A Tale of Two Axolotls

S. RANDAL VOSS, M. RYAN WOODCOCK, AND LUIS ZAMBRANO

The Mexican axolotl (Ambystoma mexicanum) is an icon of culture, a revered aquarium pet, and a highly valued animal model in biomedical research. Unfortunately, Mexican axolotls are critically endangered in their natural Xochimilco habitat in Mexico City. If axolotls go extinct, current efforts to conserve the Xochimilco ecosystem will be undermined, as will efforts to genetically manage the laboratory populations that are needed to sustain research efforts around the world. A concerted global effort is needed to protect and manage this irreplaceable species in natural and laboratory environments.

Keywords: Xochimilco, Mexican axolotl, restoration, paedomorphosis, regeneration

The Mexican axolotl, an animal of broad historical, cultural, societal, and biological significance (Bartra 1987, Reib et al. 2015), is currently seeing the best and worst of times. In research labs around the world, the axolotl is revealing clues about its ability to cheat aging and regenerate damaged body parts. However, as Dickens wrote in *A Tale of Two Cities*, there are often two sides to every story. Although axolotls appear to be thriving in domestication, the native axolotl population in Mexico is on the brink of extinction (Zambrano et al. 2007). Efforts to save the axolotl and the Xochimilco ecosystem that it inhabits have been ongoing for several decades, but during this time, axolotl numbers have decreased. With the Xochimilco population in peril, there is concern about the fate of laboratory stocks: Can they be successfully maintained in the absence of a natural source population? In this article, we review the history of wild and laboratory axolotls and identify paths to sustain these irreplaceable resources.

Axolotls and paedomorphosis

The history of the Mexican axolotl is an epic tale. It's a story of a local endemic species that becomes an icon of Mesoamerican culture, a darling of aquarists, and a model organism to scientists around the world. Understanding why the axolotl and not some other salamander became famous requires a short lesson in ecology, evolution, and development.

The Mexican axolotl (figure 1) is a member of the tiger salamander species complex (McKnight and Shaffer 1997), a group of species and subspecies that are distributed throughout North America. This group of salamanders shows variation in development and life history (Shaffer and Voss 1996). Although all tiger salamanders use aquatic habitats for laying eggs and larval development, two different patterns of postembryonic development are observed among species. The larvae in aquatic breeding habitats that are ephemeral and subject to drying typically undergo a metamorphosis. This mode of development allows the larvae to capitalize on opportunities for growth in temporary habitats that don't have fish predators. During metamorphosis, the larvae initiate developmental programs that cause the loss and gain of traits necessary to transition from the aquatic habitat to a terrestrial habitat. During their evolutionary history, metamorphic tiger salamanders are thought to have colonized relatively permanent aquatic habitats, including several lake systems that arose from geological changes in the central highlands of Mexico. Within these stable habitats, paedomorphic modes of development evolved. Paedomorphic salamanders fail to undergo a metamorphosis and retain larval traits throughout life, including external gills and tail fins. Remarkably, they mature sexually in the form of a larva. Whereas metamorphic tiger salamanders breed once a year at most, paedomorphic axolotls can breed several times a year and generate more offspring per breeding event. Therefore, the axolotl became famous among naturalists because it is a curious paedomorphic salamander with a totally aquatic life cycle and a high capacity for reproduction. These characteristics ideally suited the axolotl for human domestication and scientific study.

Deterioration of the Xochimilco ecosystem

During pre-Columbian times, aquatic habitats within the Central Valley were ideal for axolotls. The Aztecs engineered these habitats into a system of canals and wetlands that greatly increased the amount of shoreline. Axolotls thrived within these habitats and provided an abundant and readily available food source (Rojas 1998). The Aztecs not only ate axolotls, but they also included them in their art and myths of creation. For example, the axolotl was a

BioScience 65: 1134–1140.
doi:10.1093/biosci/biv153 Advance Access publication 11 November 2015

