Creature column

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The turquoise killifish

By Itamar Harel

A naturally short-lived fish opens the gates for rapid exploration of vertebrate aging.

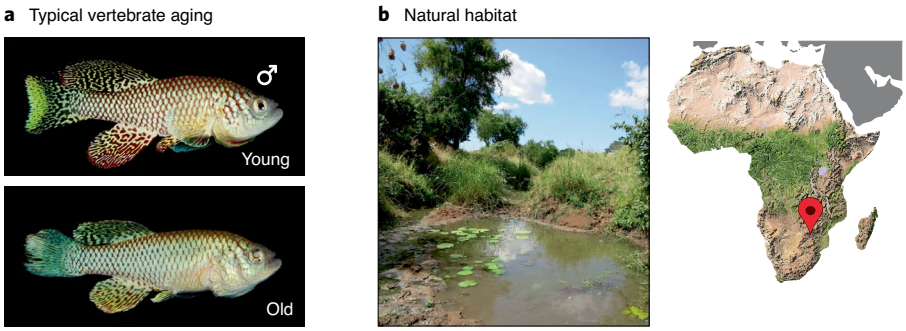

Fig. 1 | The turquoise killifish in the wild. a, A comparison between a 2-month-old male turquoise killifish (top) and a 5-month-old (bottom) shows aging much like that in humans. **b**, The natural habitat of the turquoise killifish is seasonal ponds in southeast Africa, primarily Zimbabwe and Mozambique.

As the most diverse group of vertebrates, fish species display remarkable lifespan differences ranging over 1,000-fold between extremes. For example, the rockfish and the Greenland shark can live for several centuries, while at the other extreme, the turquoise killifish and pygmy goby live for only several months. These adaptations represent an invaluable resource for exploring the mechanisms that regulate the pace of life.

The turquoise killifish (*Nothobranchius furzeri*) can be found in the dry savanna woodlands of southeast Africa, where it inhabits seasonal freshwater ponds that form during the brief rainy season (Fig. 1a,b). This unpredictable habitat produces strong selective pressures that have reshaped the killifish's life history, arming these small fish with an exceptional set of adaptations[1–3]. To mate before habitat desiccation, hatchlings undergo explosive growth and can reach sexual maturity at a record speed of 2–3 weeks. After a few months of mating, killifish undergo typical vertebrate aging, and with a lifespan of 4–6 months, they are the shortest-lived vertebrate that can be bred in the lab; their lifespan is up to ~6-fold shorter than that of mice and ~10-fold shorter than that of zebrafish (Fig. 2a).

What happens to these fish once the rainy season ends? During the dry season, the killifish population is found primarily as developing embryos that cling to the muddy soil, in a state of suspended animation called 'embryonic diapause'. Diapause is a common strategy that allows animals to 'escape in time' from unfavorable conditions[4]. And indeed, these embryos slow their pace of life and wait for months or even years, before synchronously hatching at the onset of the next rainy season (Fig. 2b). Thanks to these unique characteristics, the turquoise killifish has emerged as an experimental model system for vertebrate aging research, as a result of its naturally compressed lifespan, explosive sexual maturity, embryonic diapause and short generation time. In addition, the African turquoise killifish has been used to explore a wide range of other biological questions, including natural selection, genome evolution, ecology, nutrition and toxicology, embryonic development, regeneration and biomedical research.

The most widely used laboratory turquoise killifish strain, the GRZ, is named after the region it was first collected in 1968, the Gonarezhou National Park in Zimbabwe ('Place of Elephants' in the local Shona dialect). Since then, accumulating evidence has confirmed that killifish age in a similar way to any other vertebrate, though at a substantially faster pace. Accordingly, old killifish exhibit classical aging phenotypes, including a decline in fertility, wound healing and cognitive functions, as well as increased incidences of neoplastic lesions (reviewed in refs. [1–3]). Additionally, killifish lifespan can be manipulated by conserved lifespan interventions, such as intermittent fasting and drug treatments[5,6].

Efficient genome engineering approaches are instrumental for any experimental genetic model organism. Recently, two transformative projects were completed in the killifish: a comprehensive genome engineering platform for high-throughput CRISPR–Cas9-based genome editing[7,8] and back-to-back turquoise killifish genome projects, which provided vital information for experimental and evolutionary studies[9,10]. These resources allow the high-throughput generation of knockouts and knock-ins of various aging- and disease-associated genes, establishing the killifish as a powerful genetic model system for experimental aging research.

Under controlled laboratory conditions, the killifish developmental trajectory can be easily manipulated to favor diapause skipping by controlling incubation temperatures[1] (Fig. 2b). In the lab, this compressed life cycle allows the rapid generation of stable transgenic or genome-edited lines as quickly as 2–3 months (Fig. 2c,d). For example, we have knocked out the protein subunit of telomerase, a complex that elongates telomeres by adding TTAGGG sequences to the end of existing chromosomes. In so doing, we have generated the fastest vertebrate model for a human age-associated telomere syndrome[7]. This approach has been applied to several questions, including identification of pathways that can extend vertebrate lifespan[6].

Applying classical transgenesis approaches (for example, using the Tol2 transposase) has helped provide an elegant visualization of various processes in killifish embryonic development, such as cell cycle during diapause and tissue-specific promoters[8,11]. Similarly, the development of efficient CRISPR–Cas9-based genome engineering approaches has provided a means of functional interrogation, which was demonstrated by the proof-of-principle perturbation of over a dozen genes involved in the hallmarks of aging[7,8]. These include genes affecting cellular senescence, loss of protein homeostasis, deregulated nutrient sensing, mitochondrial dysfunction, epigenetic alterations, genomic instability, telomere attrition and intercellular communication.

