

The significance of conservation knowledge for the axolotl: understanding, importance and strategies.

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Abstract

Biodiversity loss has emerged as a global concern. Among declining populations, amphibians have experienced alarming deterioration due to human activities. Amphibians are crucial indicators of environmental stress, facing threats in both aquatic and terrestrial realms. The axolotl (*Ambystoma mexicanum*), an enigmatic amphibian species with cultural, scientific, and ecological importance, has encountered a sharp decline in population, warranting its categorization as critically endangered. Notably, axolotls exhibit remarkable regenerative abilities, enhancing their scientific and ecological significance. This paper explores the importance of conservation knowledge for the axolotl, focusing on understanding its decline, highlighting its significance, and discussing several conservation strategies. By evaluating the current conservation status of the axolotl, the urgent need for effective conservation measures is emphasized. Understanding the causes of decline and implementing targeted conservation measures are crucial for preventing the extinction of this unique species. This paper highlights the necessity of conservation knowledge and the importance of proactive conservation efforts to safeguard the axolotl and preserve its invaluable ecological and cultural legacy.

Introduction

Global biodiversity loss is a pressing issue of concern. While the exact number of species being extinct remains uncertain, the current rate of extinction is believed to surpass any observed in the past 100,000 years (Hussain & Pandit, 2012). Within the realm of declining populations, amphibians have experienced significant declines over the past 25 years worldwide caused directly and indirectly by human activities (Beebee & Griffiths, 2005). The concern surrounding amphibians stems, in part, from their role as indicators of environmental stress. As larvae, they closely interact with water, while as adults, they have contact with both aquatic and terrestrial environments, making them susceptible to stressors in both realms (Blaustein & Wake, 1995).

The increased attention towards amphibian biology and the recognition of a potential global pattern of decline and loss began in 1989 during the First World Congress of Herpetology. Historical data indicates that the decline initiated as early as the 1970s in the Western United States and northeastern Australia (Stuart et al., 2004). Amphibians face a greater threat level compared to birds and mammals, with 41% of the total 7,486 known species being globally threatened, in contrast to 13% of birds and 27% of mammals. Alarming numbers of amphibian species are teetering on the brink of extinction, with 722 species listed as critically endangered according to the International Union for Conservation of Nature (IUCN) (IUCN, 2022).

The Mexican axolotl (*Ambystoma mexicanum*), a neotenic salamander endemic to the Mexican Central Valley (MCV), has experienced a significant decline in population numbers (Contreras et al., 2009). This decline has led to the axolotl being classified as critically endangered on the IUCN Red List (IUCN SSC Amphibian Specialist Group, 2020). The axolotl holds cultural and societal importance, as it is intertwined with the Aztec mythological figure Xolotl, who defied sacrifice and transformed into the axolotl as we know it today. Additionally, the axolotl is shedding light on its remarkable abilities in anti-aging and regenerating damaged body parts (Voss et al., 2015). This unique amphibian presents a conservation paradox, as it thrives in captive settings while its wild population in Mexico faces the imminent threat of extinction (Vance, 2017).

Gaining an understanding of the reasons behind the decline in axolotl populations is crucial for explaining their vulnerability and developing strategies to prevent their extinction. In order to implement effective conservation measures, it is essential to identify and address the threats that impact the axolotl. The IUCN Red List serves as a valuable conservation tool, not only because it categorizes species based on their level of threat, but also because it provides access to a wealth of data collected to support these assessments, which is readily available and searchable online (Rodrigues et al., 2006).

This paper aims to highlight the significance of existing conservation knowledge regarding the axolotl and emphasize the importance of such knowledge. The literature used in this descriptive study was sourced from Google Scholar and SmartCat. Initially, the conservation status of the axolotl is evaluated, and the criteria used for its assessment are discussed in greater detail. To retrieve relevant papers, search terms such as "IUCN critique" and "Red List axolotl" were utilized. Subsequently, the scientific and ecological significance of the axolotl is explored to address the question of why conserving this species is of utmost

importance. Relevant papers were obtained using search terms like "importance conservation" and "importance axolotl". Finally, various conservation strategies that have been implemented are elucidated to provide a clearer understanding of the efforts already undertaken in this regard. To obtain proper papers, search terms like "conservation management (axolotl)" were used.

The axolotls' conservation status and its importance

Biodiversity loss is a global crisis and is accelerating in amplitude (Wood et al., 2000). Human modifications to ecosystems have a profound impact on life on our planet. Humans benefit in many ways of biodiversity, serving for instance as a source for medicine, food, fuel, and human well-being. Unfortunately, this destructive trend is likely to continue in the future. Evidence reveals that biodiversity also influences the characteristics of ecosystems, consequently shaping the advantages that humans derive from these natural systems (Díaz et al., 2006). The loss of biodiversity exerts a harmful influence on the functionality of ecosystems. For instance, when biodiversity declines, the efficiency of ecological communities in capturing vital resources, generating biomass, decomposing organic matter, and recycling essential nutrients is diminished. Furthermore, ecosystem stability decreases over time, and alterations within ecosystems occur at an accelerated pace as the extent of biodiversity loss increases (Cardinale et al., 2012).

Conservation biology is the science to preserve biodiversity by providing principles and tools (Soulé, 1985). In 1980, The World Conservation Strategy presented the initial definition of conservation as follows: *"The management of human utilization of the biosphere in a manner that maximizes sustainable benefits for the current generation while safeguarding its capacity to fulfill the needs and desires of future generations."* This strategy primarily emphasized the preservation of essential ecological processes and life systems, the conservation of genetic diversity, and the establishment of sustainable practices regarding species and ecosystems. However, this definition has faced criticism due to its focus on the instrumental value of nature for mankind, rather than recognizing the intrinsic value of protecting nature for its own sake (Hamblen & Canney, 2013). There is still an ongoing debate about the proper definition of conservation since scientists with different interests face each other. This debate is also referred to as the "parks vs people" debate, centering around the dilemma whether scientists should prioritize nature preservation or human well-being (Miller et al., 2011). The definition of conservation utilized in this paper originates from Chris Sanbrook, a conservation social scientist, who defines it as: *"Actions that are intended to establish, improve, or maintain good relations with nature."* This definition highlights that conservation involves active actions, aiming to establish, improve, or maintain relations with nature. It recognizes that conservation efforts can create new connections or enhance existing ones. While intentions may be good, not all conservation actions achieve their desired outcomes. This definition portrays conservation as a positive endeavor to foster harmonious relationships with nature, accommodating diverse interpretations of these relationships. It also allows for varying understandings of the entities involved, including people and non-living geodiversity (Sandbrook, 2015).

In order to be part of the conservation agenda and make applicable management decisions, it is essential to conduct analysis of the conservation status. The Red List of Threatened Species created by the International Union of Conservation of Nature (IUCN), established in 1964, is widely acknowledged as the most extensive assessment for documenting the present state of biological diversity of all groups of multicellular organisms (Dahlberg & Mueller, 2011). The IUCN Red List is a tool that plays a crucial role in informing and motivating action for biodiversity conservation and policy reform. It provides valuable information on various aspects such as species range, population size, habitat and ecology, utilization and trade, threats, and recommended conservation measures. This

comprehensive data aids in making informed conservation decisions, ensuring the protection of the essential natural resources required for our survival. The IUCN Red List currently includes over 150.300 species, out of which more than 42.100 species are threatened with extinction (IUCN, 2022).

