TECHNOLOGY FEATURE

LIVE FAST, DIE YOUNG

Research into ageing requires patience, but a small cadre of scientists is angling to speed up answers by developing the flamboyant, short-lived turquoise killifish as a new model.

SEBASTIAN KAHNERT/DPA/PICTURE ALLIANCE

The turquoise killifish (*Nothobranchius furzeri*), with its fleeting life, is helping researchers to study the mechanisms that influence human ageing.

BY AMBER DANCE

Physiologist Alessandro Cellerino has always been an aquarium enthusiast, but fish were not originally part of his research plan. One afternoon in 2000, hanging out in a tank-filled cellar in Canossa, Italy, with breeder Stefano Valdesalici, Cellerino idly asked him which fish were the shortest-lived. Valdesalici pointed to a tank with brightly speckled African turquoise killifish: "They don't make it any longer than three months."

"Are you kidding?" asked Cellerino, who works at the Scuola Normale Superiore in Pisa, Italy. "OK, I want them."

So in March 2004, Cellerino and his graduate student Dario Riccardo Valenzano found themselves bouncing through Mozambique in a four-wheel-drive truck with Valdesalici, who chairs the Italian Killifish Association in Canossa. They donned chest-high waders and gloves to net killifish from the seasonal, cow-pat-spattered mud holes where the fish live. Like the tank-bred versions, these wild strains were exceptionally short-lived. Of the many varieties of killifish, the turquoise killifish (*Nothobranchius furzeri*) has the shortest lifespan — the briefest of any vertebrate bred in captivity, ranging from 3 to 12 months depending on strain and living conditions.

Using killifish to study ageing is not a new idea. In the late twentieth century, scientists studied ageing in one species, *Nothobranchius guentheri*, that lives for about 14 months. But given techniques available at the time, they could come up with only basic descriptions of ageing features. When Cellerino encountered *N. furzeri*, timing and luck were on his side: advances in molecular analysis had set up excellent conditions in which to develop the model and investigate mechanisms behind its dotage.

The killifish's brief lifespan, relative to those of longer-lived models such as mice and zebrafish, enables ageing research to progress apace. And because the fish is a vertebrate, the research is more directly relevant to people than are studies of short-lived organisms such as fruit flies or nematodes (see 'The long and short of it').

To set up a killifish model, researchers have taken advantage of modern genomic tools and drawn techniques from well-established zebrafish protocols, rather than starting from scratch. In 2015, the publication of CRISPR–Cas9-based gene-editing techniques for the killifish[1], as well as two complementary genome-sequencing efforts[2,3], boosted the fish to the status of genetically tractable model.

The killifish — or *Notho*, as some scientists affectionately call it — is certainly gaining fans. Interest has "really exploded over the past few years", says Valenzano, who now works at the Max Planck Institute for Biology of Ageing in Cologne, Germany. He estimates that about two dozen scientists have visited his group in the past year to learn killifish husbandry. In June, about 70 *Notho* aficionados attended the second *Nothobranchius* Symposium in Jena, Germany. But the challenges of keeping killifish — such as their lack of a standardized diet — and a want for basic reagents, such as *Notho*-specific antibodies, mean that the fish has a way to go before it reaches the utility of lab mice.

The ephemeral existence that so appeals to scientists is an evolutionary adaptation to the fish's natural environment: their accelerated development enables them to live and reproduce in transient mud pools during the wet season in equatorial Africa. The eggs survive in a dormant state during the dry season, and once the rains come and pools form, they hatch. The fish have only a few weeks or months to grow up and spawn before the water dries up.

Hobbyists, attracted by the males' flashy appearance, have been collecting turquoise killifish since they were discovered in Zimbabwe in 1968. Consequently, Cellerino's first challenge was to confirm that their lifespan was not a side effect of decades of breeding in tanks. Most of the wild *N. furzeri* that Cellerino's team caught in Mozambique lived for about eight months — not as brief a time as the inbred Zimbabwe line, but still short enough to interest scientists.

