado-Díaz J. (2017). Habitat quality affects the incidence of morphological abnormalities in the endangered salamander *Ambystoma ordinarium*. PLoS ONE 12(8), 1-15. DOI: e0183573. https://doi.org/10.1371/journal. pone.0183573.

Stuart SN. (2004). Status and trends of amphibian declines and extinctions worldwide. Science 306,1783–1786.

Tejedo, M., Marangoni, F., Pertoldi, C., Richter-Boix, A., Laurila, A., Orizaola, G., and Gomez-Mestre, I. (2010). Contrasting effects of environmental factors during larval stage on morphological plasticity in post-metamorphic frogs. Climate research, 43(1-2), 31-39.

Titon Jr, B., and Gomes, F. R. (2017). Associations of water balance and thermal sensitivity of toads with macroclimatic characteristics of geographical distribution. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 208, 54-60.

Urban MC. (2010). Microgeographic adaptations of spotted salamander morphological defenses in response to a predaceous salamander and beetle. Oikos 119,646–658.

Van Buskirk, J. (2011). Amphibian phenotypic variation along a gradient in canopy cover: species differences and plasticity. Oikos, 120(6), 906-914.

Van Buskirk, J. O. S. H., and McCollum, S. A. (2000). Influence of tail shape on tadpole swimming performance. Journal of Experimental Biology, 203(14), 2149-2158.

Van Buskirk J, Schmidt BR. (2000). Predator-induced phenotypic plasticity in larval newts: trade-offs, selection, and variation in nature. Ecology 81:3009–3028.

Vega-Trejo, R., Zúniga-Vega, J. J., and Langerhans, R. B. (2014). Morphological differentiation among populations of *Rhinella marina* (Amphibia: Anura) in western Mexico. Evolutionary Ecology, 28(1), 69-88.

Velarde-Mendoza, T. (2012). Importancia ecológica y cultural de una especie endémica de ajolote (*Ambystoma dumerilii*) del lago de Pátzcuaro, Michoacán. Etnobiología 10(2), 40-49.

Velarde-Mendoza, T. (2018). Información sobre la especie importancia tradicional del achoque. Boletín Red Achoque. 2, 1-7.

Velickovic, M., and Perisic, S. (2006). Leaf fluctuating asymmetry of common plantain as an indicator of habitat quality. Plant Biosystems, 140(2), 138-145.

Wake, D. and V. Vredenburg. (2008). Are we in midst of the sixth mass extinction? A view from the world of amphibians. Proceedings of the National Academy of Sciences. 105,11466-11473.

Waye, H. L., Dolan, P. C., and Hernandez, A. (2019). White blood cell profiles in long-term captive and recently captured eastern tiger salamanders (*Ambystoma tigrinum*). Copeia,107(1), 138-143.

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

Wu, Z., Li, Y., and Murray, B. R. (2006). Insular shifts in body size of rice frogs in the Zhoushan archipelago, China. Journal of Animal Ecology, 75, 1071–1080. doi: 10.1111/j.1365-2656.2006.01126.x.

Zambrano L., Cordoval-Tapia F., Ramírez-Herrejón J. P., Mar-Silva V., Bustamante L., Camargo T. and Bustamante E. (2011). Las especies exóticas en el lago de Pátzcuaro, Michoacán, México. En Huerto Delgadillo, R., Vargas Velázquez, S., y Ortíz Paniagua, C. (eds.) Estudio Ecosistémico del lago de Pátzcuaro. Instituto Mexicano de Tecnología del Agua.

Zhelev, Z. M., Popgeorgiev, G. S., Arnaudov, A. D., Georgieva, K. N., and Mehterov, N. H. (2015). Fluctuating asymmetry in *Pelophylax ridibundus* (Amphibia: Ranidae) as a response to anthropogenic pollution in south Bulgaria. Archives of Biological Sciences, 67(3), 1009-1023.

Zhelev, Z. M., Popgeorgiev, G. S., and Georgieva, Z. K. (2014). Fluctuating asymmetry in the populations of *Pelophylax ridibundus* and *Pseudepidalea viridis* (Amphibia: Anura) in the region of the lead and zinc plant “Kardzhali” (South Bulgaria). Acta Zoologica Bulgarica, 66(1), 83-87.

Zhelev, Z. M., Tsonev, S. V., and Angelov, M. V. (2019). Fluctuating asymmetry in *Pelophylax ridibundus* meristic morphological traits and their importance in assessing environmental health. Ecological Indicators, 107, 105589.

Zippel, K., Johnson, K., Gagliardo, R., Gibson, R., McFadden, M., Browne, R., and Townsend, E. (2011). The Amphibian Ark: a global community for ex situ conservation of amphibians. Herpetological Conservation and Biology, 6(3), 340-352.