The categories of the IUCN Red List are designed to indicate the probability of a species becoming extinct within the assessed scale (i.e., regional, national, or global). The initial division in determining the threat status assessment revolves around whether a species has undergone evaluation or not (Figure 1). When there is insufficient knowledge about species, species belong in the Not Evaluated (NE) section. Species belong to Not Applicable (NA) when they are not native to the region or taxonomically uncertain. The other categories are relevant for evaluation. Data Deficient (DD) is assigned to species when there is insufficient information available to make a reliable assessment of their threat status. If a species is assigned to DD, conservation scientists can use this knowledge gap to provide more in-depth research. The category of Least Concern (LC) is used for species that are not considered threatened or near threatened. The category Near Threatened (NT) is applied to species that are not currently classified as threatened but may be at risk of becoming so in the future. On the other hand, the categories Vulnerable (VU), Critically Endangered (CR), and Endangered (EN) are assigned to species that meet the criteria for being considered threatened. The designation Regionally Extinct (RE) is used when there is certainty that the last individual of a species has disappeared from the assessed region. Species are assigned to Extinct in the Wild (EW) when they are only present in captivity (Dahlberg & Mueller, 2011; IUCN, 2012).

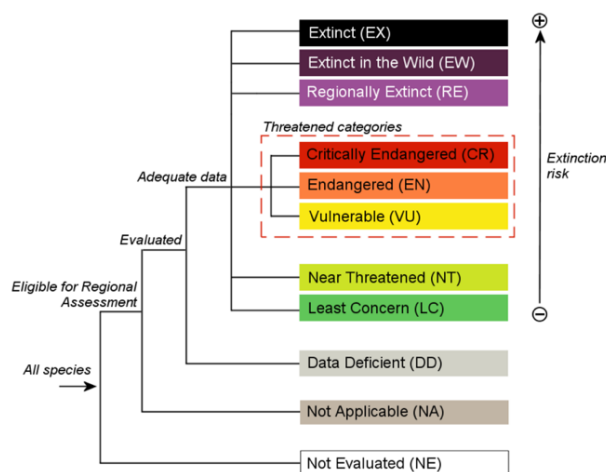


Figure 1. The structure of the IUCN categories used to evaluate species in the Red List (IUCN, 2012).

The species that contain adequate data are evaluated against five different criteria (A-E) (Figure 2). A taxon has to meet only one of the five criteria to be assigned to a level of threat. The highest threat category is used when multiple criteria are met. Criterion A is about the reduction of a species population size when a population is more than 15% declined over a time span of 10 to 50 years. Criterion B describes the reduction of their geographic range. This is evaluated with the extent of occurrence (EOO) and/or the area of occupancy (AOO) (IUCN, 2012). EOO is a measure of the geographical range occupied by a taxon and is utilized to evaluate the impact of disturbances, such as habitat degradation,

fragmentation, ongoing decline, or significant fluctuations in the taxon's population within its range (de Castro Pena et al., 2014). While EOO is a probability, AOO is the actual known area within the EOO that is occupied by the desired species (Álvarez-Martínez et al., 2018). Criterion C is about small population sizes with ongoing declines. Criterion D is about very small or restricted populations. And criterion E is met when quantitative analysis indicates the probability that a species might go extinct over a given time. A more detailed summary of the five criteria is found in the appendix.

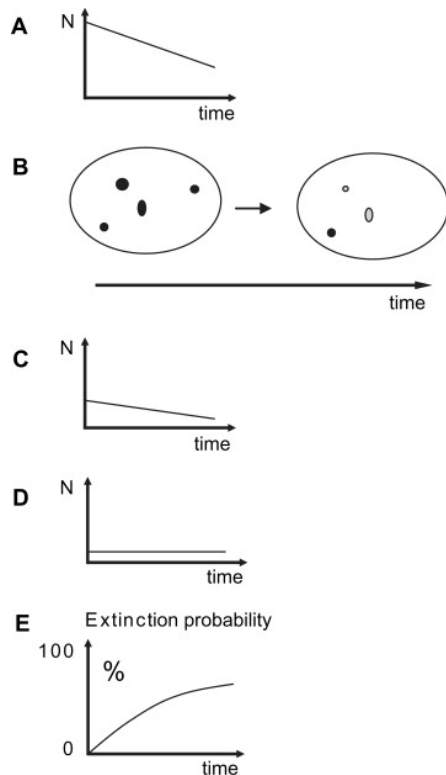


Figure 2. The five criteria in the Red List of IUCN: (A) reduction of population size (criterion A); (B) reduction of geographic range (criterion B); (C) small total population size have ongoing declines (criterion C); (D) very small or restricted populations (criterion D); (E) quantitative analysis assesses probability of species going extinct over time (criterion E) (Dahlberg & Mueller, 2011).

The axolotls' conservation status

The axolotl has been assessed by the IUCN in 2019 and is listed as critically endangered under criterion A2abce. The species is classified as critically endangered due to a significant population decline of more than 80% over the past three generations (16.5 years). This decline is attributed to the cumulative effects of urbanization, pollution of waterways resulting from urban development, as well as predation and competition from invasive species (IUCN SSC Amphibian Specialist Group, 2020).

Geographic range

The axolotl is a neotenic salamander found exclusively in the Mexican Central Valley (MCV). MCV is characterized by a network of interconnected lakes and wetlands. However, as Mexico City expanded across the valley, the lakes were reduced to a few small and disrupted remains (Contreras et al., 2019; Recuero et al., 2010). The distribution of the axolotl steadily diminished, and now it is only found in three last remaining strongholds, the

Xochimilco Lake, Chalco Lake, and Chapultepec Lake in the southeastern part of the MCV (Figure 3). These water systems provide a variety of activities and processes that have a direct influence on water quality, including traditional agriculture methods like chinampas, the presence of greenhouses, tourism, and ongoing urban development (Contreras et al., 2019). Axolotls' current EOO is estimated to be 467 km² (IUCN SSC Amphibian Specialist Group, 2020).

