Axolotls and neoteny: those who forgot to grow up

[1,2]Marian Proorocu, [1,3,4]I. Valentin Petrescu-Mag

[1] Department of Environmental Engineering and Protection, Faculty of Agriculture, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania; [2] Enviromep SRL, Colonia Făget, Cluj, Romania; [3] Bioflux SRL, Cluj-Napoca, Romania; [4] University of Oradea, Oradea, Romania. Corresponding author: I. V. Petrescu-Mag, zoobiomag2004@yahoo.com

Abstract. This article is a descriptive work on a special type of reproduction, a strangeness for vertebrates: *Ambystoma mexicanum*. The axolotl is an amphibian that does not develop lungs and retains its external gills from the larval stage. The gills allow the animal to lead an underwater life. The axolotl reaches sexual maturity without going through metamorphosis, thus presenting the phenomenon of neoteny. Sexually mature individuals live in the larval stage all their lives and are able to reproduce in the larval stage. The axolotl has a physiological peculiarity in that it lacks thyroid-stimulating hormone. In related species, this hormone is required for the thyroid to produce thyroxine for the axolotl to initiate metamorphosis. The axolotl does, however, have the ability to undergo metamorphosis if experimentally given the missing hormone. After artificially induced metamorphosis, the individual is able to lead a terrestrial life (indicating that the suppression of metamorphosis occurred relatively recently in the course of axolotl evolution). In other words, axolotls were once amphibians, just like their relatives, the salamanders. Although tens or hundreds of thousands of generations of his ancestors did not walk on land, the artificially metamorphosed axolotl did not lose the instincts of terrestrial life (locomotion, feeding, chasing prey, avoiding predators, etc). Another method of artificially inducing metamorphosis is by injecting iodine, which is used by the salamander's body in the production of thyroid hormones. A metamorphosing axolotl goes through a series of anatomical, morphological and physiological changes that help it adapt to terrestrial life. These changes include increased muscle tone in the limbs (because on land the specific gravity becomes greater and the body needs musculature to support or pull the body), absorption of gills and appendages specific to swimming, development of eyelids and a reduction in skin permeability to water, allowing the axolotl to stay hydrated longer when on land.
Keywords: *Ambystoma mexicanum*, salamander, metamorphosis, paedomorphic.

Introduction. The axolotl (*Ambystoma mexicanum*) is a paedomorphic salamander closely related to the tiger salamander (*Ambystoma tigrinum*). Axolotls are unusual among amphibians in that they reach the reproductive stage without undergoing metamorphosis. Instead of becoming morphologically adults and moving on land, adults continue to live in water, having a larval appearance throughout their lives. This article is a descriptive work on a special type of reproduction, a strangeness for vertebrates.

Mythology. The axolotl represents the Aztec god of fire and lightning, also called Xolotl, which shapeshifts himself in the form of a salamander to not be sacrificed (Tate 2010). However, this Mexican salamander is impressive enough on its own, with the ability to regenerate lost limbs and stay "young" throughout its live (www.nationalgeographic.com). The axolotl continues to play a huge cultural role in Mexico (Tate 2010) and has appeared in cartoons and murals. In 2020, it was announced that the axolotl would be featured on Mexico's new 50 peso banknote design, along with images of corn and chinampas (www.wikipedia.org).

Origins, spread. The species was originally found in several lakes in the area where Ciudad de México is now located, such as Lake Xochimilco and Lake Chalco (Valiente et al 2010). These lakes were drained by Spanish settlers after the conquest of the Aztec Empire, which led to the destruction of much of its natural habitat (Tate 2010; Valiente et al 2010). Today, the species is spread wideworld, but in aquaria only. The Xochimilco Ecological Park is specially designed to accommodate this species (Valiente et al 2010).

Body exterior, behavior, neoteny, metamorphosis. The axolotl (Figure 1) has the features of a salamander larva, with a color usually black, brown or dark brown (Newth 1960). Albinotic varieties with shades of pink, but also white varieties, often appear in captivity. It grows up to a length of 30 cm, but its average length is 15 cm (Clarke et al 1988).

It uses its four legs to move on the bottom of the water bed, and its tail to swim. The axolotl feeds on some insects, small fish and crustaceans. The species prefers cooler waters in that region, which is a region with a fairly warm climate.

The specific oddity of the axolotl is the ability to regenerate its body parts after injury or amputation. It can completely regenerate including parts of its nervous system (Clarke et al 1988), which is rare among vertebrates.

Figure 1. *Ambystoma mexicanum.* Photo by David J. Stang. First published at www.zipcodezoo.com

Normally, the axolotl remains almost inactive on the bottom of the lake during the day and rarely comes to the surface of the water to breathe. When night falls, the animal goes in search of food. Axolotls communicate with each other through chemical and visual signals, but this occurs more during mating season (Woodley & Staub 2021). Another way of communication between individuals of the species seems to be through pheromones (Woodley & Staub 2021), but this communication also takes place during the reproductive period.