Table 1. ANOVA of morphological characters in individuals of *A.dumerilii* from captivity and wildlife. Eye to Eye Distance (EED), Head Width (HW), Head length (HL), Body Width (BW), Total length (TL), Tail Width (TW), Tail Length (TLe), Femur length right and left (FLR, FLL), Tibia-Fibula length right and left (TFLR, TFLL), Radius-Ulna Length right and left (RULR, RULL), Humerus length right and left (HLR, HLL)

Character	Lake	Captivity	d. f.	*F*	*P*
HW	4.678 ± 0.389	4.033 ± 0.228	1	102.17	**0.0001**
HL	4.496 ± 0.494	3.47 ± 0.528	1	106.96	**0.0001**
BW	3.529 ± 0.388	3.624 ± 0.357	1	1.6966	0.1956
TL	24.9 ± 1.747	21.4 ± 1.002	1	142.14	**0.0001**
TW	1.418 ± 0.183	1.3064 ± 0.178	1	9.9329	**0.0021**
TLe	10.532 ± 1.445	8.482 ± 0.995	1	68.819	**0.0001**
FLR	1.465 ± 0.279	1.406 ± 0.227	1	1.3783	0.2431
TFLR	1.3385 ± 0.272	1.2991 ± 0.2219	1	0.6458	0.4234
FLL	1.4567 ± 0.259	1.332 ± 0.206	1	7.1695	**0.0086**
TFLL	1.4035 ± 0.278	1.2977 ± 0.182	1	4.084	**0.04584**
HLR	1.553 ± 0.206	1.3826 ± 0.225	1	7.9356	**0.0069**
RULR	1.515 ± 0.155	1.2717 ± 0.165	1	29.847	**0.0001**
HLL	1.555 ± 0.203	1.3028 ± 0.231	1	17.071	**0.0001**
RULL	1.499 ± 0.211	1.248 ± 0.177	1	23.017	**0.0001**
SVL	14.32 ± 1.393	13.01 ± 2.386	1	12.47	**0.0001**

Table 2. Allometric patterns of morphological characters in individuals of *A. dumerilii* from the lake and captivity in relation to standard body size. Eye to Eye Distance (EED), Head Width (HW), Head length (HL), Body Width (BW), Total length (TL), Tail Width (TW), Tail Length (TLe), Femur length right and left (FLR, FLL), Tibia-Fibula length right and left (TFLR, TFLL), Radius-Ulna Length right and left (RULR, RULL), Humerus length right and left (HLR, HLL)

Character (Log)	Lake			Captivity		
	Slope b (95% CI)	r^2	*P*	Slope b (95% CI)	r^2	*P*
HW	0.6 (0.39, 0.8)	0.36	**0.0001**	0.73 (0.05, 0.81)	0.88	**0.0001**
HL	0.58 (0.3, 0.85)	0.23	**0.0001**	0.71 (0.49, 0.9)	0.49	**0.0001**
BW	0.86 (0.59, 1.1)	0.41	**0.0001**	0.97 (0.83, 1.1)	0.82	**0.0001**
TL	0.89 (0.72 1.05)	0.67	**0.0001**	1 (0.91, 1.04)	0.95	**0.0001**
TW	0.73 (0.41, 1.05)	0.26	**0.0001**	0.86 (0.67, 1.05)	0.65	**0.0001**
TLe	0.83 (0.49, 1.16)	0.3	**0.0001**	0.95 (0.79, 1.1)	0.75	**0.0001**
FLR	0.53 (0.07, 1)	0.08	**0.025**	1 (0.85, 1.27)	0.69	**0.0001**
TFLR	0.9 (0.36, 1.4)	0.16	**0.002**	0.91(0.66, 1.15)	0.55	**0.0001**
FLL	0.44 (-0.04, 0.9)	0.06	**0.06**	1.12 (1.05, 1.49)	0.75	**0.0001**
TFLL	0.8 (0.29, 1.3)	0.15	**0.002**	1 (0.79, 1.19)	0.68	**0.0001**
HLR	0.91 (0.4, 1.4)	0.44	**0.001**	0.82 (0.51, 1.12)	0.44	**0.0001**
RULR	0.86 (0.49, 1.22)	0.58	**0.0001**	0.59 (0.34, 0.84)	0.38	**0.0001**
HLL	0.68 (0.24, 1.1)	0.35	**0.004**	0.94 (0.62, 1.2)	0.71	**0.0001**
RULL	0.78 (0.3, 1.25)	0.38	**0.001**	0.83 (0.57, 1.09)	0.55	**0.0001**
EED	0.3 (-0.08, 0.7)	0.04	0.12	0.67 (0.57, 0.77)	0.83	**0.0001**

Figures

Figure 1. Individual of *Ambystoma dumerilii* with: A) Body measurements: B) Traits measured for fluctuating asymmetry assessment.

Figure 2. PCA plot based on Procrustes coordinates of individuals from captivity and lake conditions that represents the variation in body shape between both conditions. Black circles indicate individuals from captivity, and gray circles to those from lake (A). Wireframe graph of mean body shape of individuals from captivity and lake conditions. Black contour represents the mean body shape of individuals from captivity, and gray contour to that of lake individuals (B).

Figure 1.

