THE

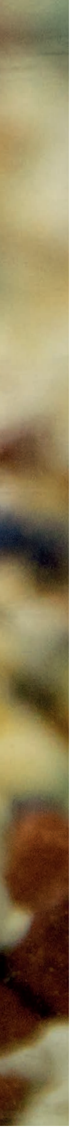
AXOLOTL PARADOX

An iconic salamander species, celebrated and studied around the world, is racing towards extinction.

BY ERIK VANCE

When biologist Luis Zambrano began his career in the late 1990s, he pictured himself working miles from civilization, maybe discovering new species in some hidden corner of Mexico's Yucatán Peninsula. Instead, in 2003, he found himself counting amphibians in the polluted, murky canals of Mexico City's Xochimilco district. The job had its advantages: he was working minutes from his home and studying the axolotl (*Ambystoma mexicanum*), a national icon in Mexico and arguably the world's most recognizable salamander. But in that first year, Zambrano couldn't wait for it to be over.

Axolotls inhabit thousands of labs and home aquariums around the world, but are vanishing from their natural habitat.



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"Let me tell you, I hated the project at the beginning," he says. For one thing, "I couldn't catch anything".

Over time, however, he did catch some axolotls. What he found surprised him — and changed the course of his career. In 1998, the first robust study to count axolotls estimated that there were about 6,000 of them per square kilometre in Xochimilco[1]. Zambrano — who now is a professor at the National Autonomous University of Mexico (UNAM) in Mexico City — discovered in 2000 that the number had dropped to about 1,000 animals per square kilometre. By 2008, it was down to 100; today, thanks to pollution and invasive predators, there are fewer than 35 animals per square kilometre[1].

The axolotl is on the brink of annihilation in the canals of Mexico City, its only natural habitat. But although there might be just a few hundred individuals left in the wild, tens of thousands can be found in home aquariums and research laboratories around the world. They are bred so widely in captivity that certain restaurants in Japan even serve them up deep-fried.

"The axolotl is a complete conservation paradox," says Richard Griffiths, an ecologist at the University of Kent in Canterbury, UK, who recruited Zambrano to the project. "Because it's probably the most widely distributed amphibian around the world in pet shops and labs, and yet it's almost extinct in the wild."

This creates a problem for biologists. Thanks to its unique physiology and remarkable ability to regenerate severed limbs, the axolotl has become an important lab model for everything from tissue repair to development and cancer. But after centuries of inbreeding, captive populations are vulnerable to disease. And the loss of genetic diversity in wild axolotls — owing to their diminishing population — means that scientists lose out on learning all they can about the animal's biology.

As lab scientists continue to study the captive animal and its large and complex genome, Zambrano and a handful of other researchers are doing their best to preserve the wild version. They are breeding and releasing axolotls into control ponds and canals in and around Xochimilco to see how they fare, and hopefully to retain some of their natural genetic diversity. The task of saving them is difficult, but should be doable given the animal's hardiness — if the Mexican government would only engage with the process.

"I've seen that in other places in the world, these kinds of huge tasks are possible," Zambrano says. "If they can do it, why can't we?"

THE CREATURE THAT NEVER GREW UP

Axolotls evolved relatively recently compared to other salamander species in the region, and they thrived along the banks of Lake Texcoco in the mountains of central Mexico. They are neotenic, meaning that the adults retain traits seen only in juveniles of similar species. Although other salamanders metamorphose into terrestrial creatures, axolotls hold on to their feathery gills and stay in the water for their entire lives. It's as if they never grow up.

Sometime in the thirteenth century, Lake Texcoco was settled by the Mexica (the people that Europeans dubbed Aztecs). They built a powerful empire controlled by an island city built in the middle of the lake. As the empire grew, so did the land, expanding much faster after the Spanish conquest in 1521. Today, all that remains of the axolotl's habitat, are about 170 kilometres of canals criss-crossing Xochimilco, a district in the southern part of Mexico City (see 'A shrinking habitat').

