An Introduction to the Mexican Axolotl (*Ambystoma mexicanum*)

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A number of unusual traits, including a remarkable capacity for wound healing and limb regeneration, make the axolotl an interesting animal model. The author provides an overview of axolotl care and use in biomedical research.

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In the middle of the 19th century, French explorers captured 34 aquatic salamanders (*Ambystoma mexicanum)* from Lake Xochimilco in Mexico and relocated them to Paris. After a few of these animals, now commonly known as Mexican axolotls, were given to herpetologist August Duméril, he introduced the animal to the scientific world[1].

Today, it would be extremely difficult to catch more than a handful of wild axolotls from Lake Xochimilco, because they and most of the native species in the lake are endangered. Currently, an extensive continuing international collaboration is aiming to preserve the biodiversity of Lake Xochimilco[2]. In addition to these efforts, the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) protects the axolotl. The CITES convention regulates the international trade of all axolotls (including laboratory-reared animals). Foreign researchers wishing to import axolotls from the United States must obtain an import CITES permit from their home-government. Likewise, an American shipper must apply for an export CITES permit from the US Department of Fish and Wildlife.

The axolotl is a salamander with an amazing capacity for healing and regeneration. Unlike mammals, the axolotl's wound-healing mechanism usually does not involve the formation of scar tissue. Instead, tissues adjacent to an amputated limb will revert to an embryonic 'stem-cell-like' state and regrow the missing tissue[3,4]. Laboratories at universities worldwide are working independently and in collaboration on projects investigating the details of the mechanisms by which this process occurs[5,6]. The key to the axolotl's ability to regenerate will perhaps one day be its most important gift to medical science.

Axolotl embryos measure ~2–3 mm in diameter. Because of their large size, a dissecting microscope easily reveals cleavage furrows allowing researchers or students to observe the transitions between each stage of development. For years, researchers have been manipulating, dissecting, implanting, and otherwise scrutinizing axolotl embryos in an attempt to describe the countless events that take place after fertilization throughout development[7–9]. Moreover, the embryos are sturdy and can hold up very well to microsurgical manipulations. Their hardiness and large size make it possible for a growing number of educators to use the axolotl as a way to allow their students to have 'hands-on' experience with the scientific method[10].

Many amphibians begin their lives by hatching from eggs as aquatic larvae and then undergo metamorphosis, a restructuring of the anatomy and physiology to modify their bodies to suit them for life on land. However, not all salamanders mature into land-dwelling animals, and axolotls are one such exception. Most axolotls live out their entire life cycle in the aquatic form they had as juveniles, a trait known as 'paedomorphosis'. Although the axolotl is not the only paedomorphic salamander, it is the most extensively studied. This metamorphic failure remains a point of interest to this day; furthermore, with advances in molecular biology and genomics techniques, we are rapidly gaining new insights about the process in general, as well as its evolutionary significance[11,12]. Recent advances made toward deciphering the genetic sequence of the axolotl may finally unlock answers to many long-studied questions.

Animal Description

Perhaps the most striking of the axolotl's physical features are their external gill

FIGURE 1. Wild-type axolotl with gill branches spread. Filaments for gas exchange are clearly visible.

FIGURE 2. Top: Male axolotl with prominent jelly gland. Bottom: Gravid female axolotl with characteristically round midsection.

structures. Three branchlike projections extend from the neck region on each side of their heads. Covering each branch are feathery filaments used for gas exchange (**Fig. 1**). They have lungs too, and will occasionally swim to the surface to gulp air, but they spend much of the time under water with their branches splayed open to increase the exposure of the filaments to water. Observers will see the animal flex the branches every few minutes to move fresh water across the filaments. Typically this behavior increases as water temperature increases, presumably because warm water holds less dissolved oxygen than cooler water.

