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Experiences with the management and breeding of Mexican axolotl (*Ambystoma mexicanum*), for research and biobanking purposes, in a vivarium

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The Mexican axolotl (*Ambystoma mexicanum*) is widely used in laboratory research around the world, even though it is classified as critically endangered in nature. We review and describe the development of effective management strategies for this species, focusing on cryopreservation and research purposes under laboratory conditions. We share our experiences and challenges in maintaining water quality, feeding, and fostering natural reproduction in captive axolotls. Results indicate that the Mexican axolotl shows promise as a model for biobanking of endangered amphibian species.

KEYWORDS

***Ambystoma mexicanum*, laboratory husbandry, amphibian, water quality, axolotl**

Introduction

It is well known that amphibian species are threatened worldwide, with more than 40% classified in some risk category (Parra-Olea et al., 2014). Mexico is not an exception, as >43% of amphibian species are threatened. It is important to take effective conservation actions for Mexican species, which may include the use of assisted reproduction techniques (ARTs) for germplasm conservation (Holt and Pickard, 1999). One of the most iconic amphibians is the Mexican axolotl (*Ambystoma mexicanum*), a urodele species that can regenerate some organs and limbs. This endemic species has also held local cultural importance since the time of the Aztecs. Mexican axolotl has become a popular pet worldwide over the past decade, even though it is classified as critically endangered in its native habitat by the International Union for Conservation of Nature (IUCN, 2020). Population declines are the result of habitat loss and the introduction of invasive species (Semarnat, 2018). In Mexico, some of the strategies currently underway for the conservation of the axolotl include environmental education,

workshops and collaborations among stakeholders, eradication of invasive species, construction of shelters, captive breeding, and research on embryo cryopreservation (Servin et al., 2023). Our research group is working on cryopreservation to conserve valuable genetic material that can be used in future reintroduction programs.

Unfortunately, information on the management of captive axolotl populations is sparse; there are a few published manuals for their management in zoos and aquariums. Regarding management of axolotls under laboratory conditions, useful older information can be found in the "Compendium of Axolotl Husbandry Methods" from the Axolotl Newsletter (Kim et al., 1997) and the optimized axolotl husbandry protocol (Khattak et al., 2014). A recent publication by Yandulskaya and Monaghan (2023) describes housing strategies for a new axolotl colony. Generally, individual laboratories establish their own consistent and controlled working conditions, but these may not be transferable to other research groups.

In 2021, an axolotl breeding area was installed at the vivarium of the Faculty of Superior Studies Cuautitlan, National Autonomous University of Mexico, for research on axolotl embryo conservation (see Servin et al., 2023). In order to maintain a population of healthy animals capable of reproducing in captivity, special conditions for housing, management, and breeding were identified and standardized beforehand. In this overview, we describe how we achieved these laboratory conditions. We review i) the management of Mexican axolotl populations in the laboratory, ii) captive breeding, and iii) the future use of cryopreservation in research and conservation.

Material and methods

Management of axolotls as a laboratory animal

Housing

As aquatic animals, axolotls are housed in tanks or aquariums. Duhon (1992) stated that an adult axolotl needs to be held in tanks >6 L. In our facility, we use plastic tanks with a capacity of 15 L.

We controlled temperature and photoperiod, and to reduce stress, we provided hiding places such as artificial or natural plants to enrich the environment. By providing such housing conditions, following recognized standards established for the use of laboratory animals (National Research Council, 2011), we maintained healthy animals that reproduce in the laboratory without hormone treatment. Our axolotl colony had produced several batches of fertilized eggs with a hatching rate of approximately 71%, similar to the patterns reported for other colonies (Servin et al., 2023).

Water quality

The main challenge to keeping axolotls under laboratory conditions is to maintain ideal water quality. Without this, animals can develop skin and metabolic diseases (Wright and Whitaker, 2001). Water quality includes chemical, physical, and biological characteristics as measured by pH; levels of nitrites, nitrates, and ammonia; and total dissolved solids (TDS). Various studies have reported a range of values for these parameters in captive and laboratory holding facilities as well as in native habitats (Table 1).