The species might have perished entirely under colonial rule, except that its odd inability to grow up caught the attention of European scientists, who puzzled over it in the late nineteenth century.

Visitors to Mexico brought the creatures back and began breeding them. The animal turned out to be ideal for research: it reproduces readily in the lab, is a hardy survivor and is easy to care for. Axolotls have large cells that simplify investigations into development. Their eggs are almost 30 times larger than a human's. And in an axolotl embryo, the neural plate cells — a precursor to the brain and spinal cord — are almost 600 times larger by volume.

Also, the pigmentation of axolotls varies greatly from one cell to the next, unlike in humans or other animals, in which cell traits tend to be uniform. This can help researchers to track which tissues in an embryo become which organs. Yet it has a large genome — roughly ten times the size of a human's — which can make it challenging to study in some respects.

"It is not a good genetic model organism, but it does regenerate — and that makes it an awesome biological model," says David Gardiner, a developmental biologist at the University of California, Irvine, who has studied axolotl regeneration for decades.

In the early twentieth century, axolotls were central to understanding how organs develop and function in vertebrates. They helped scientists to unpick the causes of spina bifida in humans — a birth defect in which the spine doesn't form properly. And they played a part in the discovery of thyroid hormones: in the 1920s, scientists fed thyroid tissue from livestock to axolotls. If the tissue had been secreting hormone, the axolotls would metamorphose, losing their gills and shedding their larval skin.

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In the 1980s, axolotls helped scientists to develop a model explaining how cells take on different forms in embryos. The 'cell state splitter' model proposes that many stem cells turn into specific tissues in the body through waves of pulling and stretching as embryos. Scientists found that they could watch the axolotl's cells squeeze and stretch before they formed tissues. More recently, in 2011, extract from axolotl oocytes has been used to stop breast-cancer cells multiplying by switching on a tumour-suppressor gene[2].

But perhaps the most fascinating contribution of the axolotl to science has been in regenerative medicine. The animals can grow back missing limbs, tails, organs, parts of the eye and even portions of the brain. Many scientists have presumed this is because, being neotenic, they retain some trait from their embryonic stages, although other salamanders seem to regenerate even as adults.

Biologists have been trying to identify the mechanisms behind their regenerative abilities for decades, says Tatiana Sandoval Guzmán, a regeneration researcher at the Technical University of Dresden, Germany. "How do they do it? What is it that they have that we don't? Or maybe the opposite — what in mammals is stopping that?"

Sandoval Guzmán is interested in bone and muscle regeneration and has taken over a long-standing axolotl laboratory in Dresden. A Mexican national who went to school not far from Xochimilco, she never thought much about the animal and certainly never considered studying it until she came to Germany. Today she is fascinated by the creature, and has shown[3] that many of the mechanisms in axolotl regeneration — such as those involving muscle-tissue stem cells — are not so different from those found in humans.

Most regeneration research focuses on the stub — or blastema — that forms over the wound of a severed limb. Whereas such a wound in humans gets covered with skin tissue, axolotls transform nearby cells into stem cells and recruit others from farther away to gather near the injury. There, the cells begin forming bones, skin and veins in almost the same way as when the animal was developing inside the egg. Each tissue contributes its own stem cells to the effort.

Researchers showed that a protein called transforming growth factor-β is key both in axolotl regeneration and in preventing scar tissue in injured human embryos during the first trimester. Adult mice and humans can regenerate digit tips, although humans lose this ability with age, suggesting that regenerative abilities could be reawakened in mammals.

"There will be a day when we as humans can regenerate," says Gardiner. His studies are not focused on rebuilding limbs, but on curing paralysis, growing healthy organs and even reversing ageing by repairing

A SHRINKING HABITAT

All around the island city of Tenochtitlan, the indigenous Mexica built floating gardens or *chinampas*, creating canals that were an ideal habitat for axolotls. After Europeans conquered the city, the lakes were drained and just a tiny sliver of axolotl habitat remains.