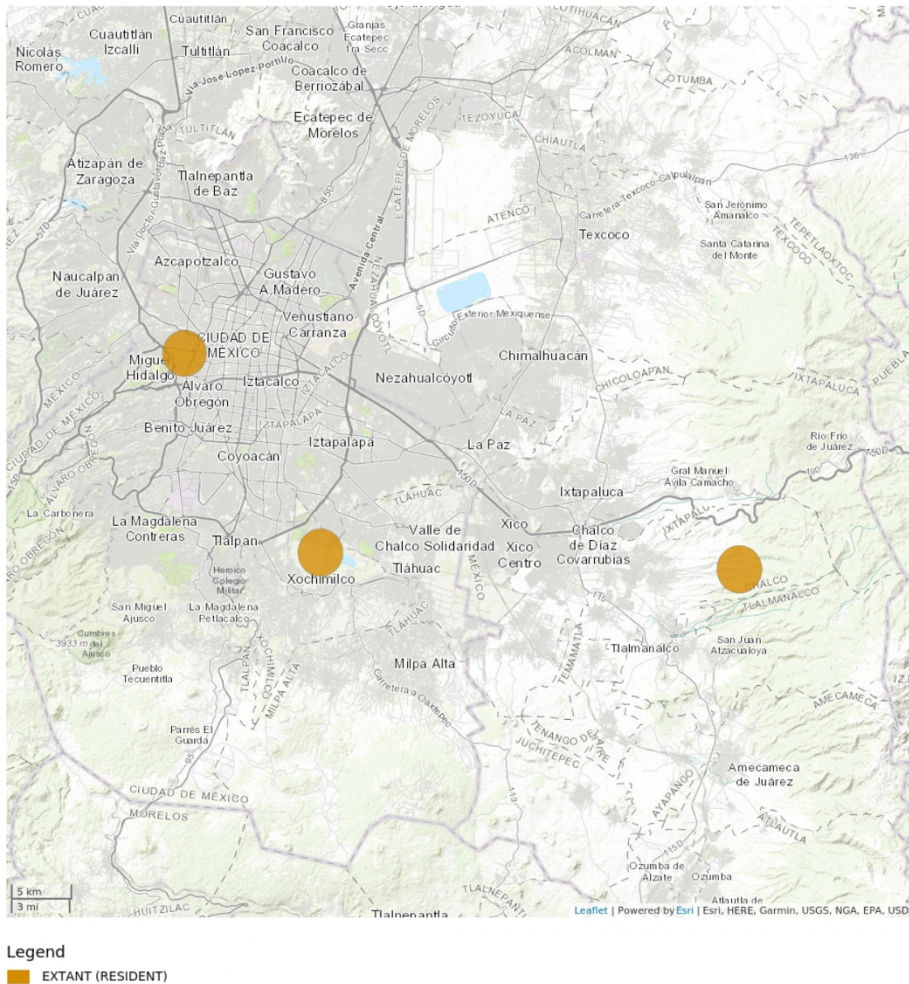


Figure 3. Distribution map of the axolotl in the Xochimilco area, Chalco Lake, and Chapultepec Lake in Mexico (IUCN SSC Amphibian Specialist Group, 2020).

Ecological niche modelling predicted 11 potential areas in six isolated and scattered sites within Xochimilco. The channels in Xochimilco exhibit high heterogeneity and spatial complexity, influenced by various variables that are affected by different levels of urbanization, land use, and water use (Table 1). The environmental variability within this relatively small area is evident in the patchiness and fragmentation of axolotl distribution, reflecting the diverse habitats and fragmented nature of their habitat within Xochimilco. Remarkably, most areas predicted as suitable for the species are associated with chinampas, which is highly heterogeneous, and is divided into small areas (Contreras et al., 2019).

Table 1. The table presents the average values of limnetic variables and the abundance of axolotls in the occurrence sites. The abundance column includes two values: the first value represents the number of organisms collected in 2002-2003, which were utilized for model construction, while the second value indicates the number of organisms observed during the validation sampling in 2006. The limnetic variables in the table are temperature (T), conductivity (Cond), and turbidity (Tur) and dissolved oxygen (DO) (Contreras et al., 2019).

Channel	Land use	Depth (m)	T (°C)	Cond ($\mu\text{S cm}^{-1}$)	pH	Tur (NTU)	DO (mg l^{-1})	NH ₄ -N (mg l^{-1})	PO ₄ -P (mg l^{-1})	NO ₃ -N (mg l^{-1})	Axolotl abundance (02-03/06)
Japon	Chinampa	0.90	22.69	0.71	7.94	63.80	5.89	0.87	6.70	4.93	1/0
Bordo	Chinampa	0.70	22.13	0.57	9.19	76.00	6.74	0.25	1.13	2.85	2/0
Urrutia	Chinampa/urban	1.00	16.17	0.94	6.97	6.10	0.07	1.56	9.20	8.93	26/0
Toro	Chinampa	0.60	20.95	0.99	8.10	30.80	3.25	0.79	11.90	0.57	1/0
Celada	Chinampa	0.60	20.95	0.99	8.10	30.80	3.25	0.79	11.90	0.57	1/0
Virgen	Chinampa	0.50	20.80	0.70	8.30	89.50	9.05	0.71	8.40	0.00	0/1

Population

The axolotl has experienced a significant decline in population size attributed to habitat transformation (Contreras et al., 2019). The population density measured in Xochimilco in 2004 is almost six times lower than in 1998 (0,0012 org/m², 0,006 org/m², respectively) (Graue, 1998; Zambrano et al., 2007). The long-term survival of this species in its natural habitat is uncertain due to several factors, including population isolation, water pollution, eutrophication, the introduction of non-native species, and overharvesting. Chemical contaminants can disrupt hormone levels and interfere with reproductive development in amphibians, leading to recruitment disruptions in natural populations. Hormonal disruption has been suggested as a potential cause for sex ratio imbalances in the Xochimilco population. Water pollution can also weaken amphibian immune systems, making them more susceptible to parasitic infections and infectious diseases. These combined factors have raised concerns about the survival of the Mexican axolotl (Recuero et al., 2010).

Population viability analysis (PVA) is a technique specifically designed to examine the dynamics of small populations. It employs simulations to project changes in population size over time and assesses the risk of extinction within a defined timeframe. PVA achieves this by tracking the fate of each individual within the population at various stages of growth. Studies employing these methods in amphibian demographics have provided valuable insights for the conservation of these species. Consequently, this type of analysis has become indispensable for species facing challenges such as small population sizes, limited distribution ranges, and multiple threats arising from highly modified environments

The population extinction probability, calculated with PVA, initially appears to be low, as depicted in Figure 4. However, a slight decline in survival rate or fecundity can significantly increase the likelihood of extinction. A minor reduction in the survival rate results in a 70% increase in the probability of extinction within 20 years, whereas a decrease in fecundity only raises the likelihood by 30%. Over a 50-year period, the probability of extinction approaches 100%. These findings underscore the sensitivity of population viability to relatively small changes in vital rates, emphasizing the need for effective conservation measures to mitigate the risk of extinction in the long term (Zambrano et al., 2007).

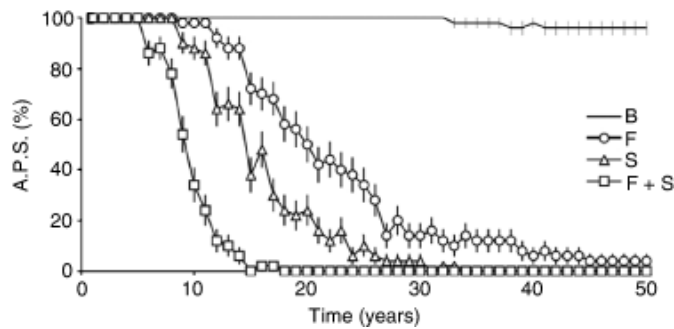


Figure 4. The extinction probabilities of the axolotl population over a 50-year period. The graph include the basic matrix model (B), a reduction in fecundity values (F), a reduction in survival probabilities (S), and a combined reduction in both fecundity values and survival probabilities (F+S). The percentages of average population survival (A.P.S.) are used to assess the extinction probabilities. The graph displays the extinction probabilities for each model, with vertical lines indicating the standard errors associated with the estimates (Zambrano et al., 2007).

No comprehensive density study has been conducted on the Chalco subpopulation. However, available evidence indicates that it is characterized by a small population. Additionally, the Chalco system is highly unstable and faces a significant risk of disappearing in the near future. From 2013 until 2019, the only site where axolotls were spotted is Chapultepec Lake, which is an artificial cement lake. Hence, it is believed that the population, with approximately 50-1000 mature individuals, has experienced a decline of 80% or more within the past three generations (2003-2019) (IUCN SSC Amphibian Specialist Group, 2020).

Threats

The primary threat to the axolotl is the fragmentation and pollution of its habitat. In the pre-Columbian era, the MCV's aquatic environments provided optimal conditions for axolotls to thrive. The Aztecs engineered these habitats, creating a network of canals and wetlands that significantly expanded the shoreline. Within these transformed habitats, axolotls flourished and served as a plentiful and easily accessible food source. Besides being a source of food, the Aztecs also worshiped the axolotl because the God Xolotl transformed itself into the axolotl to avoid being sacrificed. When the Aztec empire fell, the habitat deterioration started since urbanization provides a burden for water supply and overexploitation initiated. Since Xochimilco does not receive fresh water and less than half of the water from Mexico City is being recharged, the lake became polluted (Voss et al., 2015). Small levels of contaminants, even if they do not result in significant physiological impacts, can disrupt the food chain, and consequently modify the dynamics of predator-prey relationships and competition among prey species (Chaparro-Herrera et al., 2013).

