RNAseq Analysis of Brain Aging in Wild Specimens of Short-Lived Turquoise Killifish: Commonalities and Differences With Aging Under Laboratory Conditions

Mariateresa Mazzetto,†,1,2 Cinzia Caterino 1, Marco Groth 1, Elisa Ferrari,2 Martin Reichard 3,4,5 Mario Baumgart 1, and Alessandro Cellerino* 1,2

1Biology of Ageing, Leibniz Institute for Age Research—Fritz Lipmann Institute e.V. (FLI), Beutenbergstr. 11, 07745 Jena, Germany
2Bio@SNS, Scuola Normale Superiore, Department of Neurosciences, Piazza dei Cavalieri 7, 56126 Pisa, Italy
3Institute of Vertebrate Biology, Czech Academy of Sciences, Květná 8, 603 65 Brno, Czech Republic
4Department of Ecology and Vertebrate Zoology, University of Łódź, 90-237 Łódź, Poland
5Department of Botany and Zoology, Faculty of Science, Masaryk University, 611 37 Brno, Czech Republic
†Present address: Yale School of Medicine, New Haven, CT.
*Corresponding author: E-mail: alessandro.cellerino@sns.it.
Associate editor: Kelley Harris

Abstract

A vast body of studies is available that describe age-dependent gene expression in relation to aging in a number of different model species. These data were obtained from animals kept in conditions with reduced environmental challenges, abundant food, and deprivation of natural sensory stimulation.

Here, we compared wild- and captive aging in the short-lived turquoise killifish (Nothobranchius furzeri). These fish inhabit temporary ponds in the African savannah. When the ponds are flooded, eggs hatch synchronously, enabling a precise timing of their individual and population age. We collected the brains of wild fish of different ages and quantified the global age-dependent regulation of transcripts using RNAseq. A major difference between captive and wild populations is that wild populations had unlimited access to food and hence grew to larger sizes and reached asymptotic size more rapidly, enabling the analysis of age-dependent gene expression without the confounding effect of adult brain growth.

We found that the majority of differentially expressed genes show the same direction of regulation in wild and captive populations. However, a number of genes were regulated in opposite direction. Genes downregulated in the wild and upregulated in captivity were enriched for terms related to neuronal communication. Genes upregulated in the wild and downregulated in captive conditions were enriched in terms related to DNA replication.

Finally, the rate of age-dependent gene regulation was higher in wild animals, suggesting a phenomenon of accelerated aging.

Key words: killifish, Nothobranchius furzeri, brain aging, RNAseq, gene expression.

Introduction

Aging is characterized as an age-dependent decrease in organismal fitness that results in increased mortality risk. One important source of aging is the accumulation of molecular damage that progressively impairs cellular homeostasis. High-throughput expression profiling techniques such as RNAseq provide a global and unbiased assessment of molecular changes associated with biological phenomena and are widely applied in biomedical and ecological research. A vast body of studies is available that describes age-dependent gene expression in relation to aging in a number of different model species (Zahn et al. 2006; Aramillo Irizar et al. 2018; Schaum et al. 2020; Aging Atlas Consortium 2021). Yet, most data are derived from animals kept in highly controlled laboratory conditions with reduced environmental challenges and pathogen load and with a considerably modified diet compared with natural nutrition (Zak et al. 2022). Captive animals, on the other hand, may be deprived of natural sensory stimulation and opportunities for physical activities with negative consequences on brain development and functions (van Praag et al. 2000; Sale et al. 2014; Pedersen 2019) and—on the other hand—have a supply of energetically rich diet supporting faster growth. To what extent the differentially expressed genes and pathways identified under artificial laboratory conditions reflect aging processes under natural conditions remains unclear.

© The Author(s) 2022. Published by Oxford University Press on behalf of Society for Molecular Biology and Evolution.
This is an Open Access article distributed under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

Mol. Biol. Evol. 39(11):msac219 https://doi.org/10.1093/molbev/msac219 Advance Access publication November 1, 2022
Gene expression profiles associated with aging in natural conditions are available for wolves (Charrua et al. 2016) and bats (Huang et al. 2019) but—to our knowledge—not for model organisms such as rodents or fruit flies. In the present paper, we set out to compare age-dependent gene expression during adult life in a vertebrate model species, the annual fish *Nothobranchius furzeri*. *Nothobranchius furzeri* is a small (6 cm) annual fish that inhabits temporary ponds in Southeastern Africa subject to the monsoonal seasonality. The duration of these habitats usually varies from 1 to 4 months after which all fish die. However, fish density strongly declines, and all fish often disappear, long before desiccation (Vrtilek, Zak, Polacik et al. 2018). The lifespan of wild-derived *N. furzeri* strains raised in captivity is limited to 7–8 months (Terzibasi et al. 2018). This species has become a new model organism since a compressed adult lifespan is associated with rapid physiological decay. A large number of typical vertebrate aging phenotypes at the neuro-muscular, histological, and cellular/molecular levels are described (for systematic reviews, see Cellerino et al. 2016; Platzer and Englert 2016; Hu and Brunet 2018; Poeschla and Valenzano 2020). Examples are reduced locomotor activity and impairment in learning paradigms (Valenzano et al. 2006), accumulation of the fluorescent age pigment lipofuscin (Terzibasi et al. 2008), apoptosis (Di Cicco et al. 2011), telomere erosion (Hartmann et al. 2009), and reduced mitochondrial function (Hartmann et al. 2011). RNAseq was applied to describe the global, age-dependent regulation of transcripts in different organs (Baumgart et al. 2014, 2016). The comparison of these datasets with similar datasets obtained in zebrafish, mouse, and humans revealed a consensus vertebrate transcriptional pattern that is conserved across species (Aramillo Irizar et al. 2018).