Unlike many smaller salamander species, adult axolotls can reach a substantial 30 cm in length. Because they produce a large number of eggs, mature females are typically the more massive of the two sexes, averaging ~170–180 g. Males are usually smaller, averaging ~125–130 g. Though large, axolotls are neither cumbersome nor awkward in movement. They have powerful tails to propel them along, and their long sleek bodies are well suited to their aquatic environment, allowing them to move very effectively when threatened. For the most part, during the daytime axolotls are quite sedentary and spend much of their time on the bottom of the tank regardless of the depth of the water. The axolotl's posture during the day is generally described as 'relaxed' or 'lazy'. They usually walk slowly across the floor, surfacing only occasionally. They typically stand with their weight set evenly on all four feet, and their tails extended behind them or curved slightly to one side. Many people report increased activity in their animals during the evenings, but one should not confuse an active axolotl (swimming, exploring) with an animal under stress. When uncomfortable, they may be seen rolling, rubbing on plants, floating, or darting about rapidly. A tightly coiled tail can be another sign of discomfort.

Housing

As with all amphibians, the axolotl's skin and gills are delicate and can be easily damaged by poor water quality, rough handling, or chemicals left on a handler's gloves or hands. The water in their native Lake Xochimilco is brackish (low salinity), so laboratory-reared axolotls are generally kept in a slightly saline environment. A mild salt solution created several decades ago by the German axolotl researcher Johannes Holtfreter has become standard housing water[13]. Holtfreter's solution contains a mixture of several salts that not only help to reduce bacterial loads in the animal's environment but also help support the animals' electrolyte levels. Most city water-treatment plants add chlorine and chloramines to the public drinking-water supply. Although harmless to humans, these additives damage axolotl skin, especially with long-term use. Most very small larvae will die from chlorine exposure in a few hours. In addition to the danger posed by chlorine and chloramines, bacterial and particulate contamination can also influence water quality. If Holtfreter's solution is made with tap water from an unknown or unreliable source, one should ideally process it through a filtration system that includes carbon and particulate filters as well as ultraviolet irradiation. If this type of filtration system is lacking in an area with poor-quality tap water, it is preferable to make the Holtfreter's solution using deionized or distilled water, thus avoiding possible water-quality issues. It is important to point out that Holtfreter's recipe does not contain any dechlorinating ingredients. If the solution is made with tap water, one must remove the chlorine and chloramines chemically. Fortunately, this is easy to do by using any commercially available chlorine- and chloramine-removing agent. Many such products also have a 'stress-coat support' additive that will help the axolotl maintain the protective mucus skin-coat that makes healthy axolotl skin feel slippery. Stickiness or roughness is indicative of a skin problem, the source of which is frequently poor water quality.

If the housing water is changed daily, adult axolotls will be quite comfortable in a glass or food-grade plastic container holding a minimum of 2 liters of water.

They also thrive in glass aquaria equipped with low-power filtration. High-power filters and air pumps generate rapid currents, which tend to irritate their delicate skin after a period of time. Axolotls explore their surroundings with their noses, and anything that smells or looks like food will be eaten; this includes small pieces of gravel and broken pieces of plastic plants. As a rule, nothing with a diameter <3 cm should be accessible, or the animal may eat it. Bowel obstructions are a common cause of death in animals kept in tanks with gravel or small rocks.

Axolotls will eat live food but can easily become conditioned to eating more convenient pellet food. It is easy to entice them with strong-smelling, soft pellets that sink to the bottom of the tank. Food pellets made of whole ground fish such as anchovies are a good source of protein. Commercial fisheries sometimes sell these pellets in bulk, as do aquatics supply companies. With time and patience, one can condition axolotls to eat in one particular region of the tank or to catch pellets as they are dropped by hand. They thrive on consistent but small daily feedings rather than occasional 'binge' meals.

Axolotls are generally nonaggressive, so one can group-house similarly sized animals. If given enough room, they will generally do well as a group. Three adults will be comfortable if housed in a 10-gallon tank with adequate filtration. Occasionally when group-housed, animals will bite each other, and toes or gill branches can gradually become damaged. Plants (real or plastic), rocks, caves, or pieces of PVC pipe provide hiding places, and the animals will often settle themselves between objects. If provided with such things, an animal that is getting bitten will retreat to a safe spot. Usually the offender will not pursue.

Breeding

Maintaining breeding animals in groups is possible, but preparing the males under such circumstances will require a bit of coordination to ensure that they are ready to breed in a timely manner. When housed in groups, males often court each other and release their sperm as they would when a female is present. Individually housing males for ~2 weeks before presentation with a female helps prevent such spontaneity and also helps ensure potency when he is paired with a female. Males housed individually will also react more vigorously when presented with a female, because they are more sensitive to the presence of a tank-mate than are males that are regularly reared in groups.