TOMÁS FILSINGER

damaged and worn-out tissues. "And when they write that story, it will go back to these model organisms," he says.

By the time that day comes, however, the wild axolotl may be gone. That worries Gardiner and Sandoval Guzmán because the animals that they study, like many lab animals, are highly inbred. Scientists use an 'inbreeding coefficient' to measure how small a gene pool is. Identical twins have a coefficient of 100%; totally unrelated individuals would score close to zero. A score above 12% indicates a population in which individuals are mostly breeding with their first cousins, and is considered a serious concern by ecologists and geneticists. The famously inbred and unhealthy Spanish Habsburg kings of the seventeenth century often had a coefficient somewhere above 20%. The average axolotl inbreeding coefficient is 35%.

"These animals that we have, they still work just fine, they regenerate just fine. But they are so inbred. It's a bottleneck," Gardiner says. "Populations are very vulnerable to disease when inbred."

Their high level of inbreeding is partly a result of the bizarre historical path captive axolotls have taken. Most laboratory specimens trace their heritage back to a single group of 34 animals that were taken out of Xochimilco by a French-funded expedition in 1863. They sparked an axolotl-breeding craze across Europe by museums and naturalists.

In 1935, some of the animals travelled from a Polish laboratory back to North America, where they eventually became a breeding stock at the University of Buffalo, New York. Here, scientists brought in a series of wild axolotls to mix up the gene pool and at one point even added in tiger salamanders (*Ambystoma tigrinum*). The Buffalo population thrived and eventually moved to the University of Kentucky in Lexington, which is now the centre of global axolotl breeding. This means that, in addition to being inbred, almost all of the axolotls in labs and aquariums today are actually part tiger salamander.

"They got bottlenecked in Europe for sure and then they got bottlenecked again," says Randal Voss, head of the programme in Kentucky, which holds some 2,000 adults and 3,000–5,000 larvae.

Voss says that axolotl research today is expanding throughout the world, thanks to modern genetics and stem-cell research. In 2015, he and his group published an initial assembly of the axolotl genome[4], a Herculean task given its large size, estimated to be about 32 billion bases. But it is incomplete — the size and complexity of the genome proved too much for the computational power Voss's group could throw at it. Scientists in several centres continue to work on completing the picture.

But as they work on that, the creature's vulnerability to disease has already caused mysterious massive die-offs in Voss' facility. Scientists worry that if a new infectious disease were to race around labs worldwide, it might force them to abandon the axolotl, potentially setting research back by years.

What's more, no one can be sure that lab axolotls haven't already diverged so much from their wild counterparts that they have lost key elements of regeneration. "Going back to study the wild population can give you a different mechanism or different genes," says Sandoval Guzmán. "Losing the genetic diversity — of course it's a loss for science."

DOUBLE DIGITS

"I can't always know for sure, but the axolotls from Kentucky do have some differences," says Arturo Vergara Iglesias, staring into a tank of axolotls lazily crawling about. Although they trace their lineage to the University of Kentucky breeding facility, many of the non-native animals that he comes across will subsequently have been bred by labs, pet shops and hobbyists, which can lead to problems. "They have a lot of malformations. For example, they often have too many fingers."

Vergara Iglesias is a biologist at the Centre for Biological and Aquaculture Research (CIBAC), an axolotl breeding facility near Xochimilco that is hoping to preserve a few wild lines. On the side, he breeds his own wild axolotls to sell to labs and pet distributors. He is standing over a salamander tank on a traditional Xochimilco farm plot, or *chinampa*, that is used as an educational facility for tourists. These animals, and the others he sells, were bred from a group of 32 pulled out of the water not far from the plot. In Mexico, the axolotl is a prized pet and a source of national pride. It's the subject of countless Mexican memes and souvenirs, and is even the official emoji for Mexico City.

