Beastly beauty. With its feathery gills and quizzical face, the axolotl is plausible as an Aztec god.

ENDANGERED SPECIES

Sanctuaries Aim to Preserve a Model Organism's Wild Type

The axolotl, a salamander that retains unique evolutionary features and is a darling of biologists because it can regenerate limbs, faces adversity on two fronts

MEXICO CITY—Leaning over the *Traginera* flatboat's edge, Luis Zambrano surveys a canal floating with plastic bottles, Styrofoam cups, and a leafy carpet of invasive lilies. African tilapia fish ripple the brown water's surface and a Chinese carp lurks underneath, but Zambrano sees no signs of his elusive goal: the axolotl salamander.

"We've spotted only a few in 6 months," says Zambrano, a freshwater ecologist at the National Autonomous University of Mexico (UNAM) who is trying to count and preserve the feathery-gilled, 33-centimeter-long salamanders in their only natural habitat, the Xochimilco network of polluted canals and small lakes in and around Mexico City, the world's third largest metropolitan area.

Five hundred years ago, axolotls—named for an Aztec god who transformed into a water animal to avoid being sacrificed—were common in the lakes around the Aztec capital. But as the wetlands receded, so did the axolotls, to the point that Zambrano now estimates a population density of only 100 per square kilometer of wetland, compared with estimates 10 times higher in 2004 and another six times higher than that in the 1980s. The species, *Ambystoma mexicanum*, is now classified as critically endangered by the International Union for Conservation of Nature.

A serious threat to axolotls could prove damaging to science, for the salamander has been used for more than a century as a model organism by developmental biologists. Even though the wild type's survival is threatened, thousands of axolotls are raised in laboratories every year for use in research projects involving regeneration, stem cells, and developmental biology. For example, more than 1000 adult and juvenile axolotls are maintained in aquariums at the University of Kentucky's Ambystoma Genetic Stock Center in Lexington, which distributes between 15,000 and 20,000 axolotl embryos each year to more than 100 research labs in India, Germany, Japan, Mexico, and elsewhere.

Although they are propagated as aquarium pets and are considered easy to breed, some axolotl colonies in labs are now under threat from a puzzling disease. The Ambystoma center's director, developmental biologist Randal Voss, is concerned about what he calls a "mysterious epidemic"—it first emerged when the center was managed at Indiana University in the 1990s and re-emerged a few years ago—that has been killing some axolotl larvae. He says, "Very little is known about disease and pathogens of lower vertebrates."

Allowing axolotls to disappear from the wild would carry an immeasurable risk, researchers say: There's a danger that vulnerable lab populations might be wiped out by disease, and no one knows exactly how a loss of the wild type might diminish future studies of evolution and regeneration.

Axolotls in the lab

Ever since the Aztecs began using axolotls for medicine and in cultural ceremonies, the odd-looking salamanders have had a special significance outside their watery homes. The use of axolotls in modern science began in the 1860s, when a French expedition collected 34 of the amphibians and shipped them to the Natural History Museum in Paris, which gave six to French zoologist Auguste Duméril. Over the past century, various labs have bred them in colonies for research.

Among the naturalists fascinated by the axolotl's neoteny—its retention of larval characteristics such as gills into adulthood—was Stephen Jay Gould, who described the salamanders as "sexually mature tadpoles." His book *Ontogeny and Phylogeny* pictured an axolotl on its cover along with a closely related tiger salamander that had fully metamorphosed and lost its gills. "The axolotl is a fascinating case of what is known as heterochrony—that is, you evolve a brand-new life history by tinkering with the timing of developmental events," says H. Bradley Shaffer, director of the Center for Population Biology at the University of California, Davis. His research groups have worked since the 1970s on issues related to the evolution, ecology, and conservation of axolotls and tiger salamanders.

Because of their large egg and embryo size, susceptibility to tissue grafting, and ability to regrow severed limbs and tails, "axolotls have a long history as primary amphibian models, especially in research areas involving embryonic development," says Voss. He calls them a "re-emerging model organism" for scientists who study them with gene expression and other new tools. For example, cell and developmental biologist Elly Tanaka of the Center for Regenerative Therapies at the Dresden University of Technology in Germany says her lab was able to develop and breed transgenic axolotls, which "makes it easier to study the

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mysterious process of regeneration on a molecular level by driving gene expression in regenerating tissues."