But that begged another question: would killifish age in a way that parallels the human process? Yes, says Valenzano: the fish do get 'old' before they die. "They don't drop dead after four months," he says. "They slowly deteriorate." The fish become duller in colour, lose muscle mass and body weight, develop cancers and swim around less.

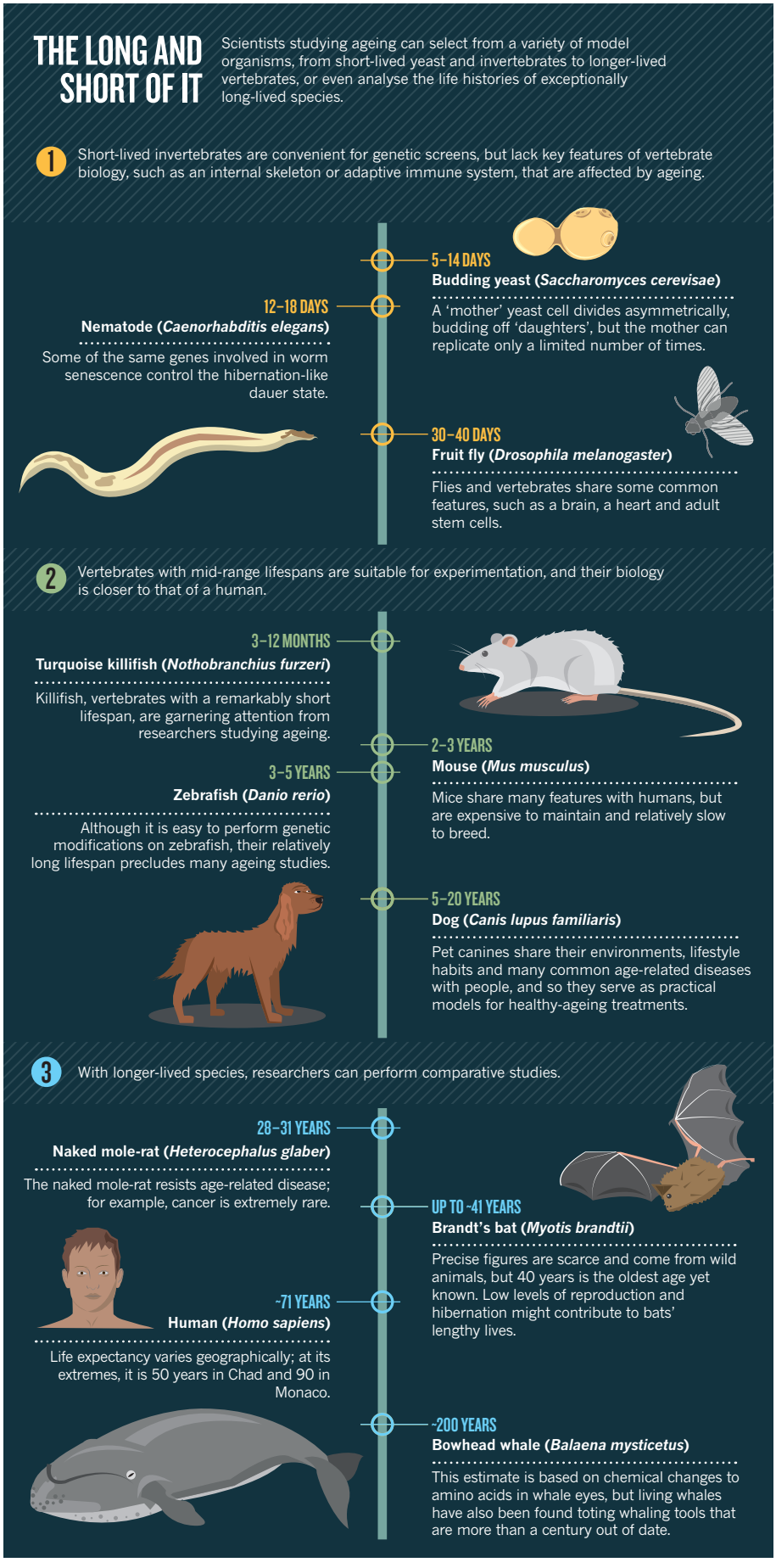
The brain shows typical signs of ageing, too, says Livia D'Angelo, an anatomist at the University of Naples Federico II in Italy. Glia — brain cells that provide support and protection for neurons — upregulate the glial fibrillary acidic protein GFAP, as happens in mammalian ageing, and age-associated, lipid-rich pigment granules called lipofuscin accumulate. Neurons degenerate and deposit amyloid molecules that aggregate, resembling the plaques seen in people with Alzheimer's disease, Valenzano adds. He has also found that old fish don't learn as well as young ones. Young fish rapidly work out how to avoid an unpleasant stimulus, such as a plastic stick swirling in their tank, whereas old-timers take longer to catch on[4]. "It's a very good model for neuroscience research," D'Angelo says.