*Figure 1. The albine axolotl (***Ambystoma mexicanum***). Photo by Daniel Manzur.*

central character in the creation myth called "The Raising of the Fifth Sun," written by Fray Bernandino de Sahagún in 1577 (Bartra 2011). To make the sun and moon move in the sky, it was necessary to sacrifice the god Xolotl, the twin of Quetzalcoatl. But this rebel twin did not want to die. Instead, Xolotl eluded capture by transforming himself into a variety of plants and animals. The last of the animals that he camouflaged himself as (before he was captured and killed) was the axolotl. It is tempting to think that the axolotl was more than a convenient disguise for Xolotl and that the association between axolotl and Xolotl had a deeper biological meaning. Xolotl also disguised himself in the form of the xoloitzcuintle, the pre-Columbian dog breed, and similarly, aquatic salamanders from around the world are referred to as "water dogs." Also, it seems possible that the Aztecs observed an axolotl undergo spontaneous metamorphosis, which they do on occasion (Johnson and Voss 2013), thereby revealing a transformation potential rivaling Xolotl's.

The aquatic habitats of Xochimilco deteriorated after the fall of the Aztec empire (Ezcurra 1990, Valek-Valdés G. 2000). The pace of deterioration was gradual and subtle until the middle of the twentieth century. At this time, axolotls seemed to be sufficiently numerous to support a fishery for local consumption. However, the rate of deterioration increased precipitously as Mexico City more than tripled in size between 1950 and 1975 (Ezcurra 1990). Rapid urbanization put an enormous burden on the water supply, a problem that continues today. Less than half of the water that Mexico City extracts from the water table is being recharged (Burns 2009). Historically, the water table was maintained by water sources from underground springs and rivers that drained mountains surrounding Mexico City and by permeable soils that have been lost to urbanization (Bojorquez et al. 2000). By the early 1950s, Xochimilco no longer received inputs from springs and rivers. In a short period of time, the valley became an endorheic basin with very little water, and the chemistry of much of the available water became alkaline, salty, and polluted (Legorreta 2009).

Initial attempts to save the Xochimilco ecosystem were not successful. Strategies were employed to manage and conserve water, but these were not optimized in ways to sustain axolotls. For example, when water is extracted from underground sources, it causes the land to sink, a process called *subsidence*. Land subsidence changes the contour of a basin and therefore the path and flow of water (Ezcurra and Mazari-Hiriart 1996). Xochimilco lands are sinking at an alarming rate, with some areas sinking 30–40 centimeters per year. As an engineering solution to subsidence, dams were built to retain water in higher-elevation areas of the ecosystem and to mitigate flooding in lower-lying areas. However, when dams are erected to manage water, they limit the ability of aquatic organisms to disperse and escape environmental conditions that cause stress and mortality (Mazari and Mackay 1993). They also fail to sustain water levels and flow through ecosystems. To sustain water input into Xochimilco, a system of seven pipes was engineered to feed Xochimilco with treated water from a single treatment plant at Cerro de la Estrella. Unfortunately, water quality from this source varies considerably throughout the year, causing fluxes in nutrients, algal blooms, and damage to the food web (Mazari-Hiriart et al. 2008).

The decline of Axolotls in Xochimilco

Starting in the 1990s, researchers began to monitor axolotls and the ecological parameters of the Xochimilco ecosystem. A catastrophic collapse was chronicled: The density of axolotls decreased from 6000 per square kilometer in 1998 to 100 in 2008 (Graue 1998, Zambrano et al. 2007, Contreras et al. 2009), and recent data suggests that there are fewer than 35 axolotls per square kilometer (figure 2). A population viability analysis predicts the possibility of extinction by 2017 (Zambrano et al. 2007). In addition to declining water quality and urbanization that continues to deplete wetlands, axolotl reductions are also associated with the overpopulation of introduced fishes that predate on the most vulnerable axolotl life stages: eggs and juveniles (Marin 2007, Zambrano et al. 2010).

The dramatic decline of axolotls has raised awareness about the deteriorating Xochimilco ecosystem. In particular, the Darwin Initiative identified the axolotl as an umbrella/flagship species in its efforts to protect Xochimilco species through conservation education and the promotion of natural tourism (Bride et al. 2008). However, the extinction clock continues to tick for the axolotl.