When a salamander regrows its severed tail, it must regenerate a portion of the spinal cord and the neurons inside. "How these particular vertebrates have kept this ability to regenerate while others have lost or blocked it fascinated me," says Tanaka, who describes the axolotl as "an interesting and important organism for studies on the evolution of vertebrate traits." Her lab has analyzed signaling pathways that control regeneration, such as "proteins that tell a regenerating cell whether it should form an upper arm or lower arm cells." In a paper last year in *Development*, her group shed light on how the axolotl's neural progenitor cells are activated to help regenerate a segment of spinal cord.

In Kentucky, Voss's group is studying gene expression in axolotls, including differences in how brain genes function during the larval development of axolotls in contrast to closely related tiger salamanders, which metamorphose beyond the larval stage. "The data show hundreds of stable gene-expression changes that presumably evolved between these species in the last few million years."

Among the stem cell scientists who use axolotls in their research is Andrew Johnson of the Institute of Genetics at the University of Nottingham in the U.K., who studies the production of primordial germ cells (PGCs) in the salamander's embryos. "Axolotls are significant in that they share a mechanism that has been conserved during the evolution of mammals, in which PGCs are produced from pluripotent stem cells," Johnson says. His group is investigating how such stem cells ignore signals that typically trigger somatic cells to differentiate.

Salamander sanctuaries

On the flatboat, Zambrano and a local fisherman cast a gillnet every 200 meters, drag it across the canal, and then search for salamanders. They seldom find any. "The eggs and larvae do not seem to be surviving," he says.

In a lab at UNAM, Zambrano and colleagues study about 40 juvenile and adult axolotls to find out more about their egg-laying habits, the most favorable breeding conditions, and their vulnerability to alien fish species, especially tilapia and carp. He attributes the axolotl decline to the rapidly increasing numbers of those predatory fish as well as changes in land use that have polluted the nearby canals.

Shaffer points out, however, that the Xochimilco's water quality has probably improved since the 1960s and 1970s, when "many thought the native axolotls were gone." The Mexican government began cleaning up parts of the canals and, in the late 1980s, local zoologist Virginia Graue began studying axolotl population trends and tried to increase their numbers. Zambrano did not work with Graue but expanded his own research after her death in 2004.

Axolotl search. Luis Zambrano (*left*) and a local fisherman search for axolotls in their natural habitat, on one of Mexico City's canals.

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Noticing that the water quality varies greatly in the canal network, Zambrano has developed a model to predict where the axolotls would be able to survive. He has also outlined a plan to create sanctuaries. The first will be located in a narrow side canal in the *Chinanpera* area near Doll Island, on which superstitious local residents have left old dolls to scare away the ghost of a drowned girl. Invasive fish and plant species will be removed and kept away by wooden gates separating the canal from the main channel.

A key player in international efforts to help preserve the wild-type axolotl has been zoologist Bob Johnson, the Toronto Zoo's amphibian and reptile curator. He used to support Graue's work and helped link scientists from the Mexican project with those at the Durrell Institute of Conservation and Ecology (DICE) at the University of Kent, Canterbury, in the U.K. They led a 5-year effort, supported by the Darwin Initiative, to develop a conservation program for the axolotl and the canal system. That project's funding has ended, but Johnson has since helped Zambrano and the Chapultepec Zoo get four new grants, including $19,000 from the Association of Zoos and Aquariums Conservation Endowment Fund, to help create canal sanctuaries.

Although everyone seems to agree that the salamander's habitat needs to be preserved, some wonder if there are enough axolotls left to repopulate the canals. A veterinarian here has suggested releasing lab-raised axolotls into the canals, but Zambrano and others fear that the captive salamanders might introduce fungal and other diseases. "Even if you take rigorous precautions, diseases can still slip through the net," says amphibian ecologist Richard A. Griffiths, who led DICE's axolotl project.

Another fear is that introducing axolotls from ingrown lab colonies would reduce the genetic diversity of the wild type. Also, Zambrano says lab-raised axolotls likely would suffer the same fate in the canals as the wild type. "It is more effective to create sanctuaries in which the existing axolotls can survive and perhaps thrive," he says.

Even scientists who see only lab colonies of the salamander worry about the wild type's future. "We are concerned about the worldwide decline not only of axolotls but of many salamander species," Tanaka says. "They represent a very important group to study in terms of evolution."

Neurobiologist Alejandro Sanchez Alvarado, who studies the molecular basis of regeneration at the University of Utah School of Medicine in Salt Lake City, says losing the wild axolotls would be a tragedy. "Wild-type populations provide us with a window, a record of how biological traits evolve genetically," he says. "Who is to say that unlocking the evolutionary mystery shrouding regenerative capacities in vertebrates will not come from studying wild axolotl gene pools?" **–ROBERT KOENIG**