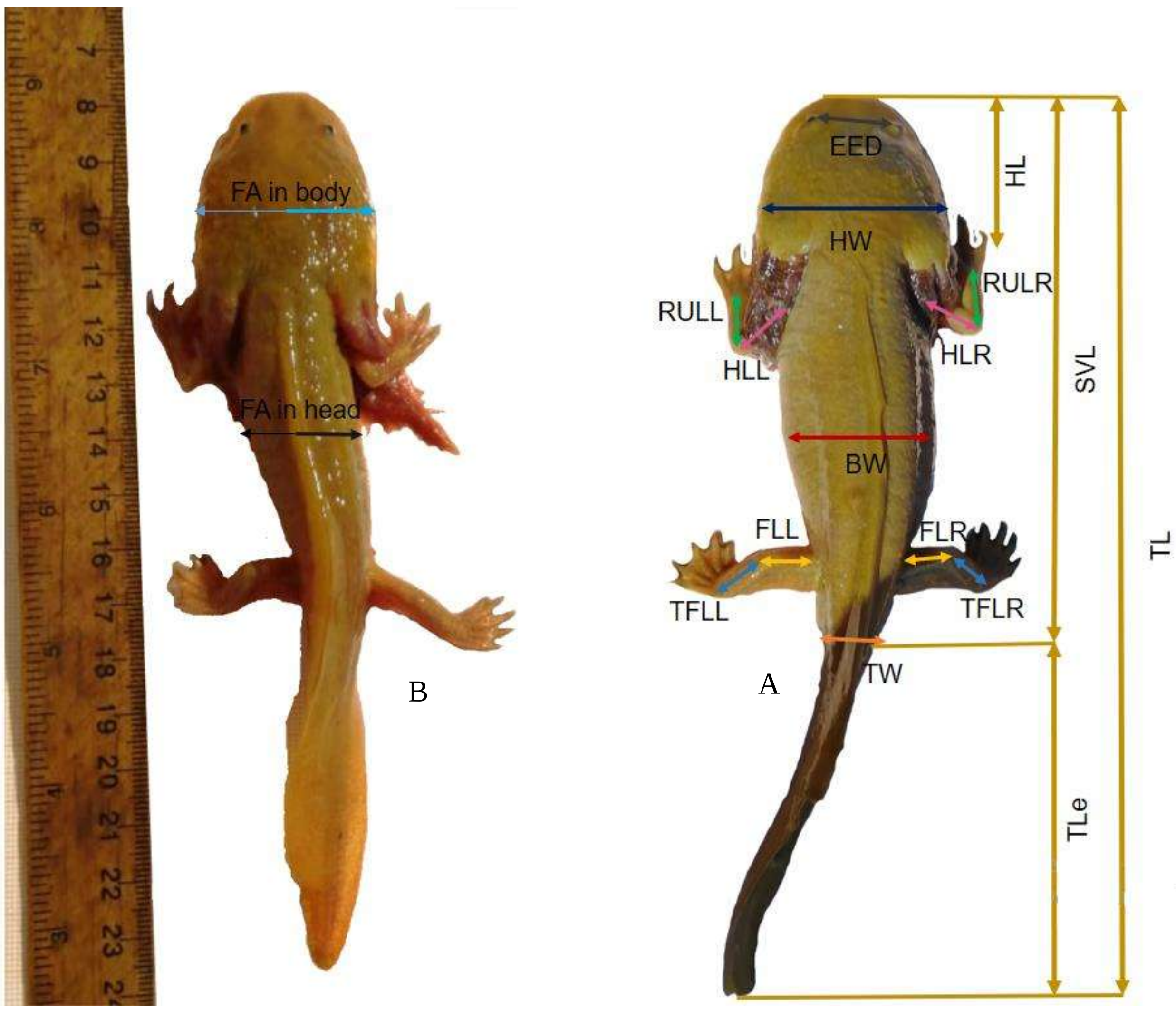


Figure 2A

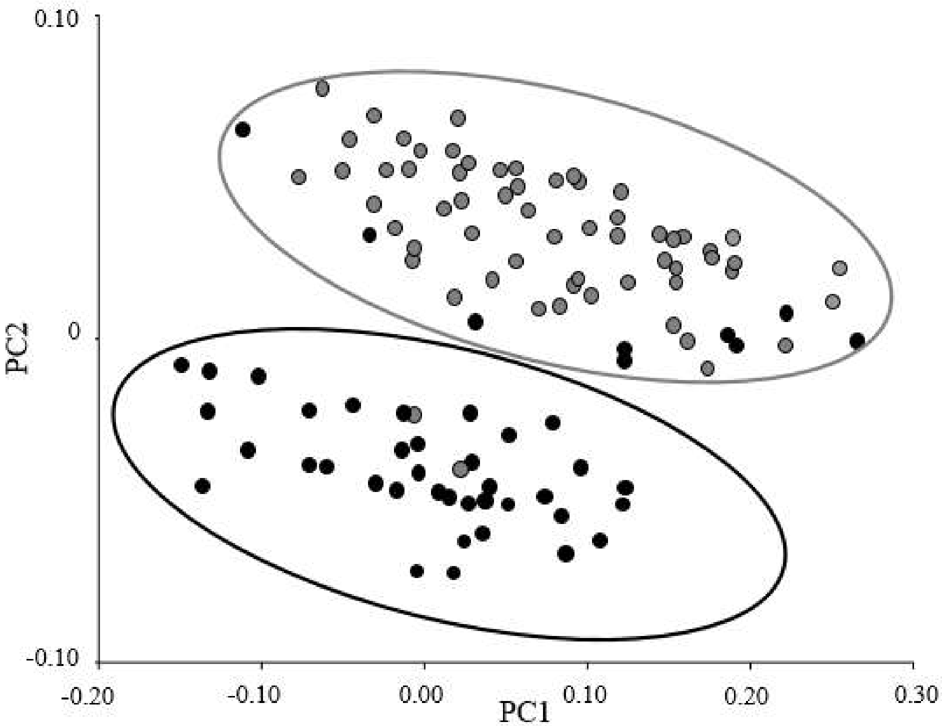
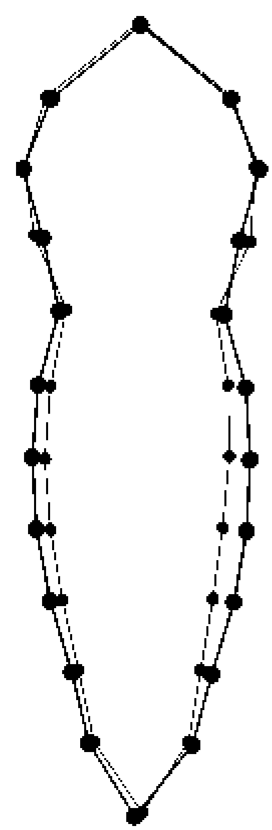