The history of laboratory axolotls

With the Xochimilco axolotl population in jeopardy, concern grows for the long-term sustainability of domestic axolotl populations. The majority of domestic axolotls in the world today trace their ancestry to a shipment of 34 axolotls from Xochimilco that arrived in Paris in 1863. These animals proved to be well suited for life in captivity. From just five males and one female, the Jardin des Plantes distributed thousands of axolotls to scientists throughout Europe

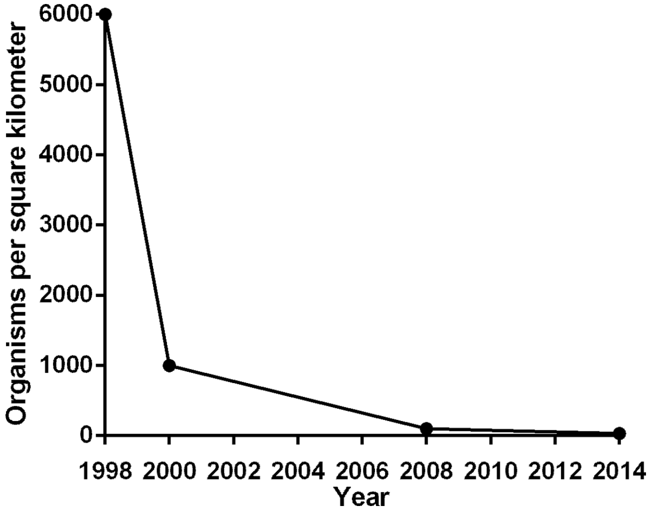

Figure 2. The Xochimilco axolotl population has declined dramatically over the last few years.

(Reib et al. 2015). Scientists quickly realized that axolotls were ideal for experimental laboratory studies because living stocks could be propagated throughout the year. Studies of axolotls played pivotal roles in elucidating fundamental concepts in developmental biology in the 1900s, and today, many consider the axolotl to be the best model for identifying the mechanisms associated with tissue regeneration (Voss et al. 2009).

In the United States, axolotls are distributed from a historic collection that has one of the deepest laboratory pedigrees of any captive bred population, tracing back to the 1863 shipment to Paris (Smith 1989, Humphrey 1976). In 1935, the famous embryologist Ross Harrison gifted axolotls he received from Krakow, Poland, to Robert Hutchison at the Morris Biological Farm of the Wistar Institute. Five white offspring from this stock were then gifted to Dr. Rufus Humphrey, who founded a population at University of Buffalo. In 1957, Humphrey relocated his axolotl collection to Indiana University, where it became a stock center funded by the National Science Foundation (NSF). Upon the retirement of George Malacinski in 2005, the axolotl collection relocated to the University of Kentucky and became the Ambystoma Genetic Stock Center (AGSC).

Since the time that Humphrey founded the AGSC population, 22 stock introductions brought new axolotls and axolotl–tiger salamander hybrids into the collection (figure 3). These introductions brought new genetic material and therefore helped to supplement genetic diversity. However, these past introductions were not performed with genetic-management objectives in mind. Instead, introductions were made to reveal new recessive phenotypes in developing embryos (Malacinski 1978). As developmental biologists have increasingly turned their attention to tissue regeneration and away from developing axolotl embryos, the practice of introducing new axolotls into the AGSC collection has all but stopped.

Inbreeding depression is a serious concern in small laboratory populations that are created from relatively few founders (Charlesworth and Charlesworth 1987). Although the AGSC population is relatively large (approximately 1200 adults), it is not sufficiently large to purge weak, deleterious mutations that accumulate over time and decrease fitness. Captive populations with inbreeding coefficients above 12.5% are considered emergency management situations; the average inbreeding coefficient for axolotls in the AGSC is 35% (figure 4). High inbreeding values trace to past management practices, including the selection for recessive alleles and good breeders, as well as factors that caused genetic bottlenecks (e.g., disease and episodes of spontaneous metamorphosis). Assessment of current genetic diversity identifies 5.82 founder genome equivalents in the AGSC population. This is similar to the Paris 1863 founder population, which yielded axolotls for labs throughout Europe. In 1880, naturalist Hans Gadow (1908) noted that whole colonies of axolotls died out or became sickly and healthy breeding pairs were difficult to acquire. Gadow attributed this deterioration to incessant inbreeding, with no import of "fresh material" to Europe. Past history predicts an ominous future for the AGSC population if genetic management strategies are not enacted. Moreover, if the Xochimilco axolotl population goes extinct, where will new sources of genetic variation come from?