Sometimes, the source of water is the main husbandry-related problem. For example, in some places in Latin America, tap water does not offer safe conditions for keeping axolotls. This is why measurement of water quality is needed in laboratories. We tested water taken at different points in the vivarium at the Faculty of Superior Studies Cuautitlan, where we house axolotls, and also monitored tap water in our laboratory. To measure the physiochemical parameters in the tanks, we used the commercial API Freshwater Master Test™ for aquaria. To measure TDS and conductivity, we employed the digital test pen KTOG™. Results showed that tap water (from the laboratory and vivarium) had very high levels of hardness and TDS (Table 2), which can be dangerous for axolotls' health (Duhon, 1992). However, the other water quality parameters were appropriate for axolotl husbandry.

We tested two methods to improve water quality before axolotls were introduced to our facilities. The first method consisted of a physical, chemical, and biological filtration system. This involved two types of external filters: a waterfall filter (AquaClear 30 Power Filter™) and a canister filter (Fluval 107™). Filters were fitted with 30 and 100 g, respectively, of activated carbon from coconut shells (Acuario Lomas™), ceramic rings (Aquajet Acuario Lomas™), and a polyurethane sponge (Black 25 PPI). In addition, we added commercial bacteria at doses of 10 mL per 40 L (BioPro Nitrobacter™). This system was tested in three 40 L aquariums, prior to introducing axolotls to the tanks. Filters were allowed to work for 1 month, without water changes. Water quality was measured once per week. In addition, a fine cotton fiber with a 0.25-µm mesh was added to the filter to catch particulates in order to reduce hardness and TDS.

In the second method, tap water was filtered using a five-step reverse osmosis filter (Rotoplas™). At this initial stage, tanks were simply aerated to determine whether water quality was constant after 1 week. Filtered water was then used as follows: i) to prepare 40% modified Holtfreter's solution (NaCl 0.059 M, KCl 0.0067 M, $CaCl_2$ 0.00076 M, and $NaHCO_3$ 0.0024 M) (Nugas and Bryant, 1996), ii) to prepare 20% modified Holtfreter's solution, iii) mixed 70%/30% (v/v) of reverse osmosis filtered water with dechlorinated tap water, and iv) mixed 50%/50% (v/v) of reverse osmosis filtered with dechlorinated tap water. Water was dechlorinated by adding 0.07 mL/L of potassium thiosulfate (Clorkill Biomaa™, 1.0 g/10 mL). The two different Holtfreter's solutions were used to hold either larvae and adults (40% solution) or embryos (20% solution) (Nugas and Bryant, 1996). Each treatment was replicated three times (Table 2).

Axolotls were acquired from five different hatcheries to ensure genetic variability. We started with a population of 12 females (from three hatcheries) and 6 males (from two different hatcheries). All animals were approximately 1 year old, and each axolotl was identified by a name and number, as well as their housing tank. Females and males were kept separately and united only when reproduction was scheduled. They were housed in plastic tanks with a capacity of 15 L with aeration and artificial plants.

TABLE 1 Physical–chemical parameters reported for Mexican axolotls (*Ambystoma mexicanum*).

Parameter	Captive reported (range)	References	Native habitat reported (range)	References
pH	7–8.4	Wright and Whitaker (2001) ; Khattak et al. (2014); Mena and Servin (2014), Robles et al. (2009)	7.05–8.84	Bojórquez et al. (2017) , Contreras et al. (2009) , Levy (2017)
Nitrate (mg/L)	0–40		0.1–14.2	
Nitrite (mg/L)	0–10		0.1–1.6	
Ammonia (mg/L)	0–1		0.1–0.9	
Temperature (°C)	15–20		16.8–25	
Alkalinity	10–350		500–610.8	
Total hardness (mg/L)	50–150		140–312	
Total dissolved solids (TDS) (ppm)	140–250		316.3	

Ambient temperature

Maintaining appropriate temperatures for axolotls in our facility has been a challenge; since 2023, there have been several heatwaves where the ambient temperature reached 28.5°C to 31°C. The usual temperature in the area is 23°C to 25°C. We have used fans, coolers, and water chillers to maintain a constant temperature of 21°C, which is just over the limit recommended for the species (Mena and Servin, 2014). Shaffer (1989) found that water temperature in the native habitat was 16°C–20°C.