Figure 2B



8. Discusión general

Diferentes factores derivados de la actividad antropogénica han afectado a lo largo del tiempo a especies, en el caso de *Ambystoma dumerilii* que presenta una fuerte presión dentro del lago derivado de la contaminación o muchas otras causas, así como en cautiverio donde se puede encontrar un mal manejo de las condiciones apropiadas al ser una especie micro-endémica con condiciones óptimas difíciles de replicar por lo que esperaríamos que esto llevara a los organismos al estrés reflejado a través de la asimetría fluctuante (Davis y Maerz, 2009; Zambrano *et al.* 2011; Michaels *et al.* 2014; Waye, 2019; Cayuela *et al.* 2020; Gangenova *et al.* 2020), nosotros encontramos que en esta especie los individuos del lago son más grandes además de presentar mayor FA.

Todas estas condiciones comprometen el sistema inmune de los organismos al generar estrés. De acuerdo a nuestros resultados, los organismos más grandes y con un mayor nivel de FA lo presentaron los organismos del lago, por lo que están sometidos a diferentes presiones a las presentes en cautiverio, dado que en relación a las pendientes de las correlaciones alométricas de casi todos los rasgos fueron más bajas en el lago, lo que indica que el SVL aumenta a una mayor velocidad en los caracteres morfológicos en comparación a los individuos de cautiverio los cuales, presentaron caracteres más pequeños y delgados. Esto contrasta con las generalidades marcadas en otros estudios donde los anfibios tienen una tasa de crecimiento menor derivado de los factores ambientales estresantes, sin embargo, los organismos del lago pueden tener una mayor disponibilidad de alimento, así como de espacio, pero con diversos factores de estrés como la depredación, la contaminación, entre otras. (Denver *et al.* 2002; Delgado-Acevedo y Restrepo, 2008; Tejedo *et al.* 2010).

Sin embargo, esto podría estar relacionado con la diferencia o la disponibilidad en de alimento, así como la cantidad de nutrientes de estos, dado que en el lago se alimentan principalmente de acociles, y otros vertebrados pequeños, además de peces e incluso de larvas de la misma especie (Milligan *et al* 2008; Fair y Breshears, 2005), lo cual podría permitir un crecimiento más acelerado (Hochwender y Fritz, 1999, Lempa *et al.* 2000; Martel *et al.* 1999). De igual manera los niveles de AF podrían indicar que los organismos del lago están sometidos a diferentes presiones de estrés como la presencia de depredadores en el lago, la disponibilidad de espacio en cautiverio y la diferencia de la dieta en ambas condiciones (Milligan *et al.* 2008, Velickovic y Perisic, 2006), dado que la AF aumenta cuando se alcanza una tasa de crecimiento óptima para su desarrollo en condiciones de lago, lo que deriva en una mayor inestabilidad del desarrollo y por tanto de la FA (Hochwender y Fritz, 1999, Lempa *et al*. 2000, Martel *et al.* 1999).

Nuestro trabajo permitió conocer las diferencias en la AF de los individuos ejercidos por las diferentes condiciones presentes en ambos ambientes como dieta, disponibilidad de espacio, así como la presencia de depredadores. Nuestros resultados nos mostraron que los organismos del lago presentaron una mayor AF por lo que estos organismos están sometidos a mayores presiones que pueden ser de diferentes índoles generando estrés en los organismos, por ello es necesario realizar mayores estudios sobre esta especie. En tanto que los individuos en cautiverio presentaron un tamaño menor que puede estar asociado a la disponibilidad del espacio o por una baja disponibilidad de nutrientes en su alimentación en comparación con los individuos silvestres.

9. Bibliografía:

Alvarado-Díaz, J., Suazo-Ortuño, I., Wilson, L. D., & Medina-Aguilar, O. (2013). Patterns of physiographic distribution and conservation status of the.

AmphibiaWeb. 2019. *Ambystoma dumerilii*: Lake Pátzcuaro Salamander, disponible en: http://amphibiaweb.org/species/3831. University of California, Berkeley, CA, EE. UU. Accedido el 23 de octubre de 2018.

Aguilar-Miguel, X., Legorreta, G., and Casas-Andreu, G. (2009). Reproducción ex situ en Ambystoma granulosum y Ambystoma lermaense (Amphibia: Ambystomatidae). Acta zoológica mexicana, 25(3), 443-454.