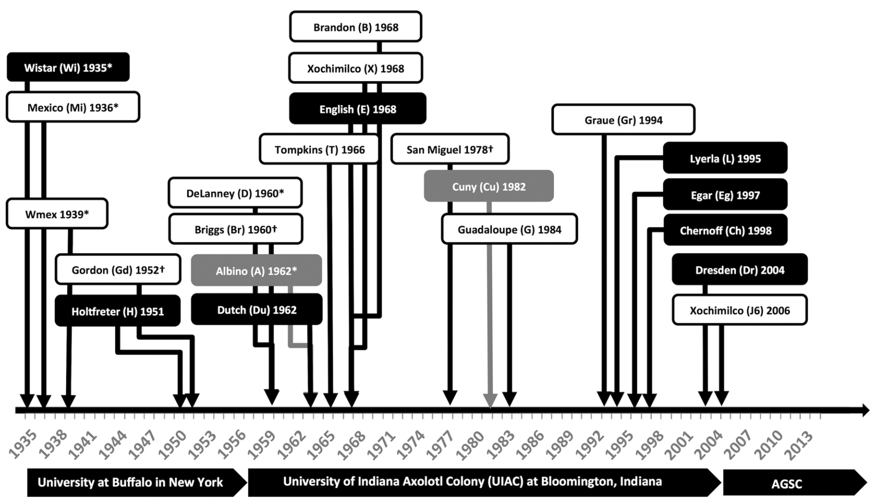

Figure 3. A timeline of 22 stock introductions into the Ambystoma Genetic Stock Center (AGSC) axolotl population. Five stock introductions contributed genetic material that is predicted to be present in the genomes of all AGSC axolotls (*). The genetic material from three stock introductions was lost and is not present in the current ASGC population (†).

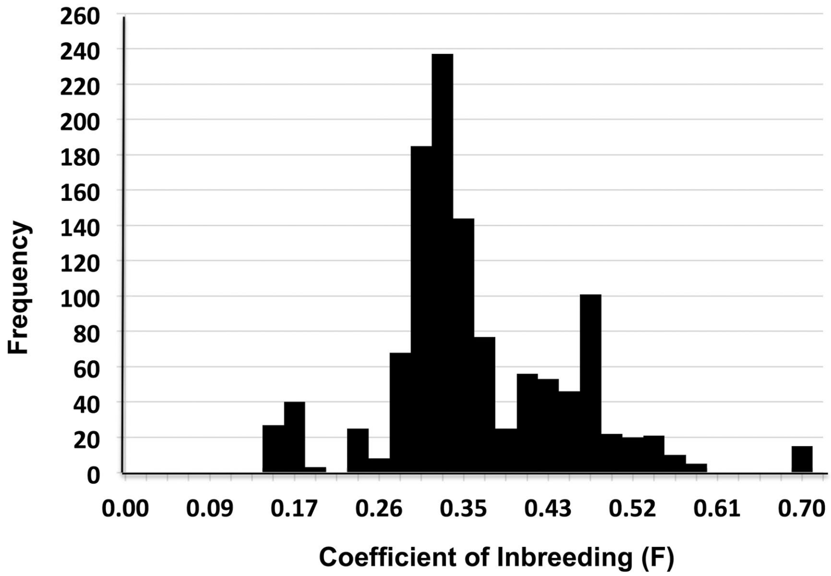

Figure 4. The coefficients of inbreeding calculated for all current Ambystoma Genetic Stock Center adult axolotls (*N *= 1206).

has increased in refuges by more than 50% (Tovar 2014). Axolotls and other native species such as crayfish and non-predatory silverside fish are thriving in these refuges (Rubio 2014, Tovar 2014), thereby increasing biodiversity and providing complexity. Early analyses of population dynamics (Manzur 2014) suggest that population growth within the refuges will counter the rate of decline previously predicted for the Xochimilco population (Zambrano et al. 2015). New techniques of organic fertilization have been developed and accepted by local producers; this has reduced pollutants that affect axolotls and other species and has generated a growing market of ecologically friendly products (Zambrano et al. 2015).