Unlike other amphibians, the axolotl does not develop lungs and retains its external gills from the larval stage. The gills allow it to lead an underwater life. The axolotl reaches sexual maturity without going through metamorphosis, thus presenting the phenomenon of neoteny. Sexually mature individuals live in the larval stage all their lives and are able to reproduce in the larval stage. The axolotl has a physiological peculiarity in that it lacks thyroid-stimulating hormone (Crowner et al 2019). In related species, this hormone is required for the thyroid to produce thyroxine for the axolotl to initiate metamorphosis.

The axolotl does, however, have the ability to undergo metamorphosis if experimentally given the missing hormone (Crowner et al 2019). After artificially induced metamorphosis, the individual is able to lead a terrestrial life (indicating that the suppression of metamorphosis occurred relatively recently in the course of axolotl evolution). In other words, axolotls were once amphibians, just like their relatives, the salamanders. Although tens or hundreds of thousands of generations of his ancestors did

not walk on land, the artificially metamorphosed axolotl did not lose the instincts of terrestrial life (locomotion, feeding, chasing prey, avoiding predators, etc). Another method of artificially inducing metamorphosis is by injecting iodine, which is used by the salamander's body in the production of thyroid hormones (Bothe et al 2021).

A metamorphosing axolotl goes through a series of anatomical, morphological and physiological changes that help it adapt to terrestrial life (Olejnickova et al 2022). These changes include increased muscle tone in the limbs (because on land the specific gravity becomes greater and the body needs musculature to support or pull the body), absorption of gills and appendages specific to swimming, development of eyelids and a reduction in skin permeability to water, allowing the axolotl to stay hydrated longer when on land (Olejnickova et al 2022). An axolotl's lungs, although present alongside the gills after reaching non-metamorphosed reproductive age, continue to develop during metamorphosis. However, this adult stage obtained by induced metamorphosis is not the natural condition of this species. Therefore, induced metamorphosis significantly shortens the lifespan of axolotls.

Reproduction. Axolotls are solitary animals. They reach sexual maturity at the age of one year old, and their spawning season in the wild is during February (www.nationalgeographic.com). Males attract females, possibly using pheromones, and with a courtship dance, also called a "hula" dance, shaking its tail and lower body (Hosie & Smith 2021). The female responds by nudging him with her snout (www.nationalgeographic.com).

The male then deposits spermatophores, or sperm packets (Hosie & Smith 2021), on the lake floor, which the female picks up with its cloaca, a body cavity, which then fertilizes the eggs (www.nationalgeographic.com).

Females lay up an average of 300 eggs (though they can lay up to 1000), on vegetation or rocks (Hosie & Smith 2021), thus being protected from predators. After two weeks of incubation the eggs hatch and, with no parental care, the larvae are off and swimming on their own (www.nationalgeographic.com).

Critically endangered. Axolotls have long fascinated the public, even more so when they were first brought from Mexico to Paris in 1864 (www.nationalgeographic.com). That was the period of expansion of aquariophilia. Europeans across the Old Continent started breeding the salamanders, the beginning of a robust pet trade in the animals, which breed easily in captivity (www.nationalgeographic.com).

Yet in most countries, the species cannot be traded across international borders, in part due to the concern that they'll be poached from the wild (Shukhova & MacMillan 2020). Axolotls are illegal to own in some U.S. states for the same reason (www.nationalgeographic.com). In some U.S. states their posession is illegal due to the risk of escaping in the wild and interbreeding with native salamanders (Woodcock et al 2017).

A. mexicanum is included in the list of endangered species with the mention "critically endangered". According to the Mexican Academy of Sciences, in 1998 there were 6000 specimens per square km, in 2003 the number had dropped to 1000, and in 2008 it was only 100 (www.wikipedia.org). In a research carried out in 2013 for four months, biologists from the National Autonomous University of Mexico failed to identify any specimens in the natural habitat, but a few months later such creatures were still discovered, and the authorities created conditions to protect them (Trujillo 2000). For example, the Xochimilco Ecological Park is home to a protected area for these amphibians that is classified as "Special Protection" by the Mexican government (www.wikipedia.org).

Research. *A. mexicanum* is also a common research subject for biologists (Adamson et al 2022), due to its ability to regenerate lost or damaged limbs, spinal cords, hearts, and even parts of their brains (Sámano et al 2021) — all without permanent scarring (www.nationalgeographic.com). Since scarring prevents tissue from regenerating, finding out how and why axolotls don't scar could unlock human's ability to regenerate cells,

tissues and organs (Adamson et al 2022). Sámano et al (2021) deciphered how the axolotl's molecules communicate to promote regeneration (www.nationalgeographic.com).

Conclusions. Unlike other amphibians, the axolotl does not develop lungs and retains its external gills from the larval stage. The gills allow it to lead an underwater life. The axolotl reaches sexual maturity without going through metamorphosis, thus presenting the phenomenon of neoteny. Sexually mature individuals live in the larval stage all their lives and are able to reproduce in the larval stage. The axolotl has a physiological peculiarity in that it lacks thyroid-stimulating hormone. In related species, this hormone is required for the thyroid to produce thyroxine for the axolotl to initiate metamorphosis.