The fish also respond to anti-ageing interventions much as some short-lived vertebrates do. Resveratrol — the stuff in red wine that prolongs life in nematodes and fruit flies — can lengthen their lifespan by up to 59%[4]. Restricting feedings to every other day creates a caloric deficit known to extend lifespan in organisms ranging from yeast to rodents, and it does the same for killifish, although the effects vary by strain[5].

GONE FISHING

Having shown that killifish decline with age, scientists now want to understand how the process occurs. One key resource is the collection of several strains from Africa whose genomes are not identical. *Notho* scientists have four main strains to choose from, Cellerino says: the original Zimbabwe line, and three derived from fish caught in Mozambique in 2004 and 2007, which have slightly longer lifespans.

By cross-breeding two strains, Cellerino and



CLAIRE WELSH/*NATURE*

his team created fish with a range of lifespans. They then compared the genomes and longevities of parent and second-generation progeny, and identified a few chromosomal regions, each with hundreds of genes that might influence ageing. Although these did not directly reveal genes involved in longevity, they suggested possible candidates. From this study, the scientists estimated that about 32% of variation in lifespan among turquoise killifish results from genetics, a figure comparable to the 20–35% estimated genetic contribution in mice[6].

From then on, the killifish's transformation into a valid research model accelerated. Anne Brunet, a geneticist studying ageing at Stanford University in California, had longed for a short-lived vertebrate and was delighted to hear about killifish when Valenzano visited Stanford for a summer course. She recruited him to her lab for a postdoc, and in 2006, Valenzano brought the killifish to California. There, he copied and modified protocols for zebrafish to transfer in foreign genes, starting with the green-fluorescent-protein gene from jellyfish[7]. In 2015, Brunet and her colleagues reported the successful use of CRISPR–Cas9 gene editing in killifish, generating fish with mutations in 13 genes involved in key ageing events such as telomere shortening and mitochondrial dysfunction[1].

As enthusiasm for *Notho* grew, two groups tackled its genome sequence: Brunet's lab at Stanford, and Cellerino and collaborators at the Leibniz Institute on Aging–Fritz Lipmann Institute in Jena, where Cellerino worked for a time and still maintains a cooperative group. Both groups published genome sequences[2,3] in December 2015. "The two papers are complementary," says molecular geneticist Matthias Platzer at the Leibniz Institute, who collaborates with Cellerino. Researchers from the teams now plan to make a consensus sequence.

Beyond the genome, scientists are exploring which genes are transcribed into RNA and used for protein production during different stages in the life cycle. Platzer and his colleagues are interrogating messenger RNA molecules — the killifish transcriptome — to find out. To put together a transcript catalogue[8], they sequenced RNA from killifish whole body, brain and skin, taken at a range of ages, from the embryonic period to 39 weeks old.

Cellerino's team used similar techniques to track what happens in tissues from the same killifish as it develops. By taking small fin clips, they let the fish live long enough to be sampled again. They found that the transcriptomes of short- and long-lived killifish differ when those fish are only ten weeks old, and identified a protein that is a key controller of lifespan[9].

Because the killifish is not a mammal, linking fish genes to human ones will require a leap. Fish genes often have a human counterpart, but these can be difficult to find. This is in part because the killifish's ancestor underwent a whole-genome duplication: where human DNA has one copy of a gene, the killifish often has two. But at the Jena meeting, geneticist John Postlethwait of the University of Oregon in Eugene offered a potential solution. The trick, he explains, is to use an intermediate genome from another fish: the spotted gar (*Lepisosteus oculatus*). The gar's ancestors diverged from the killifish's before the duplication event, so its genome is in some ways more similar to that of a mammal. Scientists may be able to find a killifish gene's counterpart in the gar, and from there, find a match in people[10].