Aguilar-Miguel, X. 2005. Ambystoma dumerilii. Algunas especies de anfibios y reptiles contenidos en el Proyecto de Norma Oficial Mexicana PROY-NOM-059-ECOL-2000. Facultad de Ciencias, Centro de Investigación en Recursos Bióticos, Universidad Autónoma del Estado de México. Bases de datos SNIBCONABIO. Proyecto W035. México. D.F. Disponible en: http://www.conabio.gob.mx/conocimiento/ise/fichasnom/Ambystomadumerilii00.pdf

Arana, M. F. 2016. Produce CIBAC hasta 5,000 ajolotes al año mediante tecnología especializada. Revista Electrónica Seminario de la UAM, 22(24), 4-6.

Benítez, H. A., and Parra, L. E. 2011. Asimetría fluctuante: una herramienta morfo-funcional para medir la estabilidad del desarrollo. International Journal of Morphology,29(4), 1459-1469.

Blaustein, A. R., Han, B. A., Relyea, R. A., Johnson, P. T., Buck, J. C., Gervasi, S. S., and Kats, L. B. 2011. The complexity of amphibian population declines: understanding the role of cofactors in driving amphibian losses. Annals of the New york Academy of Sciences,1223 (1), 108-119.

Bower, Deborah S, Karen R Lips, and Arthur Georges. 2017. “Devastating Fungal Diseases.” *Science* 357 (6350): 454–56.

Brodeur, J. C., Bahl, M. F., Natale, G. S., & Poliserpi, M. B. 2020. Biomarker and hematological fieldwork with amphibians: is it necessary to sample all night?.Environmental Science and Pollution Research, 1-10.

Buckley, C. R., Michael, S. F., & Irschick, D. J. 2005. Early hatching decreases jumping performance in a direct-developing frog, Eleutherodactylus coqui. Functional ecology, 67-72.

Cano-Martínez, A., Vargas-González, A., and Guarner-Lans, V. (2007). Temperature effect on contractile activity of the Ambystoma dumerilii heart previously treated with isoproterenol. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 147(3), 743-749.

Cayuela, H., Valenzuela-Sánchez, A., Teulier, L., Martínez-Solano, Í., Léna, J. P., Merilä, J., ... and Schmidt, B. R. (2020). Determinants and consequences of dispersal in vertebrates with complex life cycles: a review of pond-breeding amphibians. The Quarterly Review of Biology, 95(1), 1-36.

Ceballos, G., et al. 2009. Zonas críticas y de alto riesgo para la conservación de la biodiversidad de México, en Capital natural de México, vol. II: Estado de conservación y tendencias de cambio. Conabio, México, pp. 575-600.

Cohen, Jeremy M., Taegan A. McMahon, Chloe Ramsay, Elizabeth A. Roznik, Erin L. Sauer, Scott Bessler, David J. Civitello, et al. 2019. “Impacts of Thermal Mismatches on Chytrid Fungus Batrachochytrium Dendrobatidis Prevalence Are Moderated by Life Stage, Body Size, Elevation and Latitude.” *Ecology Letters* 22 (5): 817–25. https://doi.org/10.1111/ele.13239.

Cuevas-Reyes P, Gilberti L, González-Rodríguez A, Fernandes G. W. 2013. Patterns of herbivory and fluctuating asymmetry in Solanum lycocarpum St. Hill (Solanaceae) along an urban gradient in Brazil. Ecol Indic 24:557–561.

Delgado-Acevedo J., Restrepo C. (2008). The contribution of habitat loss to changes in body size, allometry, and bilateral asymmetry in two Eleutherodactylus frogs from Puerto Rico. Conservation Biology, 22: 773-782, DOI: 10.1111/j.1523-1739.2008.00930.

De Vosjoli, P. 1999. Designing environments for captive amphibians and reptiles. Veterinary Clinics of North America: Exotic Animal Practice, 2(1), 43-68.

Eisemberg, C. C., and Bertoluci, J. (2016). Fluctuating asymmetry in populations of the South American frog Physalaemus cuvieri (Leptodactylidae) in areas with different degrees of disturbance. Journal of Natural History, 50(23-24), 1503-1511.

Fairbairn, D. J. (1997). Allometry for sexual size dimorphism: pattern and process in the coevolution of body size in males and females. Annual Review of Ecology and Systematics 28, 659–687.

Fariaa, P. J., C. V. Oosterhoutb, and J. Cable. 2010. Optimal release strategies for captive-bred animals in reintroduction programs: experimental infections using the guppy as a model organism. Biological Conservation 143:35–41.

Fair, J. M. and Breshears, D. D. 2005. Drought stress and fluctuating asymmetry in *Quercus undulata* leaves: confounding effects of absolute and relative amounts of stress? Journal of Arid Environments 62:235–249

Freeman DC, Brown ML, Duda JJ, Graraham JH, Emlen JM, Krzysik AJ, Balbach H, Kovaci DA, Zak JC 2005. Leaf fluctuating asymmetry, soil disturbance and plant stress: a multiple year comparison using two herbs, Ipomoea pandurata and Cnidoscolus stimulosus. Ecol Indic 5:85–95. https://doi.org/10.1016/j.ecolind.2004.05.002

Fox, G. A., Cooper, A. M., and Hayes, W. K. 2015. The dilemma of choosing a reference character for measuring sexual size dimorphism, sexual body component dimorphism, and character scaling: cryptic dimorphism and allometry in the scorpion Hadrurus arizonensis. PLoS One, 10(3), 1-23.