Our hope is that the iterative approach of transforming canals, one at a time, into high-quality environments and involving the local community in these efforts will ultimately transform Xochimilco into a sustainable ecosystem. To gain community support, incentives are being developed to encourage traditional agricultural approaches that have been part of the landscape for more than two thousand years. For example, one current plan rewards farmers that use water from rejuvenated canals (that contain axolotls) for crop irrigation and washing. The water is assumed to be of sufficient quality to gain a farmer an "axolotl friendly" certification for products grown. This certification identifies the farmer's products as high quality, making them more likely to be purchased by customers. It is encouraging that local people are beginning to take ownership of the axolotl refuges and are gaining from this ownership. Politicians and officials are beginning to show their support for the refuge program because it is beginning to show positive results (Zambrano et al. 2015).

Axolotl habitat restoration

Restoration of the entire Xochimilco ecosystem to pre-Colombian conditions would be difficult at this point. The wetlands are surrounded by densely populated urban areas. The ecosystem has been so drastically altered that any contemporary change in hydrodynamics would increase flooding in most of the city (Perez and Blanco 2010). Urbanization and varied agricultural and economic enterprises within the wetlands make it difficult to enact an ecosystem-level restoration project. Furthermore, local political leaders only serve three-year terms and cannot be re-elected; this makes it difficult to accomplish environmental planning at any scale.

Given these constraints, a bottom-up restoration approach was recently initiated to restore local habitats within the Xochimilco ecosystem. High-quality niches are being recreated to support axolotl growth and reproduction while preventing predatory attacks of exotic fish (figure 5). What is unique about this strategy is that it is aligns the axolotl's future with the local economy. Water availability and quality are as important to axolotls as they are to local people who grow crops within Xochimilco. Many different food crops are grown on islands (called *chinampas*) between the canals in Xochimilco. A high-quality water source is needed for irrigation and to rinse crops at harvest. To ensure that the water from the local canals is high quality, researchers are working with farmers to create local axolotl refuges. Semipermeable barriers are positioned at the entrance of canals to reduce the flow of sediment and to block the entrance of exotic fishes (Valiente and Zambrano 2010). Axolotls are relocated to these refuges, and their presence and persistence are associated with water-quality metrics that positively increase as the refuge matures. Thus, a symbiosis is born: High-quality water benefits axolotls and humans. Water transparency

Management of laboratory axolotl stocks

With a strategy in place to protect and expand axolotl populations in Xochimilco, there is hope that wild genetic material will be available for import into the AGSC to increase genetic diversity. Although most axolotls in the world trace their ancestry to the AGSC, it is also possible that axolotls from domestic axolotl collections around the world harbor significant genetic variation that is not present in the AGSC population. Whether new sources of genetic variation arise from Xochimilco or domestic stocks, we see a need to characterize axolotl genetic variation at a global scale using DNA typing. Empirical and existing pedigree data could then be integrated to inform genetic-management decisions, similar to how zoos work collaboratively to manage small, captive populations. The early steps in this process are underway: The AGSC is developing a panel of single nucleotide

Figure 5. Refuges for axolotls in Xochimilco canals. Canals surround rectangular islands used by local farmers with traditional agriculture system (Chinampa). The land is naturally fertilized; therefore, there is no need for agrochemicals pesticides, reducing pollution in the water.

polymorphisms that will allow the empirical assessment and prioritization of axolotls for import and sharing.