The axolotl does, however, have the ability to undergo metamorphosis if experimentally given the missing hormone. After artificially induced metamorphosis, the individual is able to lead a terrestrial life (indicating that the suppression of metamorphosis occurred relatively recently in the course of axolotl evolution). In other words, axolotls were once amphibians, just like their relatives, the salamanders. Although tens or hundreds of thousands of generations of his ancestors did not walk on land, the artificially metamorphosed axolotl did not lose the instincts of terrestrial life (locomotion, feeding, chasing prey, avoiding predators, etc). Another method of artificially inducing metamorphosis is by injecting iodine, which is used by the salamander's body in the production of thyroid hormones.

A metamorphosing axolotl goes through a series of anatomical, morphological and physiological changes that help it adapt to terrestrial life. These changes include increased muscle tone in the limbs (because on land the specific gravity becomes greater and the body needs musculature to support or pull the body), absorption of gills and appendages specific to swimming, development of eyelids and a reduction in skin permeability to water, allowing the axolotl to stay hydrated longer when on land.

Conflict of interest. Authors declare that there is no conflict of interest.

References

Adamson C. J., Morrison-Welch N., Rogers C. D., 2022 The amazing and anomalous axolotls as scientific models. Developmental Dynamics 251(6):922-933.

Bothe V., Mahlow K., Fröbisch N. B., 2021 A histological study of normal and pathological limb regeneration in the Mexican axolotl *Ambystoma mexicanum*. Journal of Experimental Zoology Part B: Molecular and Developmental Evolution 336(2):116-128.

Clarke J. D. W., Alexander R., Holder N., 1988 Regeneration of descending axons in the spinal cord of the axolotl. Neuroscience Letters 89(1):1-6.

Crowner A., Khatri S., Blichmann D., Voss S. R., 2019 Rediscovering the axolotl as a model for thyroid hormone dependent development. Frontiers in Endocrinology 10:237.

Hosie C. A., Smith T. E., 2021 Behavioral Biology of Amphibians. In: Behavioral Biology of Laboratory Animals (pp. 345-359). CRC Press.

Newth D. R., 1960 Black axolotl, and white. American Scientist 48(3):300-310.

Olejnickova V., Kolesova H., Bartos M., Sedmera D., Gregorovicova M., 2022 The tale-tell heart: evolutionary tetrapod shift from aquatic to terrestrial life-style reflected in heart changes in axolotl (*Ambystoma mexicanum*). Developmental Dynamics 251(6):1004-1014.

Sámano C., González-Barrios R., Castro-Azpíroz M., Torres-García D., Ocampo-Cervantes J., A., Otero-Negrete J., Soto-Reyes E., 2021 Genomics and epigenomics of axolotl regeneration. International Journal of Developmental Biology 65(7-8-9):465-474.

Shukhova S., MacMillan D. C., 2020 From tigers to axolotls: Why people keep exotic pets in Russia. People and Nature 2(4):940-949.

Tate C. E., 2010 The axolotl as food and symbol in the basin of Mexico, from 1200 BC to today. Pre-Columbian foodways: interdisciplinary approaches to food, culture, and markets in ancient Mesoamerica. Springer, New York, pp. 511-533.
Trujillo I., 2000 Xochimilco The Struggle for Survival. Voices of Mexico.
Valiente E., Tovar A., González H., Eslava-Sandoval D., Zambrano L., 2010 Creating refuges for the axolotl (*Ambystoma mexicanum*). Ecological Restoration 28(3):257-259.
Woodcock M. R., Vaughn-Wolfe J., Elias A., Kump D. K., Kendall K. D., Timoshevskaya N., Timoshevskiy V., Perry D. W., Smith J. J., Spiewak J. E., Parichy D. M., Voss S. R., 2017 Identification of mutant genes and introgressed tiger salamander DNA in the laboratory axolotl, *Ambystoma mexicanum*. Scientific Reports 7(1):6.
Woodley S. K., Staub N. L., 2021 Pheromonal communication in urodelan amphibians. Cell and Tissue Research 383:327-345.
***www.nationalgeographic.com, https://www.nationalgeographic.com/animals/amphibians/facts/axolotl [Last view: December 2022].
*** www.wikipedia.org, https://ro.wikipedia.org/wiki/Axolotl [Last view: December 2022]
*** www.zipcodezoo.com [Last view: December 2022]

Received: 17 November 2022. Accepted: 12 December 2022. Published online: 21 December 2022.
Authors:
Marian Proorocu, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Agriculture, Department of Environmental Engineering and Protection, 3-5 Calea Mănăştur Street, 400372 Cluj-Napoca, Romania, e-mail: mproorocu@yahoo.com
Ioan Valentin Petrescu-Mag, SC Bioflux SRL Cluj-Napoca, 54 Ceahlau Street, 400488 Cluj-Napoca, Romania, e-mail: zoobiomag2004@yahoo.com
How to cite this article:
Proorocu M., Petrescu-Mag I. V., 2022 Axolotls and neoteny: those who forgot to grow up. ELBA Bioflux 14(1):12-16.