Some annual killifish embryos can shut down and survive months with no oxygen.

PHOTO: JASON PODRABSKY AND CLAIRE RIGGS

PHYSIOLOGY

A fish back from the dead

Understanding how killifish survive months without oxygen could help stroke victims

By **Elizabeth Pennisi**, *in Portland, Oregon*

Deprive a human of oxygen for 5 minutes or more and she will turn blue, pass out, and may die. Suffocate the embryo of a Venezuelan annual killifish, however, and it survives for months, emerging unscathed to complete its development. Pinkie-sized and nondescript, the killifish is "a champion of anoxia tolerance among vertebrates," says Daniel Warren, a comparative physiologist at St. Louis University in Missouri.

At the annual meeting of the Society for Integrative and Comparative Biology here last month, Jason Podrabsky offered a look at the killifish's bag of tricks. The comparative physiologist from Portland State University in Oregon and his students are studying the fish to explore the extremes of vertebrate physiology—and to find clues to treating stroke, heart attack, or trauma, which can starve tissues of oxygen. "If we can figure out the mechanisms that animals naturally use to keep them tolerant of anoxia, the potential is there to adopt and use them in a surgical or trauma setting," says W. Wesley Dowd, a comparative physiologist at Loyola Marymount University in Los Angeles, California.

Researchers have identified two other vertebrates, Europe's crucian carp and North America's Western painted turtle, that can live without oxygen just as long as the killifish. But those organisms experience anoxia when the lakes they live in freeze, and the cold helps them survive by slowing their metabolism. In contrast, killifish survive anoxia at tropical temperatures, a much harder task.

The killifish inhabit temporary pools that form during the rainy season and often vanish before the fish can complete their life cycle. Their eggs may be marooned in the clay-laden mud, sealed off from oxygen, for weeks or months until the rains return. Podrabsky's studies revealed that changes in temperature help trigger the embryos to enter "diapause," or dormancy. Rainy seasons, when the ponds fill and killifish thrive, are warm. But when Podrabsky cooled the embryos to 25°C, they stopped developing and entered diapause. At that point, he reported, the fish's heart stopped in a matter of hours, mitochondria—the cell's power plants—reduced their production of the energy molecule ATP, and metabolism shut down.

Over the past several years, Podrabsky and his students have searched for the genetic basis of this shutdown by sequencing the killifish genome and isolating RNAs it produces. Graduate student Amie Romney, for example, has followed both the protein-coding RNA packaged by the mother in her eggs and small pieces of RNA produced by the embryo. The two types of RNA appear to tussle for control over diapause, she reported. The maternal RNA carries the instructions for initiating diapause, but high temperatures—meaning wet conditions—seem to stimulate the embryo's production of small noncoding RNAs that may counter those instructions. One such small RNA, mir430, is known to inactivate maternal RNA in the embryos of another fish, the zebrafish. In killifish embryos that skip diapause, mir430 increases in abundance and stays plentiful, Romney reported.

Many other RNAs seem to help the fish deal with low or no oxygen, graduate student Claire Riggs found. She reported that some RNAs isolated from killifish embryos resemble small RNAs seen in mammal cells temporarily deprived of oxygen, and many are concentrated in the brain, an organ especially sensitive to hypoxia. Even so, Dowd says researchers can map "only 10% [of RNAs] … to a genomic sequence that we know something about."

Riggs and others are also comparing the killifish's adaptations with those of other anoxia-tolerant champions. Unlike killifish, anoxic turtles continue making ATP, Warren has found; the turtles rely on an anaerobic pathway, like a human sprinter outrunning her oxygen supply. That causes extreme lactic acid buildup, which the turtles buffer with carbonate taken from their shell and other bones. They also slow down their heart. The crucian carp, in contrast, keeps its heart beating strongly, even after 5 days without oxygen, Göran Nilsson, a physiologist at the University of Oslo, has found. The carp also generates energy anaerobically, but it produces alcohol instead of lactic acid. And it continues to glean oxygen from its environment by increasing the surface area of its gills sevenfold.

The three anoxia champions do have some common strategies. Over the past few decades, Nilsson has shown that levels of GABA, a chemical that inhibits nerve activity in humans, increases in the brains of carp and painted turtles during anoxia, perhaps slowing brain energy consumption. Podrabsky also found that GABA increases in his killifish—to concentrations "far beyond any vertebrate that's been looked at," he says. Killifish embryos die if they can't build up GABA when they are deprived of oxygen.

Now Riggs, with the help of other anoxia researchers, is examining small RNAs from all three species, to see whether they share the same rapid cellular switches that seem to shut down metabolism. If so, Dowd says, "that would be a remarkable example of convergent evolution"—and a hint that similar switches could protect other organisms from hypoxia, perhaps even us. ■