To complement efforts in building a global axolotl DNA registry, it will be important to develop methods for cryopreserving sperm and to implement long-term genetic planning using proven pedigree analysis and genetic-management tools. For example, simulations using PMx (Ballou et al. 2010) estimate that 89% of current genetic variation in the AGSC can be maintained for a period of 100 years if mean kinship is used to guide the breeding program. Our strategy will be to manage separate axolotl subpopulations within the AGSC and select breeding pairs to reduce inbreeding. Establishing separate subpopulations of sufficient and constant size has the potential to retain more genetic variation than a larger continuous population (Lacy 1987, Margan et al. 1998). The overall management strategy outlined above will ensure the conservation of irreplaceable axolotl stocks while allowing the AGSC to continue operations as an international genetic stock center for biological research.

A path forward

The title of our article traces to Charles Dickens' *A Tale of Two Cities*. That book's overarching message was to comment on eighteenth-century society and politics. French and English societies were breaking down under the weight of inequality, injustice, and poverty; the time was ripe for social and political change. The pressures that threaten one of Mexico's most treasured natural resources are equally weighty and, like *A Tale of Two Cities*, have complex political and social components.

Although the Mexican government has enacted plans to improve the Xochimilco ecosystem and water conservation, these plans have been difficult to implement. Partly, this reflects a lack of understanding about the resilience of urban wetlands and the need for consistent, long-term actions that prioritize environmental health and conservation ethics. The same local government that enacted policies to protect Xochimilco by implementing garbage-cleaning programs on canals is now supporting the construction of a highway over the wetlands (Zambrano et al. 2013). If this highway were built, it would almost certainly increase water and air pollution and facilitate the further urbanization of the area. To compensate, they have promoted conservation actions based on the liberation of farmed axolotls into deteriorated canals without studies of the genetics, health, or demography of these introduced animals. Xochimilco cannot be resurrected if policy decisions do not recognize what's at stake: Xochimilco and the natural species it contains are irreplaceable resources of incalculable value to Mexico and others around the world.

As for the axolotls in the AGSC, their future is also precarious. Will it be possible to import axolotls globally with the growing concern about the spread of infectious diseases that are decimating some salamander species (Yap et al. 2015)? Another concern is how AGSC axolotls will be sustained into the future. The NSF funded the AGSC axolotl collection for several decades. However, the NSF so radically changed their funding mechanism (NSF 13-557) for maintaining living stocks that it no longer provided a sustainable means of support for axolotls and other animal models. Fortunately, funding was secured from the National Institutes of Health in 2015 to extend support of the AGSC through 2020. However, future funding will depend on the need and relevance of the axolotl as a model in biomedical research. In a climate where funding is declining and researchers are aligning their interests with immediate, clinical outcomes, the axolotl may not receive the type of long-term support it will take to unlock the secret of regeneration. Currently, there is no safety net for the AGSC collection if scientific review committees do not prioritize the axolotl for future research funding.

What is clear to us is that we cannot walk separate paths in our efforts to conserve wild and domestic axolotls. Scientists and citizens who value the axolotl for different reasons need to become better connected. It is our hope that this article will motivate different stakeholders to embrace the axolotl's epic tale and through this connection build a stronger coalition. Countries around the world need to understand that the value of axolotls extends beyond specific interests and national borders. The axolotl is not only a flagship species for the Xochimilco ecosystem (Bride et al. 2008); it is also a highly prized aquarium pet and animal model in tissue-regeneration research. The long-term sustainability of domestic axolotl stocks rests on the conservation of the natural population in Mexico.

Acknowledgments

Studies of the axolotl in Xochimilco were funded by Autoridad de Xochimilco Tlahuac y Milpalta, the Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO), the Instituto Nacional de Ecología (INE), and the Mexico City Natural Resources Commission (CORENA). The Ambystoma Genetic Stock Center is funded by the National Institutes of Health (OD019794). The Army Research Office (W911NF1410165) is providing support to develop a genetic-management plan for the AGSC.

References cited

Ballou JD, Lacy RC, Pollak JP. 2010. PMx: Software for Demographic and Genetic Analysis and Management of Pedigreed Populations. Chicago Zoological Society. (7 October 2015; *www.vortex10.org/PMx.aspx*)

Bartra R. 1987. La Jaula de la Melancolía. Grijalvo.