It's hard to know exactly how many axolotls are left in the wild there. Zambrano guesses that during his last survey, in 2014, there were fewer than 1,000 in total, and perhaps fewer than 500. But he can't be more specific — in the past two years, he's been unable to raise the money to do any follow-up studies. That he can't obtain funding for a simple census does not bode well for conservation efforts.

Zambrano says that to save the wild axolotl, policymakers must address its two primary threats. The first is non-native fish such as the common carp (*Cyprinus carpio*) and tilapia (*Oreochromis niloticus*). Ironically, these were introduced to Xochimilco in the 1970s and 1980s through programmes run by the Food and Agriculture Organization of the United Nations, with the aim of getting more protein into local diets. Zambrano says he has mapped the areas where axolotls still remain; he envisions a team of local fishers being paid to sweep them of fish on an ongoing basis. Although this wouldn't remove all the fish, for a few hundred thousand

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Biologist Luis Zambrano (centre, seated) and his students tour the canals of Xochimilco, Mexico City, in the early morning.

dollars it might give the salamanders a window in which to re-establish themselves. His work has shown that axolotls are most vulnerable to carp when they are at the egg stage, and to tilapia when they are juveniles, but reveals that if they can grow beyond a certain size, they might still thrive[5].

The second threat is trickier. Every time a powerful storm fills the city's ageing sewer system, treatment facilities release human waste into Xochimilco, carrying with it ammonia, heavy metals and untold other toxic chemicals. Amphibians, which breathe in part through their highly permeable skin, are vulnerable to these regular pollution dumps. It's a testament to the animal's resilience that it exists in the wild at all.

IT SEEMED POINTLESS TO SEND AXOLOTLS OFF TO THEIR DEATHS.

These are complex issues, but they are not unsolvable. So far, however, there have been no efforts to save the wild axolotl beyond a few halfhearted outreach programmes and some photo opportunities. In 2013, CIBAC released a few thousand axolotls for a behavioural study; some of them survived and even seemed to breed the following year. This suggests that lab-bred salamanders might be able to thrive in the wild if they are raised in captivity to a certain size. But biologists caution that this doesn't mean Mexico should start releasing them into canals.

"There's probably not much point in doing releases into the wild until you can neutralize the threats," says Griffiths. "You just might be increasing the fish population by just chucking out more fish food."

When Griffiths first started working in Xochimilco in 2000, his plan was to create a breeding programme aimed at releasing axolotls into the wild. But he and his Mexican partners quickly abandoned the idea once they saw the condition of the ecosystem, which was polluted and teeming with predators. It seemed pointless to send axolotls off to their deaths. Successful reintroductions, such as those of the pool frog (*Pelophylax lessonae*) in Britain or the hellbender salamander (*Cryptobranchus alleganiensis*) in the United States, require managing the ecosystem as a whole and working with the community.

"If we had a million dollars per year for ten years, we would save Xochimilco. Which is nothing compared with the amount of money that is spent in this city," says Zambrano.

One afternoon in October, Zambrano and a group of volunteers gather by the ponds near the UNAM campus to release ten lab-raised wild axolotls into a protected pond. If the animals survive and breed, they might someday act as a sort of genetic bank for the organism. Zambrano has been sporadically releasing and tracking animals here over two years to understand their behaviour and habitat preferences. His work so far suggests that the salamanders prefer fairly dirty ponds over the most pristine ones — another sign that axolotls might still thrive in Xochimilco if other pressures are removed. Similarly, CIBAC is breeding wild-type animals in an effort to preserve the axolotl's genetic diversity. But if axolotls do not have a suitable home, most researchers say that their extinction in the wild might be inevitable, no matter what they do.

"I would be frustrated if I saw it in that way," says Zambrano. "I see it with another view — that I am doing my best to keep that from happening." ■

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CLARIFICATION

A quote in 'The axolotl paradox' (*Nature* **551,** 286–289; 2017) implies that animals obtained from a breeding facility in Kentucky have a high rate of malformations. This is not the case. The animals referred to may trace their lineage to the facility, but have been bred and potentially inbred elsewhere.