Feeding

Axolotls are strict carnivores, which means dietary protein levels must be high. Few reports exist regarding nutritional requirements for the species in captivity. We used the recommendations found in Manjarrez-Alcívar et al. (2022) of >45% protein in juvenile axolotl's diet. Live food, such as teleost fishes, *Tubifex tubifex*, *Artemia salina*, and *Daphnia* sp., can be used as an option; however, under laboratory conditions, these organisms can be difficult to obtain or impractical for feeding a large number of individuals. Pelleted food may be the best option. Some brands of trout or turtle pellets can be used (Duhon, 1992; Khattak et al., 2014).

One of the problems when axolotls are fed pellets is that animals have to learn how to eat them. The use of stainless steel long tweezers (feeding tongs) helps solve this problem. We put a pellet at the tip of the feeding tongs, and we positioned it near the mouth of the axolotl. We found it advisable to employ operant conditioning, teaching the axolotl to associate feeding tongs with food and to eat pellets without stress. Axolotls can learn to eat pellets on their own, but care has to be taken to avoid uneaten food polluting holding tanks.

In our laboratory, axolotls are fed turtle pellets (Tortuguetas Normal™) containing >30% crude protein, 5% crude fat, 2% crude fiber, and 10% moisture; also, we offer live and freeze-dried tubifex (Tubifex Azoo™) containing >52% crude protein, 12% crude fat, 5% crude fiber, 12% crude ash, and 5% moisture. In addition, *Daphnia* and *Artemia* are offered to young axolotls <9 months old. With this diet, axolotls are kept healthy and can express natural behavior capturing small live food.

Captive breeding of the Mexican axolotl

The Mexican axolotl naturally reproduces in winter when temperatures range between 12°C and 14°C, day length is approximately 11 h of light and 13 h of darkness, and water quality is good and stable (see Table 2). In the laboratory, the environment for reproduction relies on artificial systems and activities, such as timers to control daylight hours, efficient filtering systems, programmable water chillers, and constant monitoring of physical–chemical parameters. In our facility, high temperatures due to climate change and several unusual heat waves created an additional challenge.

The reproduction tank must be large, preferably allowing for 40 L of water per individual. It must have bottom substrate, such as small stones for the placement of spermatophores, and natural or artificial plants to allow the collection of embryos or oocytes for cryopreservation. The breeding tank should be prepared several days, or weeks, prior to the collection of biological samples.

The future use of cryopreservation in research and conservation

ARTs may be a useful tool for the conservation of germplasm of endangered species (Wildt et al., 2001). Creation of biobanks, allowing the storage of genetic material, is of great importance for the future of conservation in cases, such as the Mexican axolotl, where it is not currently possible to reintroduce a species to its natural habitat owing to habitat destruction and the presence of invasive species. Cryopreservation of oocytes, sperm, and embryos has helped to preserve several endangered species. These include programs for the Amur tiger (*Panthera tigris altaica*) (Swanson et al., 2007), the Mexican wolf (*Canis lupus baileyi*) (Rosales, 2015), and the California condor (*Gymnogyps californianus*) (Gee et al., 2004). Sperm cryopreservation, in particular, helps maintain a genetic bank for future captive breeding efforts; however, the application of this technique to amphibians is relatively recent. Therefore, many techniques for

TABLE 2 Physical–chemical parameters of tap water from the laboratory and vivarium, at the Faculty of Superior Studies Cuautitlan, National Autonomous University of Mexico, before and after different treatments tested to find appropriate water quality conditions for holding Mexican axolotls.

Parameter	Tap water laboratory and bioterium	Physical, chemical, and biological filtration	Inverse osmosis filtration	Inverse osmosis filtration to prepare 40% modified Holtfreter's solution	Inverse osmosis filtration to prepare 20% modified Holtfreter's solution	Inverse osmosis filtration 70%, dechlorinated tap water 30%	Inverse osmosis filtration 50%, dechlorinated tap water 50%	Water quality for captive axolotls reported by Mena and Servín (2014)
pH	7.8	7.8	6.1	7.1	7.1	7.3	7.8	7–8.4
Nitrate (mg/L)	40	10	0	0	0	0	0	0–40
Nitrite (mg/L)	0	0.5	0	0	0	0	0	0.1–1.6
Ammonia (mg/L)	0	0.25	0.25	0.25	0.25	0.25	0.25	0–1
Temperature (°C)	23	23	23	21	21	21	21	15–20
Alkalinity	600	540	40	675	500	450	300	100–350
Total hardness (mg/L)	475	450	0	750	475	30	50	50–150
Total dissolved solids (TDS) (ppm)	315	378	1–5	1,272	687	105	150	140–250

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obtaining samples and protocols for cryopreservation are under development in various parts of the world (Clulow et al., 2022).