———. 2011. Axolotiada. Fondo de Cultura Económica.

Bojorquez-Tapia LA, Ezcurra E, Mazari-Hiriart M, Diaz S, Gomez P, Alcantar G, Megarejo D. 2000. Basin of Mexico: A history of watershed mismanagement. Pages 129–137 in Ffolliott PF, Baker MB Jr., Edminster CB, Dillon MC, Mora KL, eds. Land Stewardship in the Twenty-First Century: The Contributions of Watershed Management US Department of Agriculture. US Department of Agriculture, Forest Service, Rocky Mountain Research Station.

Bride IG, Griffiths RA, Melendez-Herrada A, McKay JE. 2008. Flying an amphibian flagship: Conservation of the axolotl *Ambystoma mexicanum* through nature tourism at Lake Xochimilco, Mexico. International Zoo Yearbook 42: 116–124.

Burns E. 2009. Repensar la Cuenca: Gestion de Ciclos del Agua en el Valle de México. UAM-Centli México.

Charlesworth D, Charlesworth B. 1987. Inbreeding depression and its evolutionary consequences. Annual Review Ecology Systematics 18: 237–268.

Contreras V, Martine-Meyer E, Valiente-Riveros E, Zambrano L. 2009. Recent decline and potential distribution in the last remnant area of the microendemic mexican axolotl (*Ambystoma mexicanum*). Biological Conservation 142: 2881–2885.

De Sahagún B. 1577. 2011. Xolotl el dios que le temía a la muerte. Pages 48–58 in Bartra R, ed. Axolotiada: Vida y Mito de un Anfibio Mexicano. Fondo de Cultura Económica.

Ezcurra E. 1990. De las Chinampas a la Megalopolis: El Medio Ambiente en la Cuenca de México. Fondo de Cultura Económica.

Ezcurra E, Mazari-Hiriart M. 1996. Are mega cities viable? A cautionary tale from Mexico City. Environment: Science and Policy for Sustainable Development 38: 6–35.

Gadow H. 1908. Through Southern Mexico: Being an account of the travels of a naturalist. Witherby.

Graue V. 1998. Estudio Genético y Demográfico de la Población del Anfibio *Ambystoma mexicanum* (Caudata: Ambystomatidae) del Lago de Xochimilco. PhD dissertation. National Autonomous University of Mexico, Mexico City, Mexico.

Humphrey RR. 1976. History of the Indiana University axolotl colony. Axolotl Newsletter 1: 3–8.

Johnson CK, Voss SR. 2013. Salamander paedomorphosis: Linking thyroid hormone to life history and life cycle evolution. Pages 229–258 in Yun-Bo S, ed. Animal Metamorphosis. Academic Press. Current Topics in Developmental Biology, vol. 103.

Lacy RC. 1987. Loss of genetic diversity from managed populations: Interacting effects of drift, mutation, immigration, selection, and population subdivision. Conservation Biology 1: 143–158.

Legorreta J. 2009. Ríos, Lagos, y Manantiales del Valle de México. Universidad Autónoma Metropolitana.

Malacinski GM. 1978. The mexican axolotl, *Ambystoma mexicanum*: Its biology and developmental genetics, and its autonomous cell-lethal genes. American Zoologist 18: 195–206.

Manzur DT. 2015. Evaluación de Refugios de Axolote (*Ambystoma mexicanum*) por Medio de Matrices Poblacionales. BS dissertation. National Autonomous University of Mexico, Mexico City, Mexico

Margan SH, Nurthen RK, Montgomery ME, Woodworth LM, Lowe EH, Briscoe DA, and Frankham R. 1998. Single large or several small? Population fragmentation in the captive management of endangered species. Zoo Biology 17: 467–480.

Marin AI. 2007. Preferencia de Plantas en la Ovoposición del Ajolote *Ambystoma mexicanum*, en Condiciones de Laboratorio. BS dissertation. National Autonomous University of Mexico. Mexico City, Mexico.

Mazari M, Mackay DM. 1993. Potential for groundwater contamination in Mexico City. Environmental Science and Technology 27: 794–802.