García-Feria, L. M., Hernández-Jauregui, D. M. B., Bravo, D. V., and Olivares, R. A. C. 2017. El comercio de anfibios y la presencia de *Batrachochytrium dendrobatidis* en vida libre: ¿dispersión en círculo vicioso?. Neotropical Biology and Conservation, 12(1), 30-36.

Goater, C.P., 1994. Growth and Survival of postmetamorphic toads—interactions among larval history, density, and parasitism. Ecology 75, 2264–2274.

Gangenova, E., Giombini, M. I., Zurita, G. A., and Marangoni, F. 2020. Morphological responses of three persistent native anuran species after forest conversion into monoculture pine plantations: tolerance or prosperity?. Integrative zoology, 15(5), 428-440.

Grenyer, R., Orme, C. D. L., Jackson, S. F., Thomas, G. H., Davies, R. G., Davies T J., Jones K. E., Olson V. A., Ridgely R. S., Rasmussen P. C., Ding Tzung-su., Bennett P. M., Blackburn Tim M., Gaston, K. J., Gittleman, J. L., Owens, Ian P F. 2006. Global distribution and conservation of rare and threatened vertebrates. Nature, 444(7115), 93-96. Vol: 444| doi:10.1038/nature05237.

Guo, Rui, Wenya Zhang, Shiwei Ai, and Liang Ren. 2017. "Fluctuating Asymmetry Rather than Oxidative Stress in Bufo Raddei Can Be an Accurate Indicator of Environmental Pollution Induced by Heavy Metals." https://doi.org/10.1007/s10661-017-5991-6

Hawkins, D.K., Quinn, T.P., 1996. Critical swimming velocity and associated morphology of juvenile coastal cutthroat trout (Oncorhynchus clarki clarki), steelhead trout (*Oncorhynchus mykiss*), and their hybrids. Can. J. Fish. Aquat. Sci. 53, 1487–1496.

Henle, K., Dubois, A., & Vershinin, V. (2017). A review of anomalies in natural populations of amphibians and their potential causes. Studies on Anomalies in Natural Populations of Amphibians Untersuchungen zu Anomalien in naturlichen Populationen von Amphibien, 57-164.

Hill, R. W., G. A. Wyse, and M. Anderson. 2016. Animal Physiology. Fourth edition. Sinauer Associates, Inc., Sun- derland, Massachusetts.

Hochwender, C. G. and Fritz, R. S. 1999. Fluctuating asymmetry in a Salix hybrid system: the importance of genetic versus environmental causes. Evolution 53:408–416

Huacuz-E. D. C. 2002. Programa de Conservación y Manejo de Ambystoma dumerilii: El A. dumerilii del Lago de Pátzcuaro. UMSNH. Morelia, Mich. Mex. Pp. 138.

Huacuz-Elías, D. C. 2005. Anfibios y reptiles. La Biodiversidad en el Estado der Michoacán. Estudio de Estado, México DF: Comisión Nacional por el Conocimiento y Uso de la Biodiversidad, Gobierno del Estado de Michoacán, Universidad Michoacana de San Nicolás de Hidalgo, 99-100

Iglesias-Carrasco, M., Martín, J., and Cabido, C. 2017. Urban habitats can affect body size and body condition but not immune response in amphibians. Urban Ecosystems,20(6), 1331-1338.

Johnson PTJ, Lunde KB, Ritchie EG, Reaser JK, Launer AE. 2001. Morphological abnormalities patterns in a California amphibians community. Herpetologica, 57: 336±352.

Johnson, P. T., and Lunde, K. B. 2005. Parasite infection and limb malformations: a growing problem in amphibian conservation. Amphibian declines: the conservation status of United States species. University of California Press, Berkeley, 124-138.

Kupferberg, S. J., Catenazzi, A., Lunde, K., Lind, A. J., and Palen, W. J. 2009. Parasitic copepod (Lernaea cyprinacea) outbreaks in foothill yellow-legged frogs (*Rana boylii*) linked to unusually warm summers and amphibian malformations in Northern California. Copeia, 3: 529-537.

Langerhans, R Brian, Regina Vega-trejo, and J Jaime Zu. 2014. "Morphological Differentiation among Populations of *Rhinella marina* (Amphibia: Anura) in Western Mexico." *Evol Ecol* 28: 69–88. https://doi.org/10.1007/s10682-013-9667-6.

Leamy LJ, Klingenberg CP. 2005. The genetics and evolution of fluctuating asymmetry. Annu Rev Ecol Evol Syst 36:1–21. https://doi.org/10.1146/annurev.ecolsys.36.102003.152640

Lempa, K., Martel, J., Koricheva, J., Haukioja, E., Ossipov, V., Ossipova, S., and Pihlaja, K. (2000). Covariation of fluctuating asymmetry, herbivory and chemistry during birch leaf expansion. Oecologia, 122(3), 354-360.

Maldonado-López, Y., Vaca-Sánchez, M. S., Canché-Delgado, A., García-Jaín, S. E., González-Rodríguez, A., Cornelissen, T., & Cuevas-Reyes, P. (2019). Leaf herbivory and fluctuating asymmetry as indicators of mangrove stress. Wetlands Ecology and Management, 27(4), 571-580.

Márquez, E. J., Cabeen, R., Woods, R. P., and Houle, D. 2012. The measurement of local variation in shape. Evolutionary biology, 39(3), 419-439.