Another threat to the axolotl is illegal trading. In the 19th century, French explorers discovered and captured 34 aquatic salamanders, now known as axolotls, from Lake Xochimilco in Mexico and transported them to Paris to introduce them to the scientific world. Currently, the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) regulates the trade of all axolotls, including those destined for science (Gresens, 2004). It is now believed that the species used for international pet trade are captive bred (IUCN SSC Amphibian Specialist Group, 2020).

Furthermore, the introduction of the exotic Nile tilapia fish (*Oreochromis niloticus*) and common carp (*Cyprinus carpio*) poses as another threat. They affect the axolotls' population size by competing for the same resources, reducing water quality, and predated on their eggs, larvae, and juveniles (Alcaraz et al., 2018; Chaparro-Herrera et al., 2013).

Another factor associated with axolotl decline is due to the chytrid fungus *Batrachochytrium dendrobatidis*. *Batrachochytrium dendrobatidis* impacts the keratinized skin of adult amphibians, leading to an osmotic imbalance that can result in the death of these organisms. The emergence of chytridiomycosis has been responsible for population declines and even extinctions of nearly 200 amphibian species. The success of chytrids within amphibian hosts is influenced by various environmental factors, including pH levels, temperature, salinity, oxygen concentration, and other factors that can affect their growth (Parra-Olea et al., 2022). It is thought that axolotls are currently not susceptible to the disease but might be in the future (IUCN SSC Amphibian Specialist Group, 2020).

The axolotls' regenerative ability

The axolotl has the ability to completely regenerate missing body parts (e.g., limbs, tail, eye, brain, and internal organs) as an adult throughout its life. One hypothesis explaining the regenerative capabilities of the Urodele group of amphibians, including salamanders and newts, is that the divergence in regenerative ability may be linked to the retention of juvenile characteristics (such as the axolotl) even after they reach sexual maturity. This phenomenon, known as paedomorphosis, suggests that the axolotl's cells retain some embryonic-like properties due to the absence of complete metamorphosis, enabling them to regenerate throughout their lifetime (McCusker et al., 2015). This idea aligns with observations in anuran amphibian species, like the African clawed frog (*Xenopus laevis*), which exhibit robust regeneration at early larval stages but lose this ability progressively during differentiation and metamorphosis (Suzuki et al., 2006).

Another hypothesis for explaining the difference in regenerative capacity suggests that the Urodele group possesses a simpler adaptive immune system. It is proposed that their regenerative ability may depend on a weak inflammatory response. However, conflicting observations on multiple organisms suggest different roles between the immune system and the regenerative processes (McCusker et al., 2015).

Limb regeneration in axolotls is triggered by injury, which initiates a wound healing process. In the presence of pro-regenerative signals, cells in the vicinity of the wound are mobilized and come together to form a specialized structure called a blastema. The blastema then undergoes growth and pattern formation to regenerate the missing limb structures (McCusker et al., 2015). In a study done by Kragl et al. (2009), a green fluorescent protein (GFP) was injected in the axolotl to track the limb tissues during regeneration. They found that the blastema contained progenitor cells and therefore retain the memory of their embryonic origin, instead of pluripotent cells that was originally thought.

Anthropogenic stressors may influence the regenerative ability of axolotls. Although there is not done a study about this, there are studies done for other species that are either from the same group of species or also have regenerative abilities. The introduction of heavy metals and organic compounds to several crustaceans can inhibit molting and limb

generation, leading to severe consequences. The inability to regenerate lost limbs would likely have negative consequences on feeding, social status, and mating success, ultimately impacting overall fitness (van Son & Thiel, 2007).

Another study was done where the effect of artificial light at night (ALAN) on tail regeneration of the eastern redbacked salamander (*Plethodon cinereus*) was examined. Their findings revealed that even minimal levels of ALAN had a negatively significant impact on tail regeneration in the salamander. However, the relationship between ALAN and tail regeneration was not straightforward but rather exhibited a more intricate pattern (Figure 5). Regeneration rates were highest in the dark control (0.0001 lx) and 1 lx treatment groups, while they were significantly lower in the 0.01 and 100 lx light treatment groups.

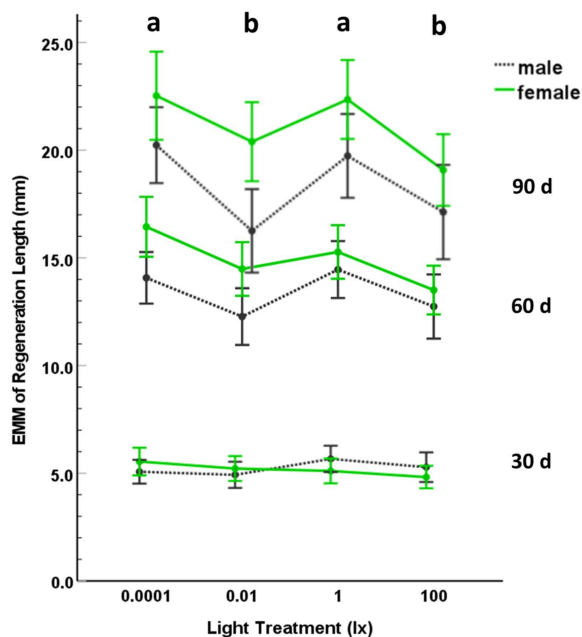


Figure 5. The graph displays the estimated marginal means (EMM) of regeneration length (mm) for male and female salamanders after being exposed to nocturnal ambient lighting of 0.0001, 0.01, 1, or 100 lx for 30, 60, or 90 days. The error bars represent the 95% confidence intervals. Treatments with different letters indicate significant differences (Wise et al., 2022)

The tail serves various functions in salamanders, such as energy storage, signaling and territorial defense, antipredator behavior, and more. The ability to regenerate the tail rapidly can have implications for survival and reproductive success due to the importance of these functions. They propose that the intricate, non-linear relationship between tail regeneration and varying levels of ALAN observed in their study is not influenced by prey behavior. Instead, they suggest two potential factors: (1) physiological mechanisms regulated by hormones such as melatonin, serotonin, prolactin, and corticosterone, and (2) genetic factors where distinct sets of genes may be activated under different scotopic illuminations, leading to variations in endocrine responses to different levels of ALAN (Wise et al., 2022).

The importance of conserving the axolotl

Many species, including humans, lose the ability to regenerate complex structures when reaching adulthood. There are many age-related pathologies such as increased DNA damage and cellular senescence, decreased metabolism, and depletion of adult stem cell pools in

humans. Axolotls exhibit extraordinary regenerative abilities that persist throughout their lifespan. While their regenerative capacity may decline with age, they still possess remarkable regenerative capabilities that surpass those of humans and other mammals. Studying regeneration in the axolotl can help us about understanding and minimizing aging pathologies in humans. Knowledge about the axolotls can for instance be applied to wound healing or the understanding of age-related oncogenesis in humans (Vieira et al., 2020).

Axolotls are primarily detritivores, with Zooplankton is the main food of the larvae. Although adults are also known to consume fish, making them also carnivores (Chaparro-Herrera et al., 2013; Zambrano et al., 2010). Although there is not done any study about the ecological importance of solely the axolotl, it is common knowledge that detritivores are an indispensable pathway of energy and nutrient flux in ecosystems. They modify habitat structures and resource availability since they biologically influence the sediments. A study done with a detritivores fish (*Prochilodus mariae*) showed that the excluding of this fish negatively changed the community structure and function of its ecosystem (Flecker, 1996).