One can determine from a female's body shape if she is ready to be mated. As she becomes heavily egg-laden, her midsection becomes round (**Fig. 2**). Mature males lack this round body shape and appear slimmer. Around his cloaca (the shared opening for the digestive and reproductive tracts), a sexually mature male axolotl has a swelling that is due to the presence of an enlarged gland that produces jelly to package and protect his sperm. Females possess this gland but lack the swelling.

These animals require very few special conditions for successful breeding. After being presented with a female, the male will deposit his sperm, which is packaged into cone-shaped packets called spermatophores. The tip of the spermatophore is white, and the wide end of the cone is clear. These packages are adhesive, and the male attaches them to rocks and plants in his environment. Because spermatophores do not adhere well to smooth surfaces such as aquarium glass, a rough surface such as rocks should be provided. The female picks up the spermatophores with her cloaca and stores the sperm internally until she is ready to shed her eggs ~24 h later. She fertilizes the egg with the stored sperm, then wraps it in a clear mucus as it is being shed. The eggs are 'glued together' by still more of the mucus, and in the wild this helps them adhere to nearby plants and rocks. A single female can release ≥100–400 eggs over the course of ~24 h. The average spawn size is 175–200 eggs.

Axolotls are long-lived, reaching sexual maturity at ~1 year of age. If healthy and properly cared for, they will stay in breeding condition for as long as 8 years of their ≥10-year laboratory lifespan. Females at their peak of health can be bred approximately every 4–5 months; the quality and quantity of the eggs are largely contingent on the female's health and nutrition. Males are more prolific and can be mated one to four times a month depending on age, experience, and health.

Larvae Care

Axolotl eggs require ~2 weeks to hatch when kept at 20 °C. Their development can be drawn out a month or longer if they are kept refrigerated at ~7 °C (ref. 14). In captivity, because the eggs must be kept in containers, their space is limited and they have a tendency to form clumps. Unlike frog eggs frequently found in large clutches, axolotl eggs are healthiest when they are permitted to rest in a single layer. This allows them to receive adequate oxygen during this delicate life phase. Dead or defective embryos, debris, and excess jelly are especially harmful to the eggs in the laboratory setting, because the surviving eggs have no natural water currents to oxygenate them. To cope with this problem, one must manually break up the jelly clumps and replace the Holtfreter's solution regularly.

Larval axolotls have an internal yolk sac that will sustain them for approximately a week after they hatch. Animals smaller than ~5 cm thrive on a live diet of small crustaceans and other small organisms. Freshly hatched brine shrimp, not much larger than grains of sand, are the

FIGURE 3. A bowl of wild-type axolotl larvae large enough to begin the transition from brine shrimp to food pellets.

TABLE 1. Treatments for ectopic infections in axolotls

Drug	Instructions[16]
Noniodized NaCl	25 g NaCl per liter of distilled or deionized water; 10-min bath every other day
10% Formalin	1.5 ml formalin per liter of distilled or deionized water; 10-min bath every other day
Malachite green (m.g.)	0.15 mg m.g. per liter of distilled or deionized water; 1-h bath every 24 h
Amikacin	5 mg/kg intraperitoneal injection; every other day for three treatments

perfect size, and it is very easy to set up and maintain a shrimp hatchery. Daily feedings are best, but without changes of water to accompany those feedings, the animals' water will quickly become foul[15]. Once the animals have grown to ~5 cm in length (nose to tail tip), their mouths are large enough for them to be weaned onto a pellet food source. Not all larvae will make this transition easily, and sometimes they stop eating altogether when pellets alone are provided. Adding back some of their original live food source sometimes helps them make the transition. Live *Tubifex* worms can also be part of the diet during this transition stage. Many pet stores sell a freeze-dried *Tubifex* product that should be avoided; if an animal is refusing to eat pellet food, it will probably refuse freeze-dried foods as well (**Fig. 3**).