McCallum, M. L. (2015). Vertebrate biodiversity losses point to a sixth mass extinction. Biodiversity and Conservation, 24(10), 2497-2519.

Mendoza-Almeralla, Cinthya, Patricia Burrowes, and Gabriela Parra-Olea. 2015. "La Quitridiomicosis En Los Anfibios de México: Una Revisión." Revista Mexicana de Biodiversidad 86 (1): 238–48. https://doi.org/10.7550/rmb.42588.

Márquez-García, M., Correa-Solis, M., Sallaberry, M., & Méndez, M. A. (2009). Effects of pond drying on morphological and life-history traits in the anuran Rhinella spinulosa (Anura: Bufonidae). Evolutionary Ecology Research, 11(5), 803-815.

Møller A, Shykoff JA. 1999. Morphological developmental stability in plants: patterns and causes. Int J Plant Sci 160:135–146

Mattoon, A. 2000. El declive de los anfibios. Worldwatch Institute, 23, 11-21.

Michaels, C. J., Gini, B. F., and Preziosi, R. F. 2014. The importance of natural history and species-specific approaches in amphibian ex-situ conservation. The Herpetological Journal,24 (3), 135-145.

Milligan, J. R., Krebs, R. A., and Mal, T. K. (2008). Separating developmental and environmental effects on fluctuating asymmetry in *Lythrum salicaria* and *Penthorum sedoides*. International Journal of Plant Sciences, 169(5), 625-630.

Myers, Norman, Russell A Mittermeier, Cristina G Mittermeier, Gustavo A B Fonseca, and Jennifer Kent.

2000. “Biodiversity Hotspots for Conservation Priorities” 403: 853–58.

Niemeier, S., Mueller, J., and Roedel, M. O. (2019). Fluctuating asymmetry—Appearances are deceptive. Comparison of methods for assessing developmental instability in european common frogs (Rana temporaria). Salamandra, 55(1), 14-26.

O’Donnell, K. M., Messerman, A. F., Barichivich, W. J., Semlitsch, R. D., Gorman, T. A., Mitchell, H. G., ... and Walls, S. C. (2017). Structured decision making as a conservation tool for recovery planning of two endangered salamanders. Journal for Nature Conservation, 37, 66-72.

Palmer, A.R., Strobeck, C. 2003. Fluctuating asymmetry analyses revisited. In: Developmental Instability: Causes and Consequences. Polak, M., Ed., Oxford University Press, USA. 171, 279-319.

Parra-olea, Gabriela, and Oscar Flores-villela Cinthya Mendoza-almeralla. 2014. “Biodiversidad de Anfibios En México-Biodiversity of Amphibians in Mexico.” https://doi.org/10.7550/rmb.32027.

Papoulias, D. M., Schwarz, M. S. and Mena, L. 2013. Gonadal abnormalities in frogs (Lithobates spp.) collected from managed wetlands in an agricultural region of Nebraska, USA.Environmental Pollution,172, 1-8.

Ramírez-Hernández, G., Suazo-Ortuño, I., Alvarado-Díaz, J., Escalera-Vázquez, L. H., Maldonado-López, Y., & Tafolla-Venegas, D. (2019). Effects of habitat disturbance on parasite infection and stress of the endangered Mexican stream salamander Ambystoma ordinarium. SALAMANDRA, 55(3), 160-172.

Ramírez-Herrejón J. P., Zambrano L., Mercado-Silva N., Torres-Téllez A., Pineda-García F., Caraveo-Patiño J. y Balart E. F. 2014. Long term changes in the fish fauna of Lago de Pátzcuaro in Central México. Lat. Am. J. Aquat. Res., 42(1): 137-149, 137 DOI: 103856/vol42-issue1-fulltext-11

Ruiz, A.M., Maerz, J.C., Davis, A.K., Keel, M.K., Ferreira, A.R., Conroy, M.J., Morris, L.A., Fisk, A.T., 2010. Patterns of development and abnormalities among tadpoles in a constructed wetland receiving treated wastewater. Environmental Science and Technology 44, 4862-4868.

Santini l., Benítez-López A., Ficetola G. F. and Huijbregts M. A. J. 2018. Length–mass allometries in amphibians. Integrative Zoology; 13: 36–45. doi: 10.1111/1749-4877.12268

Slight, D. J., Nichols, H. J., and Arbuckle, K. 2015. Are mixed diets beneficial for the welfare of captive axolotls (*Ambystoma mexicanum*)? Effects of feeding regimes on growth and behavior. Journal of Veterinary Behavior, 10(2), 185-190

SEMARNAT, 2018. Programa de Acción para la Conservación de las Especies *Ambystoma spp*, SEMARNAT/CONANP, México.

Servín, Z. E. 2011. Manual de mantenimiento en cautiverio y medicina veterinaria aplicada al ajolote de Xochimilco (Ambystoma mexicanum) en el Zoológico de Chapultepec (Doctoral dissertation, Tesis de Licenciatura. Facultad de Medicina Veterinaria y Zootecnia. UNAM).

Söderman, F., Van Dongen, S., Pakkasmaa, S., & Merilä, J. 2007. Environmental stress increases skeletal fluctuating asymmetry in the moor frog Rana arvalis. Oecologia, 151(4), 593-604

Soto-Rojas C, Suazo-OrtuñoI, Montoya Laos JA, Alvarado-Díaz J. 2017. Habitat quality affects the incidence of morphological abnormalities in the endangered salamander Ambystoma ordinarium. PLoS ONE 12(8): e0183573. https://doi.org/10.1371/journal. pone.0183573

Stuart SN, Chanson JS, Cox NA, Young BE, Rodrigues AS, Fischman DL, Waller RW. 2004. Status and trends of amphibian declines and extinctions worldwide. Science. 306 (5702):1783-6.