Amphibians in general play crucial roles in ecosystems, serving as prey, predators, and herbivores. Axolotls' dual roles as detritivores and carnivores make their presence and interactions within trophic networks vital for shaping the overall dynamics of these ecosystems. Therefore, the loss of axolotls is likely to have far-reaching impacts on other organisms that depend on them for food, regulation of populations, and ecosystem functioning (Hussain& Pandit, 2012).

Conservation strategies

The conservation of endangered species involves a three-phase approach: identification, protection, and recovery. During the identification phase, the focus is on determining which species are at risk of extinction. In the protection phase, short-term measures are implemented to halt the decline of the species. Finally, in the recovery phase, long-term measures are implemented to rebuild the population and remove the species from the threat of extinction. To develop an effective protection plan, two key pieces of information are essential. Firstly, it is crucial to understand the specific threats faced by the endangered species. Secondly, knowing the geographic distribution and land ownership of the species' habitat is vital in guiding conservation strategies.

The IUCN has extensively assessed the conservation status of birds, mammals, and amphibians, providing a comprehensive overview of global threats to these groups (Figure 6). According to the IUCN, habitat destruction is the most pervasive threat to amphibians, affecting 88% of imperiled amphibian species. This knowledge of threats and distribution helps inform conservation efforts, enabling targeted protection and recovery actions. By addressing habitat destruction and implementing appropriate measures, it is possible to mitigate the risks and work towards safeguarding endangered species for future generations.

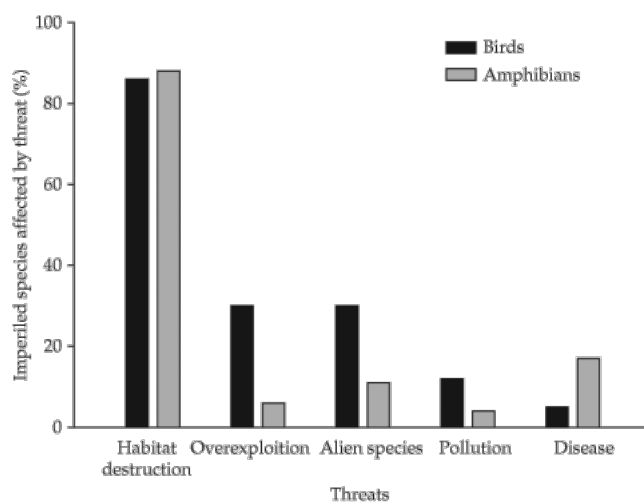


Figure 6. The graph presents the percentage of imperiled birds (black bars) and amphibians (grey bars) worldwide that face various threats, as determined through comprehensive analyses conducted by the IUCN. The total percentages for each group surpass 100% because many species are threatened by multiple factors (Baillie et al., 2004).

The objective of the recovery phase is to ensure the long-term survival of the endangered species by rebuilding their populations, restoring their habitats, and reducing threats to a point where they are no longer at risk of extinction. The management of endangered species implements a set of actions aimed at mitigating the threats faced by the species, ultimately leading to the establishment of populations that no longer require special protection (Sodhi & Ehrlich, 2010). Management strategies can be focused on either the species/population level, involving activities such as translocation, captive breeding, and predator control, or at the habitat level, which entails initiatives such as restoring habitat quality and acquiring additional habitat (Hoekstra et al., 2002). A balance between science, policy, economics, and sociology is necessary for creating the best outcome in endangered species

management (Sodhi & Ehrlich, 2010). Society has tried several management strategies for the conservation of the axolotl as elaborated below.

Flagship species

In 2002, a collaborative conservation project, called The Darwin Initiative Project, was launched by the Durrell Institute of Conservation and Ecology and Universidad Autónoma Metropolitana Unidad Xochimilco with the aim of preserving the biodiversity of Lake Xochimilco. The project focused on conservation education and the promotion of nature tourism, utilizing the Axolotl as a flagship species (Bride et al., 2008). Flagship species are iconic species that are often used to raise funds and awareness for conservation projects. While the effectiveness of flagship species as a conservation tool has been debated, this project demonstrated positive outcomes (Bowen-Jones & Entwistle, 2002).

The project successfully raised awareness about the axolotl, garnering support and funding for conservation efforts. The axolotl served as a rallying point, drawing attention to the broader issue of conserving the Xochimilco system. Although the project did not aim to immediately eliminate the threats to the axolotl and the lake, which include habitat degradation, pollution, introduced fish, and illegal collection, addressing these challenges requires the active involvement of local communities.

Recognizing that the livelihoods of local people are intertwined with the lake, the project sought to engage the community rather than impose solutions. By increasing awareness and fostering positive experiences among tourists, it is hoped that they will share their knowledge and enthusiasm with their fellow peers, thereby generating a ripple effect of greater awareness and engagement. While the project's scope was limited by time and budget, it successfully demonstrated the strategic importance of using flagship species to raise public awareness and mobilize support for conservation efforts (Bride et al., 2008).

Relocation

The use of proactive conservation techniques, such as translocations or relocations into created and restored wetlands, has gained popularity in recent years. However, the success of these techniques can be hindered by factors like high predation rates and low environmental adaptability (Griffin et al., 2000). A study is done to determine whether La Cantera Oriente, ponds within an artificial wetland, can serve as a temporary refuge for viable populations of axolotls. Additionally, a comparative analysis of the ponds was conducted to assess their suitability for axolotl reproduction and the survival of offspring. This preliminary study revealed that two out of four ponds exhibit favorable abiotic and biotic conditions for various critical stages of the axolotl life cycle. Based on these findings, future conservation plans for La Cantera Oriente entail the release of adult axolotls into those ponds, followed by continuous monitoring of their behavior and habitat utilization. This monitoring will help identify variables that can be modified in Xochimilco to enhance population growth and support the long-term conservation of the axolotl species (Ramos et al., 2021).

Habitat restoration

Over fifteen years ago, a restoration program was initiated in Xochimilco with the dual objective of restoring the wetland area and mitigating the risk of future urbanization. The restoration of this wetland holds significant importance due to the potential consequences such as disappearance, increased urban temperatures, reduced biodiversity, and heightened vulnerability to water-related disasters in the city. The axolotl played a crucial role in identifying the major threats faced by the entire ecosystem, namely the introduction of exotic fish, deteriorating water quality, and ongoing urban development (Von Bertrab & Zambrano, 2010). In response to these threats, a restoration plan called "Axolotl Refuge" was proposed, utilizing the interconnected canal system created by chinampas construction in the wetland. The strategic placement of water filters along these canals acted as barriers, preventing the entry of exotic species and improving water quality specifically for the axolotls. Consequently, these canals served as safe havens, protecting the axolotls from the identified threats.

Over a six-year period from 2010 to 2016, more than 20 experimental refuges were established, varying in length and width. This initial restoration effort demonstrated the high resilience of Xochimilco's canals, as they gradually returned to their natural state with the elimination of exotics and improvement in water quality. The axolotls responded positively to the improved conditions, exhibiting increased body weight and successful reproduction without the risk of predation by exotic fish, suggesting a potential population increase within the refuges (Zambrano et al., 2020).