Mazari-Hiriart M, Ponce de León S, López-Vidal Y, Islas-Macías P, Amieva-Fernández RI, Quiñones-Falconi F. 2008. Microbiological implications of periurban agriculture and water reuse in Mexico City. PLOS ONE 3 (art. e2305).

McKnight ML, Shaffer HB. 1997. Large, rapidly evolving intergenic spacers in the mitochondrial DNA of the salamander family Ambystomatidae (Amphibia: Caudata). Molecular Biology and Evolution 14: 1167–1176.

Pérez V, Blanco LJ. 2010. Evaluación de amenazas por inundaciones en el centro de México: El caso de Iztapalapa, Distrito Federal (1998–2005). Investigaciones Geográficas 73: 22–40.

Reib C, Olsson L, Hobfeld U. 2015. The history of the oldest self-sustaining laboratory animal: 150 years of axolotl research. Journal of Experimental Zoology 324: 393–404.

Rojas TR. 1998. La Cosecha del Agua en la Cuenca de México. Ciesas.

Rubio MS. 2014. Capacidad de Carga de Refugios Experimentales para *Ambystoma mexicanum* en Xochimilco, México. Master's thesis. National Autonomous University of Mexico, Mexico City, Mexico.

Shaffer HB, Voss, SR. 1996. Phylogenetic and mechanistic analysis of a developmentally integrated character complex: Alternate life history modes in ambystomatid salamanders. American Zoologist 36: 24–35.

Smith HB. 1989. Discovery of the axolotl and its early history in biological research.

Pages 3–12 in Malacinski GM, Armstrong JB, eds. Developmental Biology of the Axolotl. Oxford University Press.

Tovar A. 2014. Determinación de la Estructura Trófica de Refugios Experimentales en Xochimilco, México. Master's thesis. National Autonomous University of Mexico, Mexico City, Mexico.

Valek-Valdés G. 2000. Agua Reflejo de un Valle en el Tiempo. Universidad Nacional Autónoma de México.

Valiente E, Zambrano L. 2010. Creating refuges for the axolotl (*Ambystoma mexicanum*). Ecological Restoration 28: 257–259.

Voss SR, Epperlein HH, Tanaka EM. 2009. *Ambystoma mexicanum*, the axolotl: A versatile amphibian model for regeneration, development, and evolution studies. Cold Spring Harbor Protocols 8 (art. pdb.emo128).

Zambrano L, Vega E, Herrera G, Prado EA, Reynoso VH. 2007. A population matrix model and population viability analysis to predict the fate of an endangered species in a highly managed water system. Animal Conservation 10: 297–303.

Zambrano L, Valiente E, VanderZanden MJ. 2010. Stable isotope variation of a highly heterogeneous shallow freshwater system. Hydrobiologia 646: 327–336.

Zambrano L, Cordova-Tapia F, Ayala CAA, Gálvez KL, Ortíz HGA, Navarro-Pérez de Leon NM, Manzur-Trujillo D, Pacheco RM, Acosta SSD, Aguilar AL. 2013. Opinión Sobre los Posibles Impactos de la "Autopista Urbana Oriente Tramo Muyuguarda-Bilbao." Unión de Científicos Comprometidos con la Sociedad. (7 October 2015; *www.uccs.mx/images/library/file/Observatorio_socioambiental/MIAS/Impactos%20ambientales_AUO_GAMIA.pdf*)

Zambrano L, Mena H, Ayala C, Merlo A, Tovar A, Sumano C, Navarro M, Rubio M, Trejo M. 2015. Plan de Acción para la Conservación de *Ambystoma mexicanum*. Laboratorio de Restauración Ecológica, Instituto de Biología, National Autonomous University of Mexico, Convenio con la Autoridad de la Zona Patrimonio de Xochimilco, Tláhuac y Milpa Alta.

S. Randal Voss and M. Ryan Woodcock are affiliated with the Department of Biology at the University of Kentucky, in Lexington. Luis Zambrano (zambrano@ib.unam.mx) is affiliated with the Departamento de Zoología at the Instituto de Biologia at the Universidad Nacional Autonoma de Mexico.