Stuart, S. N. (Ed.). (2008). Threatened amphibians of the world. Lynx Edicions.

Tejedo, M., Marangoni, F., Pertoldi, C., Richter-Boix, A., Laurila, A., Orizaola, G., ...and Gomez-Mestre, I. (2010). Contrasting effects of environmental factors during larval stage on morphological plasticity in post-metamorphic frogs. Climate research, 43(1-2), 31-39.

Tomasini Ortiz, C., Bravo Inclán, L., Sánchez Chávez, J., and Moeller Chávez, G. (2016). Monitoreo de descargas de aguas residuales y su impacto en el lago de Pátzcuaro, México (2006-2011), 9(1), 61-74.

Tucić, B., Budečević, S., Manitašević Jovanović, S., Vuleta, A., and Klingenberg, C. P. 2018. Phenotypic plasticity in response to environmental heterogeneity contributes to fluctuating asymmetry in plants: first empirical evidence. Journal of Evolutionary Biology, 31(2), 197-210.

Thammachoti P, Khonsue W, Kitana J, Varanusupakul P, Kitana N. 2012, Morphometric and gravimetric parameters of the rice frog Fejervarya limnocharis living in areas with different agricultural activity. Journal of Environmental Protection, 3(10), 1403-08.

Van Buskirk J, Schmidt BR. 2000. Predator-induced phenotypic plasticity in larval newts: trade-offs, selection, and variation in nature. Ecology 81:3009–3028

Velarde-Mendoza Tzintia. 2012. Importancia ecológica y cultural de una especie endémica de ajolote (Ambystoma dumerilii) del lago de Pátzcuaro, Michoacán. Etnobiología 10(2):20-47.

Velickovic, M., and Perisic, S. 2006. Leaf fluctuating asymmetry of common plantain as an indicator of habitat quality. Plant Biosystems, 140(2), 138-145

Wang, X., Huang, Y., Zhong, M., Yang, S., Yang, X., Jiang, J., & Hu, J. 2019. Environmental stress shapes life-history variation in the swelled-vented frog (Feirana quadranus). Evolutionary Ecology, 33(3), 435-448.

Wake, D. and V. Vredenburg. 2008. Are we in midst of the sixth mass extinction? A view from the world of amphibians. Proceedings of the National Academy of Sciences 105(Supplement 1),11466-11473.

Waye, H. L., Dolan, P. C., and Hernandez, A. 2019. White blood cell profiles in long-term captive and recently captured eastern tiger salamanders (Ambystoma tigrinum). *Copeia*,*107*(1), 138-143.

Zhang, Wenya, Rui Guo, Shiwei Ai, Ying Yang, Jian Ding, and Yingmei Zhang. 2018. "Ecotoxicology and Environmental Safety Long-Term Heavy Metal Pollution Varied Female Reproduction Investment in Free-Living Anura, *Bufo Raddei." Ecotoxicology and Environmental Safety* 159 (April): 136–42. https://doi.org/10.1016/j.ecoenv.2018.05.001.

Zambrano L., Cordoval-Tapia F., Ramírez-Herrejón J. P., Mar-Silva V., Bustamante L., Camargo T. y Bustamante E., 2011. Las especies exoticas en el Langlois, V.S., Carew, A.C., Pauli, B.D., Wade, M.G., Cooke, G.M., Trudeau, V.L. Low levels of the herbicide atrazine alter sex ratios and reduce metamorphic success in Rana pipiens tadpoles raised in outdoor mesocosms. Environmental Health Perspectives 118, 552.

Zakharov, V. M., E. Pankakoski, B. I. Sheftel, A. Peltonen & I. Hanski, 1991. Developmental stability and population dynamics in the common shrew, Sorex-Araneus. Amer. Nat. 138: 797–810.

Zhelev, Z. M., Popgeorgiev, G. S., Arnaudov, A. D., Georgieva, K. N., and Mehterov, N. H. 2015. Fluctuating asymmetry in Pelophylax ridibundus (Amphibia: Ranidae) as a response to anthropogenic pollution in south Bulgaria. Archives of Biological Sciences, 67(3), 1009-1023.

Zhelev, Z. M., Popgeorgiev, G. S., and Georgieva, Z. K. 2014. Fluctuating asymmetry in the populations of Pelophylax ridibundus and Pseudepidalea viridis (Amphibia: Anura) in the region of the lead and zinc plant "Kardzhali" (South Bulgaria). Acta Zoologica Bulgarica, 66(1), 83-87.

Zhelev, Z. M., Tsonev, S. V., and Angelov, M. V. 2019. Fluctuating asymmetry in Pelophylax ridibundus meristic morphological traits and their importance in assessing environmental health. Ecological Indicators, 107, 105589.

Zippel, K., Johnson, K., Gagliardo, R., Gibson, R., McFadden, M., Browne, R., *et al*. and Townsend, E. 2011. The Amphibian Ark: a global community for ex situ conservation of amphibians. Herpetological Conservation and Biology, 6(3), 340-352.