We work with amphibian embryos, which is a challenge due to the presence of yolk and reduced permeability of the gelatinous layers that surround the embryo. In our ongoing work, we have cooled axolotl embryos to subzero temperatures (−6°C) and obtained hatching rates from 18% to 53% (unpublished data). Stopping or slowing embryonic development may be useful for translocation projects and to conduct research where embryo manipulation is necessary.

Results

Water quality

Water quality parameters for the vivarium, measured before and after different treatments, allowed for assessment as to whether water conditions were suitable for axolotls (Table 2). The first method—physical, chemical, and biological filtration—did not reduce hardness and TDS, but it improved water quality with regard to nutrients (such as nitrates, nitrites, and ammonia) and concentrations of organic matter (Table 2).

In the second method, water filtration using reverse osmosis, hardness and TDS dropped drastically, as well as nutrients (Table 2). Values for water quality metrics taken directly from the filter and water kept in the tank for 1 week were similar; however, none of these parameters were ideal for holding axolotls. Application of modified Holtfreter's solution (40%) resulted in neutral pH, nitrites, nitrates, and ammonia in the appropriate range, but there was a dangerous rise in hardness and TDS. The reduction of concentrations to 20% produced better results, but values for TDS and hardness were still inappropriate. We tested the mix 70/30 (v/v) with dechlorinated tap water and found that the ranges of nutrients and pH were acceptable; however, hardness and TDS were lower than expected (Table 2). The mix 50%/50% (v/v) of reverse osmosis filter water with dechlorinated tap water provided appropriate water quality values, similar to those reported in Mena and Servin (2014). This water was adopted for routine housing of axolotls, and tank water was changed three times per week.

Feeding

We fed axolotls turtle pellets (Tortuguetas Normal™) and live and freeze-dried tubifex (Tubifex Azoo™). In addition, *Daphnia* and *Artemia* were offered to young axolotls <9 months old. With this diet, axolotls remained healthy and were able to express natural feeding behavior. We observed a low prevalence of disease, with just five cases in 3 years.

Breeding

Axolotls in our colony reproduced successfully in a 3-year period. We recorded 24 cases of egg laying that led to hatching. We started with a population of 12 females and 6 males; at present, the population has grown to 64 adult axolotls. Reproductive behavior was observed 24 to 72 h after pairs were placed in the breeding tank, and egg laying was observed 48 to 72 h after courtship. Most of the embryos produced were used for research on cryopreservation.

Discussion

Keeping and breeding animals for conservation purposes is always a challenge. Diverse biological information is required in order to recognize optimal conditions and then maintain animals within these environmental constraints. Although the Mexican axolotl is a species housed in research laboratories in many parts of the world, we faced many issues in establishing a new colony for research. Our general approach and the findings reported here should prove useful for establishing programs to rear other species of aquatic salamanders under laboratory conditions for conservation initiatives. For example, the Amphibian Conservation Action Plan (2024) notes that applied *ex-situ* research populations could be linked to *ex-situ* and *in-situ* conservation and research programs (IUCN, 2024). Such *ex-situ* populations can help aquatic salamanders, such as Chinese giant salamanders (*Andrias* spp.), where reproduction and housing in captivity could conserve genetic diversity and significantly aid conservation strategies (Jing et al., 2024).

Works such as those of Marcec et al. (2014) and Silla and Langhome (2022) have shown that cryopreservation may be a viable alternative for the genetic conservation of endangered amphibian species, and the technique deserves further study. The Mexican axolotl could serve as a model species for cryopreservation research and as a "flagship species" for the conservation of Mexican amphibian species employing captive breeding.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was approved by the Internal Committee for the Care and Use of Experimental Animals (CICUAE) of the National Autonomous University of Mexico. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

ES: Writing – original draft, Writing – review & editing. AM: Writing – review & editing, Writing – original draft. AA-R: Writing – original draft, Writing – review & editing.

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Conflict of interest

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