While both local chinampa farmers (chinamperos) and academics shared the general goal of preserving Xochimilco, their specific objectives differed. The chinamperos primarily aimed to enhance their agricultural productivity, while scientists focused on generating ecological knowledge (Von Bertrab, 2013). As a result, the involvement of chinamperos in the refuge program was limited, as they were primarily enlisted to fulfill the scientific research objectives without addressing their own needs. The restoration program centered around the axolotl, treating the local farmers merely as guardians of the habitat to support the amphibian population. This approach proved unsustainable as it neglected the critical variable of the farmers' economic and practical considerations. In response to this realization, the project underwent a name change to "The Chinampa-Refuge program", reflecting a shift in focus. Its success hinges on establishing a sufficient number of refuges to transition the entire system from a perturbed state to a restored one. Achieving this shift requires a structural and cultural transformation within Mexico City society, involving all stakeholders, including the scientific community, chinamperos, city government, and financial supporters (Zambrano et al., 2020).

Removing predation

Tilapia and carp were introduced as exotic predator fishes for aquaculture purposes in Xochimilco, leading to negative consequences for the axolotls (Alcaraz et al., 2015). These introduced species not only prey upon axolotls and compete for food but also degrade water quality by increasing turbidity (Chaparro-Herrera et al., 2020). Currently, tilapia and carp account for over 98% of the vertebrate biomass in the ecosystem. To improve the axolotl habitat, the first step taken was the removal of tilapia and carp populations, which

began in 2004. In collaboration with local officials, scientists engaged local fishers to catch these fish, which were then sold to a company producing fishmeal (Staff, 2008).

During a period spanning several months between 2006 and 2008, approximately 7.5 tons of fish were harvested from Xochimilco per week. However, despite the intensive removal of carp and tilapia, the decline of the axolotl population has not been halted, and there may be time delays in observing ecosystem-level changes. Other factors, such as poor water quality, may also contribute to the axolotl decline. While the removal of exotic species is likely to be beneficial, additional strategies are necessary. One potential approach is the establishment of areas free from exotic species, serving as refuges for axolotls. It is crucial to implement a comprehensive restoration program that goes beyond exotic species removal to address the multiple factors influencing the axolotl's survival and promote favorable conditions within the food web (Zambrano et al., 2010).

Sperm collection

Conservation Breeding Programs (CBPs) play a crucial role in the sustainable management of endangered amphibian species. Incorporating spermatozoa in CBPs offers several benefits, including the preservation of male genetic diversity, cost reduction, enhanced biosecurity, decreased reliance on large captive populations, the ability to fertilize a single female's eggs using sperm from multiple genetically diverse males, and minimized necessity for animal transportation (Browne et al., 2019). By collecting spermatophores of axolotls, genetic variation is promoted, and ex situ mating activities is provided (Figiel, 2020).

Discussion

This study highlights the significance of existing conservation knowledge related to the axolotl and emphasizes its importance. Through an evaluation of the conservation status, criteria used for assessment, and examination of the scientific and ecological significance of the species, it becomes evident that preserving the axolotl is crucial. The findings underscore the need for concerted efforts to protect this unique amphibian species. The exploration of various conservation strategies further reinforces the importance of proactive measures already undertaken. Moving forward, it is essential to continue building upon the existing knowledge base and implementing effective conservation practices to ensure the long-term survival of the axolotl and maintain the integrity of the ecosystems in which it plays a significant role.

To be included in conservation efforts and facilitate informed management decisions, it is crucial to analyze the conservation status of species. The IUCN Red List is widely recognized as the most comprehensive assessment of the current state of biodiversity. The axolotl is classified as critically endangered under criterion A2abce. This classification is attributed to a significant population decline exceeding 80% over the past three generations (approximately 16.5 years). The decline of the axolotl is primarily attributed to the cumulative impact of urbanization, pollution of waterways resulting from urban development, as well as predation and competition from invasive species.

Complexities and limitations associated with assessing extinction risk using criterion A arise. Criterion A relies on assessing population decline over a specific time period, often the most recent 10 years or three generations. This criterion allows even widespread and common species with large populations to be considered threatened if they are experiencing rapid declines. This is justified by the understanding that continued decline, even in large populations, could eventually lead to extinction. Accurately measuring species population trends is challenging because imperfect sampling and short-term stochastic variations in population levels caused by environmental variability introduce observation errors and process errors, respectively. Incorrect measurements can result in incorrect Red List classifications, including false positives (incorrectly classifying a species as threatened) and false negatives (failing to classify a species that should be listed as threatened). When process errors and observation errors increase, the reliability of detecting population declines decreases (Fox et al., 2019).

Besides this, criterion A2 states that the cause of decline is not well understood. Thorough analysis of the possible threats is necessary since the shape of the decline-rate curve depends on it (Mace et al., 2008). Examining these causes of reduction to the last detail and accurately measurements will be necessary to perform more specific and better conservation strategies. I propose a more comprehensive investigation into the impact of habitat deterioration on axolotls, considering the thriving conditions they experienced during the Aztec era when their environment was utilized differently. Examining the historical context and analyzing the shifts in habitat usage over time can provide valuable insights for conservation efforts aimed at preserving the axolotl species.

The scientific and cultural significance of the axolotl is quite well understood contrary to their ecological importance. Amphibians in general are of important ecological value

because of their contribution to trophic dynamics. No articles yet exist on what happens if the axolotl goes extinct, nevertheless losing an amphibian in general will change the nutrient influx and therefore altering the ecosystem structure. Investigating the ecological importance of solely the axolotl is convenient to understand the impact on the ecosystem when they are extinct.

Even though the current axolotl population is still declining, conservation strategies that were formerly and are currently used may have had some positive impact in preserving the axolotl (Bride et al., 2008). Changing the habitat by removing exotic species and/or increasing water quality are positively influencing the axolotls' living conditions. Studies looking for alternative habitats are on-going. The success rate of relocation, repatriation and translocation programs in amphibians and reptiles are lower than for birds and mammals. Nevertheless, these conservation strategies are still useful because the chance that it will work is present (Dodd Jr & Seigel, 1991). Since there are multiple threats, one conservation strategy on its own is not enough to save the axolotl from the brink of extinction. Different strategies must work together to tackle all the different threats to ensure a safe environment and survival.

Conclusion

The issue of biodiversity loss remains a critical concern globally, necessitating urgent conservation efforts. Understanding and assessing the conservation status of species is crucial in order to prioritize conservation actions effectively. The use of the IUCN Red List has provided a comprehensive framework for documenting the present state of biological diversity across various taxa. The Red List categories, based on quantitative thresholds, assist in classifying species into threat categories, including the critically endangered status of the axolotl. The axolotl exemplifies the importance of conservation knowledge. Its assessment and classification on the Red List shed light on the significant population decline and the urgent need for conservation measures. The criteria used in evaluating the axolotl's conservation status provide a quantitative basis for understanding the severity of its decline and the risks of extinction it faces.

To combat the decline of species like the axolotl, various conservation strategies have been implemented. These efforts aim to address the underlying causes of population decline, including urbanization, pollution, and competition from invasive species. By identifying and addressing these threats, conservation initiatives can work towards preserving the unique ecological and cultural value that species like the axolotl hold.

As has been demonstrated in this paper, safeguarding biodiversity, and understanding the conservation status of species through initiatives like the IUCN Red List are essential for effective conservation action. The case of the axolotl highlights the importance of conservation knowledge in identifying and implementing strategies to protect and preserve endangered species for future generations.