Handling and Health Concerns

Given their sedate nature, axolotls won't lunge for a dangling finger (unless mistaken for food), so most caretakers find them generally easy to maintain. In fact, axolotls will eventually respond to regular, gentle handling. With enough time and patience, caretakers can get most adult animals accustomed to stroking and even carrying for short distances. They have tiny, blunt, cone-shaped teeth that are better suited to gripping than tearing, so even an accidental bite will not harm a caretaker's hands.

Most of the labor required to maintain healthy animals revolves around delivering high-quality food and keeping the animals in clean water. They are hardy, and once fully grown they generally live most of their lives without major illness. In the interest of minimizing antibiotic resistance, there are several options available before resorting to traditional pharmaceuticals, should illness occur. Depending on how far an infection has progressed, sometimes it helps to merely refrigerate the animal. Placing an axolotl in a refrigerator or cold room (~7–8 °C) slows down their basal metabolism enough to inhibit most movement and nonvital processes, inducing an artificial torpor. The cold temperature presumably helps to create an inhospitable environment for the infectious agent. Axolotls can be kept in this state for a month or more if necessary, but most infections that can be resolved by cold treatment will begin to show improvement after about a week.

Regular water changes, frequent feedings, and a reliable quarantine program can frequently minimize skin parasites and other ectopic infections. If the occasion arises when treatment for such an infection is needed, baths of noniodized sodium chloride (NaCl), formalin, or malachite green are usually best as a first line of defense[16] (**Table 1**). For recurring skin and gill infections, animals can live in a dilute formalin–malachite green mixture for several months with no apparent side effects. To make the dilute solution, add 0.015 ml 10% formalin and 0.05 mg malachite green per liter of Holtfreter's solution[17].

It is not uncommon to find juvenile axolotls floating upside-down with distended midsections full of air. Presumably, the most common cause is gas produced by the immature gut during digestion of their high-protein diet. Often, reducing their portion size when feeding helps to alleviate this problem. Two smaller meals are sometimes easier to digest, and the gas is less troublesome. Lowering the animal's water level helps as well, so they do not have to fight the extra buoyancy created by the air bubble. As the animal's gut matures, this problem usually resolves itself. Another possible reason for floating can require the administration of antibiotics: tiny rupture(s) in the pulmonary system allow air to escape into the extrapulmonary space, causing distention. A series of antibiotic injections may prevent an infection, and one can remove the air with a syringe or allow it to resolve itself on its own[18]. Whatever the reason for the gas bubble's presence, under no circumstances should an animal be anesthetized and 'burped' by squeezing the air out. Depending on where the air bubble is located, this manipulation can cause damage to the skin, connective tissue, internal organs, or all of these.

FIGURE 4. An animal room at the IUAC. Animals in white bowls are individually housed: animals in gray tubs are housed in groups of three.

FIGURE 5. An adult female, wild-type axolotl with the characteristically mottled coloration.

FIGURE 6. An adult male white axolotl.

FIGURE 7. Adult melanoid axolotl. Notice the marked decrease in mottling as compared with the wild-type animal in Fig. 4.

Injections of enrofloxacin (Baytril) or amikacin often produce marked effects on most bacterial infections, whereas tetracycline and other 'cyclic' antibiotics like oxytetracycline and doxycycline are not good choices, because they can cause severe skin irritation at the injection site. Although commercially available, over-the-counter fish medications may be used, one must usually calculate higher dosages. The concentrations necessary to treat fish are usually too low; therefore, many of these preparations will be ineffective axolotls if used per the package directions[19].

Ambystoma tigrinum virus (ATV), a ranavirus, has in the past infected laboratory-reared axolotls[20]. Initial presentation shows milky-white skin blisters the size of a pinhead. As infection progresses, these skin blisters can break open and bleed profusely. In advanced cases, the animals' limbs and neck region may appear bloated. As is the case with all viruses, antibiotics are ineffective in treatment and the infection must run its course in affected animals. Because ATV is always fatal, liberal use of bleach on surfaces and early euthanasia of symptomatic animals is recommended, to prevent the possibility of spread to nearby animals. It is important, though, to mention that skin blisters are not automatically indicative of ATV. Poor water quality and ectopic skin parasites can also cause blistering to occur.