Other intriguing biological processes linked to killifish physiology were recently explored.

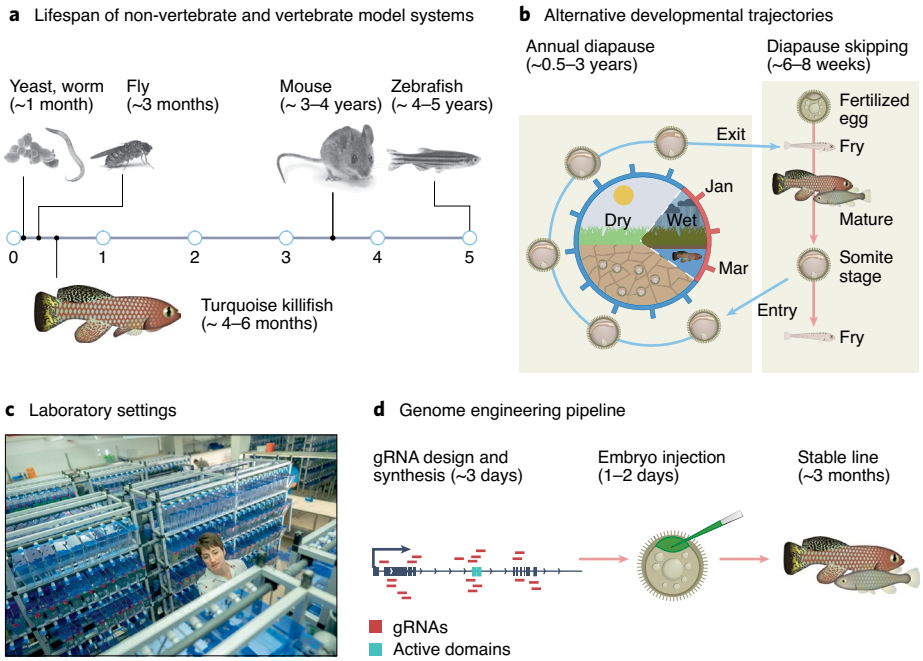

Fig. 2 | The turquoise killifish, an emerging genetic model of aging. a, Lifespan of classical genetic models (non-vertebrate and vertebrate) used for biomedical research (top), compared to the lifespan of the turquoise killifish (bottom). **b**, The turquoise killifish life cycle can proceed along very different trajectories. In the wild (left), embryos from the previous year hatch and fry rapidly reach sexual maturity, then lay a new batch of eggs. These embryos will then enter diapause, which protects the embryos during the dry season. In the lab, the 'diapause skipping' trajectory (right) is largely preferred for practical reasons. **c**, In the lab, fish are housed in commercial water-recirculating systems. **d**, Rapid and efficient genome engineering approaches for the short-lived turquoise killifish.

For example, *cbx7*, an epigenetic regulator of transcription, was linked to the long-term preservation of diapause[12], and the identification of regeneration-specific enhancers provided insights into vertebrate regeneration[13]. Large-scale characterizations have also suggested drivers of aging and disease. For instance, proteomic approaches identified a role for the proteasome[14] and for naturally occurring prion-like proteins[15] (such as *ddx5*), and genomic comparison of 45 killifish species suggested an involvement of increased mutational load[16]. Finally, AMP biosynthesis regulates metabolic health and vertebrate lifespan in a sex-specific manner[6]. These studies demonstrate how the killifish can be used to explore developmental processes, identify degenerative and pro-longevity pathways, and elucidate the mechanisms that mediate sex differences in biological aging.

Studying non-canonical animal models may open up a window to exceptional evolutionary adaptations. Such models allow researchers to tap into molecular mechanisms that govern a wide range of specialized traits, including naturally occurring cancer resistance, organ regeneration, and the ability to manipulate the pace of life. Owing to its unique adaptations, the African turquoise killifish has emerged as a promising model for exploring a wide range of biological questions, further supported by a lively and collaborative international community that meets biennially at the Nothobranchius Symposium.

As a relatively recent model system that lacks key organizational resources as of yet, the community could benefit from establishing an international resource center, which will support the growing community (offering, for example, preservation of fish lines and tailored health services). Furthermore, standardization of husbandry and experimental approaches will be important for reproducibility, as demonstrated by the lifespan variance reported by different labs. It should be noted that these differences could also reflect the natural variability in aging studies, as seen in the well-established mouse model and even across human populations.

Genome engineering, particularly CRISPR–Cas9 approaches, is an expanding field that will undoubtedly improve existing tools and open further possibilities. Thus, we can expect the existing genetic toolkit tailored to killifish to be further expanded, to include reversible and conditional gene regulation (such as the Gal4–UAS, Cre–*loxP* and Tet-ON systems), as well as the more recent CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa), which allow reversible manipulation of endogenous genes. Finally, there are more than 1,200 distinct killifish species that inhabit every continent except Australia and Antarctica[17]. Therefore, developing genetic approaches for these other species, which may have developed unique adaptation strategies (for example, hermaphroditism), could enrich the state-of-the-art research in this field.

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Competing interests

The author declares no competing interests.