Bibliography

- Alcaraz, G., López-Portela, X., & Robles-Mendoza, C. (2015). Response of a native endangered axolotl, *Ambystoma mexicanum* (Amphibia), to exotic fish predator. *Hydrobiologia*, 753, 73-80.
- Álvarez-Martínez, J. M., Jiménez-Alfaro, B., Barquín, J., Ondiviela, B., Recio, M., Silió-Calzada, A., & Juanes, J. A. (2018). Modelling the area of occupancy of habitat types with remote sensing. *Methods in Ecology and Evolution*, 9(3), 580-593.
- Baille, J. E. M., Hilton-Taylor, C., and Stuart, S. N., eds (2004). *IUCN Red List of threatened species: a global species assessment*. IUCN, Gland, Switzerland.
- Beebee, T. J., & Griffiths, R. A. (2005). The amphibian decline crisis: a watershed for conservation biology?. *Biological conservation*, 125(3), 271-285.
- Blaustein, A. R., & Wake, D. B. (1995). The puzzle of declining amphibian populations. *Scientific American*, 272(4), 52-57.
- Bowen-Jones, E., & Entwistle, A. (2002). Identifying appropriate flagship species: the importance of culture and local contexts. *Oryx*, 36(2), 189-195.
- Bride, I. G., Griffiths, R. A., MELÉNDEZ-HERRADA, A., & McKay, J. E. (2008). Flying an amphibian flagship: conservation of the Axolotl *Ambystoma mexicanum* through nature tourism at Lake Xochimilco, Mexico. *International Zoo Yearbook*, 42(1), 116-124.
- Browne, R. K., Silla, A. J., Upton, R., Della-Togna, G., Marcec-Greaves, R., Shishova, N. V., ... & Clulow, S. (2019). Sperm collection and storage for the sustainable management of amphibian biodiversity. *Theriogenology*, 133, 187-200.
- Cardinale, B. J., Duffy, J. E., Gonzalez, A., Hooper, D. U., Perrings, C., Venail, P., ... & Naeem, S. (2012). Biodiversity loss and its impact on humanity. *Nature*, 486(7401), 59-67.
- de Castro Pena, J. C., Kamino, L. H. Y., Rodrigues, M., Mariano-Neto, E., & de Siqueira, M. F. (2014). Assessing the conservation status of species with limited available data and disjunct distribution. *Biological Conservation*, 170, 130-136.
- Chaparro-Herrera, D. D. J., Nandini, S., & Sarma, S. S. S. (2013). Effect of water quality on the feeding ecology of axolotl *Ambystoma mexicanum*. *Journal of Limnology*, 72(3), e46.
- Chaparro-Herrera, D. D. J., Nandini, S., & Sarma, S. S. S. (2020). Turbidity effects on feeding by larvae of the endemic *Ambystoma mexicanum* and the introduced *Oreochromis niloticus* in Lake Xochimilco, Mexico. *Ecohydrology & Hydrobiology*, 20(1), 91-101.
- Contreras, V., Martínez-Meyer, E., Valiente, E., & Zambrano, L. (2009). Recent decline and potential distribution in the last remnant area of the microendemic Mexican axolotl (*Ambystoma mexicanum*). *Biological conservation*, 142(12), 2881-2885.
- Dahlberg, A., & Mueller, G. M. (2011). Applying IUCN red-listing criteria for assessing and reporting on the conservation status of fungal species. *Fungal ecology*, 4(2), 147-162.
- Díaz, S., Fargione, J., Chapin III, F. S., & Tilman, D. (2006). Biodiversity loss threatens human well-being. *PLoS biology*, 4(8), e277.
- Dodd Jr, C. K., & Seigel, R. A. (1991). Relocation, repatriation, and translocation of amphibians and reptiles: are they conservation strategies that work?. *Herpetologica*, 336-350.
- Figiel, C. R. (2020). Cold storage of sperm from the Axolotl, *Ambystoma mexicanum*. *Herpetological Conservation and Biology*, 15(2), 367-371.
- Flecker, A. S. (1996). Ecosystem engineering by a dominant detritivore in a diverse tropical stream. *Ecology*, 77(6), 1845-1854.
- Fox, R., Harrower, C. A., Bell, J. R., Shortall, C. R., Middlebrook, I., & Wilson, R. J. (2019). Insect population trends and the IUCN Red List process. *Journal of Insect Conservation*, 23(2), 269-278.
- Graue, W.V. (1998). Estudio genético y demográfico de la población del anfibio *Ambystoma mexicanum* (Caudata: Ambystomidae) del Lago de Xochimilco. Instituto de Ciencias del Mar y Limnología. UNAM, Mexico.

- Gresens, J. (2004). An introduction to the Mexican axolotl (*Ambystoma mexicanum*). *Lab animal*, 33(9), 41-47.
- Griffin, A. S., Blumstein, D. T., & Evans, C. S. (2000). Training captive-bred or translocated animals to avoid predators. *Conservation biology*, 14(5), 1317-1326.
- Hambler, C., & Canney, S. M. (2013). *Conservation*. Cambridge University Press.
- Hoekstra, J. M., Clark, J. A., Fagan, W. F., & Boersma, P. D. (2002). A comprehensive review of Endangered Species Act recovery plans. *Ecological Applications*, 12(3), 630-640.
- Hussain, Q. A., & Pandit, A. K. (2012). Global amphibian declines: a review. *International Journal of Biodiversity and Conservation*, 4(10), 348-357.
- IUCN. (2012). Guidelines for Application of IUCN Red List Criteria at Regional and National Levels: Version 4.0. Gland, Switzerland and Cambridge, UK: IUCN. iii + 41pp.
- IUCN. (2012). IUCN Red List Categories and Criteria: Version 3.1. Second edition. Gland, Switzerland and Cambridge, UK: IUCN. iv + 32pp.
- IUCN. (2022). The IUCN Red List of Threatened Species. Version 2022-2. <https://www.iucnredlist.org>. Accessed on 04-06-2023.
- IUCN SSC Amphibian Specialist Group. 2020. *Ambystoma mexicanum*. *The IUCN Red List of Threatened Species 2020*: e.T1095A53947343. <https://dx.doi.org/10.2305/IUCN.UK.2020-3.RLTS.T1095A53947343.en>. Accessed on 04 June 2023.
- Kragl, M., Knapp, D., Nacu, E., Khattak, S., Maden, M., Epperlein, H. H., & Tanaka, E. M. (2009). Cells keep a memory of their tissue origin during axolotl limb regeneration. *Nature*, 460(7251), 60-65.
- Mace, G. M., Collar, N. J., Gaston, K. J., Hilton-Taylor, C. R. A. I. G., Akçakaya, H. R., Leader-Williams, N. I. G. E. L., ... & Stuart, S. N. (2008). Quantification of extinction risk: IUCN's system for classifying threatened species. *Conservation biology*, 22(6), 1424-1442.
- McCusker, C., Bryant, S. V., & Gardiner, D. M. (2015). The axolotl limb blastema: cellular and molecular mechanisms driving blastema formation and limb regeneration in tetrapods. *Regeneration*, 2(2), 54-71.
- Miller, T. R., Minter, B. A., & Malan, L. C. (2011). The new conservation debate: the view from practical ethics. *Biological Conservation*, 144(3), 948-957.
- Parra-Olea, G., Calzada-Arciniega, R. A., Jiménez-Arcos, V. H., & Hernández-Ordóñez, O. (2022). The Axolotl of Alchichica. In *Lake Alchichica Limnology: The Uniqueness of a Tropical Maar Lake* (pp. 273-288). Cham: Springer International Publishing.
- Ramos, A. G., Mena-González, H., & Zambrano, L. (2021). The potential of temporary shelters to increase survival of the endangered Mexican axolotl. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 31(6), 1535-1542.
- Recuero, E., Cruzado-Cortes, J., Parra-Olea, G., & Zamudio, K. R. (2010). Urban aquatic habitats and conservation of highly endangered species: the case of *Ambystoma mexicanum* (Caudata, Ambystomatidae). In *Annales Zoologici Fennici* (Vol. 47, No. 4, pp. 223-238). Finnish Zoological and Botanical Publishing Board.
- Rodrigues, A. S., Pilgrim, J. D., Lamoreux, J. F., Hoffmann, M., & Brooks, T. M. (2006). The value of the IUCN Red List for conservation. *Trends in ecology & evolution*, 21(2), 71-76.
- Sandbrook, C. (2015). What is conservation?. *Oryx*, 49(4), 565-566.
- Sodhi, N. S., & Ehrlich, P. R. (Eds.). (2010). *Conservation biology for all*. Oxford University Press.
- van Son, T. C., & Thiel, M. (2007). Anthropogenic Stressors and Their Effects on the. *Evolutionary Ecology of Social and Sexual Systems: Crustaceans as Model Organisms*, 413.
- Soulé, M. E. (1985). What is conservation biology?. *BioScience*, 35(11), 727-734.
- Staff, L. A. D. B. (2008). Scientists Campaign to Save Rare Species of Salamander.