The Indiana University Axolotl Colony (IUAC)

In 1934, Dr. Rufus R. Humphrey began his own research with the axolotl in the anatomy department at the University of Buffalo (now part of the State University of New York system). Upon his retirement in 1957, he accepted an emeritus position at Indiana University, where he founded a small breeding colony with axolotls that he used in his own researh[21]. The descendants of these animals remain at Indiana University to this day, and have been joined by animals donated from other research facilities as well as animals obtained during subsequent collections from Lake Xochimilco. After many such acquisitions and years of purposeful breeding, Indiana University's stock has become the largest self-sustaining population in the world (**Fig. 4**).

Since 1969, the National Science Foundation has continuously funded the IUAC. The IUAC staff maintains an average breeding population of 600–700 adults as well as a highly variable number of hatchlings, juveniles, and nonbreeding adults. Colony staff obtains spawns regularly throughout most of the year, and sends weekly shipments of embryos, larvae, adults, and supplies to researchers worldwide. On average, colony staff ships ~300 eggs each week to different institutions throughout the United States, although this number fluctuates during the course of the academic year. All materials are packaged in clear plastic bags that have been heat-sealed to contain a large

FIGURE 8. Adult female albino axolotl. These animals lack the ability to produce black pigment.

air bubble and water. The bags are placed in Styrofoam coolers, packaged with ice, and shipped via overnight courier.

The IUAC maintains several lines of axolotl pigment varieties. Some are naturally occurring, whereas others are artificial introductions. The most commonly requested variety is the wild type, which are closest in appearance to what one would see in an animal caught in Lake Xochimilco today. In these animals, the pigment cells migrate normally out of the neural crest to give the skin its color[22]. Wild-type animals have three different types of major pigment cells: black (melanophores), yellow (xanthophores), and iridescent (iridophores)[23,24]. There is a great deal of variety in body coloration with wild-type axolotls, but overall they have a grayish green base color with black spots and reflective flecks throughout, and their irises are a reflective ring of gold (**Fig. 5**).

Animals exhibiting the recessive, white (*d*) mutation have skin that lacks dark pigment cells. They produce these cells, but they exhibit a mutation causing these pigment cells to be trapped before they ever reach the skin[22]. The name of the mutation can be misleading, because these animals actually have pink skin. These animals are commonly mistaken for being albino because of the lack of skin color (**Fig. 6**). Returning briefly to the axolotl's origins in Europe, it is noteworthy that the white gene is designated with the letter *d* in honor of the French herpetologist Auguste Duméril, the first person to describe the phenotype[25].

Melanoid animals show a recessive mutation (*m*) that increases the production of dark pigment cells while decreasing the yellow and reflective pigment[24]. These animals tend to be charcoal in coloration and lack the reflective iris. Many people describe them as 'velvety'. This mutation occurred spontaneously in the lab, and purposeful breeding has maintained it[24–26] (**Fig. 7**).

The albino mutation (*a*) results in axolotls that lack the ability to produce dark pigment cells[24]. They still possess yellow and reflective pigments, so these animals are generally golden-yellow in appearance. Like their wild-type counterparts, most albinos have speckled body patterns, but the absence of melanophores means that there are no greenish and black colors (**Fig. 8**). As expected, their eyes are red like albinos of other species. Unlike the white and melanoid mutations, albinism is not of spontaneous origin. Researchers intentionally introduced it into the axolotl from a close relative, the common tiger salamander (*Ambystoma tigrinum*), using hybridization, cloning, and tissue transplant techniques[27].

In addition to supplying axolotls and husbandry materials, the IUAC has a long history of sharing its years of combined expertise with the axolotl community. Toward this goal, a great deal of IUAC staff time is spent in direct correspondence with researchers and teachers worldwide, conducting facility tours and workshops, and maintaining an elaborate website (http://www.indiana.edu/~axolotl). The IUAC also edits an informal annual publication, *Axolotl News*, formerly known as the *Axolotl Newsletter*, wherein axolotl users are invited to publish articles, preliminary experimental results, new techniques, and any other axolotl-related news. The mission of the IUAC is to serve biology research programs and educators by providing experimental material and expertise, and by encouraging and facilitating the exchange of information and ideas.

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