"The killifish work clearly is very innovative and potentially could be a really valuable model," says Matt Kaeberlein, a molecular biologist at the University of Washington in Seattle who studies ageing. But he is unsure how popular the fish could become, noting that its adoption will depend on how difficult it is to work with and whether killifish scientists can obtain sufficient funding. Ron Kohanski, programme officer at the US National Institute on Aging in Bethesda, Maryland, says that the agency is not funding killifish research, but is interested in the fish: "The killifish constitutes a good model for ageing on several levels," he says.

> ***"You need to have a 'blue thumb'. You need one person who is 100% of the time taking care of these fish."***

BLUE THUMBS

Yet the African fish has its disadvantages. For one, it's not as easy to keep in a lab as other fish, such as zebrafish. "You need to have a 'blue thumb'," says Cellerino. "You need at least one person who is 100% of the time taking care of these fish." They also need more space than zebrafish, which thrive in crowded conditions; killifish males sometimes fight and might interfere with each other's growth. Because killifish develop so quickly, they eat a lot — and so produce a lot of waste, leading to water-quality challenges. "One of the things we joke about is we don't keep fish, we maintain biofilters," says Mickie Powell, a comparative physiologist at the University of Alabama at Birmingham.

Killifish spawn readily; a couple can produce 20–40 eggs a day. But then things get tricky, because the eggs need to develop in a fairly dry place. Scientists often transfer the eggs to peat for a couple of weeks, but the eggs don't hatch at the same time, so require a watchful eye.

Many researchers feed their killifish bloodworms, but the quality of that foodstuff varies with season and by supplier. Food matters, points out Powell, who is working on a standardized killifish food; for example, diet affects epigenetic markers that in turn influence longevity. She thinks that food choice might explain why some labs report different killifish lifespans.

Researchers also need a better understanding of how to keep lab collections healthy. Brunet's lab was blindsided in 2008 when several fish started to act weirdly, rolling awkwardly instead of swimming straight. A veterinary surgeon diagnosed the parasite *Glugea*, which the scientists suspect came in with other species of killifish that they bought from a fish store. "That was the lowest point," Brunet says. "We had to bleach everything and start from scratch."

Scientists still hanker after tools that are easily obtainable for other model systems. Valenzano and Brunet wish for antibodies to study fish proteins, and Valenzano also dreams of more strains and a stock centre to provide them. These will probably come as the community of killifish researchers grows.

That community is growing beyond those who study ageing, Platzer says. Developmental biologists are interested in the suspended animation, or diapause, that the eggs undergo, and evolutionary geneticists are intrigued by the killifish's use of XY chromosome sex selection. Many other fish use mechanisms such as population density, ambient temperature or ZW chromosomes, in which the egg, not sperm, determines offspring gender. The Jena meeting attracted scientists interested in using killifish to study epigenetics during blood formation, toxicology and shift-worker biology, says co-organizer Christoph Englert of the Leibniz Institute.

Valenzano says that discussions among *Notho* researchers have shifted from tool development to biology. For example, in a study posted on the preprint server bioRxiv[11], Cellerino and his colleagues describe how a microRNA involved in controlling excessive iron levels is upregulated in ageing killifish to protect the brain from iron accumulation. The human version of this microRNA is associated with Alzheimer's, a condition in which high iron levels have been implicated, he adds.

"The fun part is just about to start," says Valenzano. ■

Amber Dance *is a freelance science writer in Los Angeles, California.*

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CORRECTION

In the Toolbox article 'The visualizations transforming biology' (*Nature* **535,** 187–188; 2016), the CellPACK image was labelled as an HIV-1 particle, rather than a *Mycoplasma mycoides* cell. Also, the text implied that Nico Scherf is leader of a cell-biology research group, whereas he is a postdoc studying bioinformatics.