- Stuart, S. N., Chanson, J. S., Cox, N. A., Young, B. E., Rodrigues, A. S., Fischman, D. L., & Waller, R. W. (2004). Status and trends of amphibian declines and extinctions worldwide. *Science*, *306*(5702), 1783-1786.
- Suzuki, M., Yakushiji, N., Nakada, Y., Satoh, A., Ide, H. & Tamura, K. (2006). Limb regeneration in *Xenopus laevis* froglet. *The Scientific World JOURNAL*, 6 Suppl 1, 26– 37.
- Vance, E. (2017). The axolotl paradox. *Nature*, *551*(28), 289.
- Vieira, W. A., Wells, K. M., & McCusker, C. D. (2020). Advancements to the axolotl model for regeneration and aging. *Gerontology*, *66*(3), 212-222.
- Von Bertrab, A. (2013). De tilapias, chinampas y ajolotes: discursos sobre la restauración ecológica en el lago de Xochimilco. PhD dissertation. Universidad Nacional Autónoma de México.
- Von Bertrab, A., & Zambrano, L. (2010). Participatory monitoring and evaluation of a Mexico City wetland restoration effort. *Ecological restoration*, *28*(3), 343-353.
- Voss, S. R., Woodcock, M. R., & Zambrano, L. (2015). A tale of two axolotls. *BioScience*, *65*(12), 1134-1140.
- Wise, S. E., Rohacek, A., Scanlon, A. E., Cabrera, T., & Buchanan, B. W. (2022). The Effects of Artificial Night Lighting on Tail Regeneration and Prey Consumption in a Nocturnal Salamander (*Plethodon cinereus*) and on the Behavior of Fruit Fly Prey (*Drosophila virilis*). *Animals*, *12*(16), 2105.
- Wood, A., Stedman-Edwards, P., & Mang, J. (Eds.). (2000). *The root causes of biodiversity loss*. Earthscan.
- Zambrano, L., Rivas, M. I., Uriel-Sumano, C., Rojas-Villaseñor, R., Rubio, M., Mena, H., ... & Tovar-Garza, A. (2020). Adapting wetland restoration practices in urban areas: Perspectives from Xochimilco in Mexico City. *Ecological Restoration*, *38*(2), 114-123.
- Zambrano, L., Valiente, E., & Vander Zanden, M. J. (2010). Food web overlap among native axolotl (*Ambystoma mexicanum*) and two exotic fishes: carp (*Cyprinus carpio*) and tilapia (*Oreochromis niloticus*) in Xochimilco, Mexico City. *Biological Invasions*, *12*, 3061-3069.
- Zambrano, L., Vega, E., Herrera M, L. G., Prado, E., & Reynoso, V. H. (2007). A population matrix model and population viability analysis to predict the fate of endangered species in highly managed water systems. *Animal Conservation*, *10*(3), 297-303.

Appendix

Table 2. Summary of the five criteria (A-E) used to evaluate if a taxon belongs in an IUCN Red List threatened category (IUCN, 2012).

A. Population size reduction. Population reduction (measured over the longer of 10 years or 3 generations) based on any of A1 to A4			
	Critically Endangered	Endangered	Vulnerable
A1	≥ 90%	≥ 70%	≥ 50%
A2, A3 & A4	≥ 80%	≥ 50%	≥ 30%
<p>A1 Population reduction observed, estimated, inferred, or suspected in the past where the causes of the reduction are clearly reversible AND understood AND have ceased.</p> <p>A2 Population reduction observed, estimated, inferred, or suspected in the past where the causes of reduction may not have ceased OR may not be understood OR may not be reversible.</p> <p>A3 Population reduction projected, inferred or suspected to be met in the future (up to a maximum of 100 years) [(a) cannot be used for A3].</p> <p>A4 An observed, estimated, inferred, projected or suspected population reduction where the time period must include both the past and the future (up to a max. of 100 years in future), and where the causes of reduction may not have ceased OR may not be understood OR may not be reversible.</p>	<p>based on any of the following:</p>		<p>(a) direct observation [except A3]</p> <p>(b) an index of abundance appropriate to the taxon</p> <p>(c) a decline in area of occupancy (AOO), extent of occurrence (EOO) and/or habitat quality</p> <p>(d) actual or potential levels of exploitation</p> <p>(e) effects of introduced taxa, hybridization, pathogens, pollutants, competitors or parasites.</p>
B. Geographic range in the form of either B1 (extent of occurrence) AND/OR B2 (area of occupancy)			
	Critically Endangered	Endangered	Vulnerable
B1. Extent of occurrence (EOO)	< 100 km ²	< 5,000 km ²	< 20,000 km ²
B2. Area of occupancy (AOO)	< 10 km ²	< 500 km ²	< 2,000 km ²
AND at least 2 of the following 3 conditions:			
(a) Severely fragmented OR Number of locations	= 1	≤ 5	≤ 10
(b) Continuing decline observed, estimated, inferred or projected in any of: (i) extent of occurrence; (ii) area of occupancy; (iii) area, extent and/or quality of habitat; (iv) number of locations or subpopulations; (v) number of mature individuals			
(c) Extreme fluctuations in any of: (i) extent of occurrence; (ii) area of occupancy; (iii) number of locations or subpopulations; (iv) number of mature individuals			
C. Small population size and decline			
	Critically Endangered	Endangered	Vulnerable
Number of mature individuals	< 250	< 2,500	< 10,000
AND at least one of C1 or C2			
C1. An observed, estimated or projected continuing decline of at least (up to a max. of 100 years in future):	25% in 3 years or 1 generation (whichever is longer)	20% in 5 years or 2 generations (whichever is longer)	10% in 10 years or 3 generations (whichever is longer)
C2. An observed, estimated, projected or inferred continuing decline AND at least 1 of the following 3 conditions:			
(a) (i) Number of mature individuals in each subpopulation	≤ 50	≤ 250	≤ 1,000
(ii) % of mature individuals in one subpopulation =	90–100%	95–100%	100%
(b) Extreme fluctuations in the number of mature individuals			
D. Very small or restricted population			
	Critically Endangered	Endangered	Vulnerable
D. Number of mature individuals	< 50	< 250	D1. < 1,000
D2. Only applies to the VU category Restricted area of occupancy or number of locations with a plausible future threat that could drive the taxon to CR or EX in a very short time.	-	-	D2. typically: AOO < 20 km ² or number of locations ≤ 5
E. Quantitative Analysis			
	Critically Endangered	Endangered	Vulnerable
Indicating the probability of extinction in the wild to be:	≥ 50% in 10 years or 3 generations, whichever is longer (100 years max.)	≥ 20% in 20 years or 5 generations, whichever is longer (100 years max.)	≥ 10% in 100 years