A large amount of information is available concerning *N. furzeri* ecology (reviewed in the study by Reichard and Polacik 2019), including detailed data on demographic parameters from wild populations. These studies demonstrated that the growth rate of *N. furzeri* in natural habitats is dependent on population density (Vrtilek et al. 2019) and is faster than in captivity, with animals in low-density habitats able to reach sexual maturity in 12 days (Vrtilek, Zak, Psenicka et al. 2018). *Nothobranchius furzeri* therefore represents an ideal species to compare age-dependent gene expression in captivity and in natural conditions.

For this study, we selected the population A41 (termed as Ch1 in [Vrtilek, Zak, Polacik et al. 2018]), which belongs to the Chefu phylogeographic lineage that comprises also the MZM-0410 captive strain (originally collected from a pond only 21 km apart) for which datasets of age-dependent genome-wide transcriptome regulation are available. In 2016, wild individuals of *N. furzeri* from population A41 were collected over the entire season, and their birth date was estimated from daily otolith increments (Vrtilek, Zak, Polacik et al. 2018). The collection of fish tissues from individuals of specified ages was completed in the field to be compared with captive animals of similar age points. An important characteristic of this wild population is that no growth was observed during the observation period (sample collection started at age of 39 days, when body size reached the asymptote; supplementary fig. S1, Supplementary Material online). Therefore, age-dependent expression was not confounded with growth-dependent expression. We focused on gene expression in the brain because cellular composition in the *N. furzeri* brain does not change during aging (Kelmer Sacramento et al. 2020) and the brain tissue is expected to respond strongly to environmental stimuli. Since studies of sex-dependent gene expression are lacking for *N. furzeri*, we also took the opportunity to compare age-dependent gene expression in male and female individuals.

**Results**

**Effects of Age and Sex on Brain Gene Expression in the Wild**

We sequenced RNA extracted from the brains of wild *N. furzeri* collected at 3 time points (39, 73, and 108 dph) from both sexes for a total of 22 samples. These ages are similar to the age of 35 and 94 dph that were previously studied in captive populations (Baumgart et al. 2014, 2016), so we can compare gene expression in animals of similar chronological age. In captive animals, these ages correspond to the life stages of young fish soon after sexual maturation and young adulthood. Due to the more rapid growth and sexual maturation in wild conditions, similar chronological ages do not imply similar "biological" ages or life stages. We computed differentially expressed genes (DEGs) in all the pairwise comparisons (73 vs. 39 dph, 108 vs. 73 dph, and 108 vs. 39 dph) for each sex separately, and we detected no major sex-related differences in the number of DEGs (fig. 1A and supplementary table S1, Supplementary Material online). We then computed putative aging biomarkers defined as upregulated genes with a monotonic trend for each sex independently. Markers detected in one sex had very similar expression profiles in the opposite sex (fig. 1B and supplementary table S2, Supplementary Material online), demonstrating that sex has little influence on the genome-wide regulation of transcripts by age in the brains of wild animals.

A generalized linear model, as implemented in DESeq2 (Love et al. 2014), was applied to separate the effects of sex and age on gene expression. Only 36 sex-dependent DEGs were detected (supplementary table S3, Supplementary Material online). Significantly regulated genes such as SYBU (syntabullin), which is the nearest-neighbor gene of GDF6 (growth differentiation factor 6), the sex-determining locus of *N. furzeri* (Reichwald et al. 2015) (fig. 1C), and Cytochrome P450 Family 19 Subfamily A Member 1, a gene that catalyzes the formation of aromatic C18 estrogens from C19 androgens (Corbin et al. 1988; Baravalle et al. 2017) are related to mechanisms of sex differentiation.

Two aging-related contrasts were performed in the wild animals: for each sex separately and combining male and
We analyzed genes expressed in the brain that were detected as differentially expressed both during early- (73 vs. 39 dph) and late-adulthood (108 vs. 73 dph) and plotted the log2 (fold change) for the first contrast on the X-axis and the log2 (fold change) for the second contrast on the Y-axis. Genes DE in both comparisons showed a positive correlation in their fold-changes, and the data points were more concentrated in quadrants I and III (i.e., genes with either negative or positive monotonic trend, Fisher’s exact test: \( P < 2.2 \times 10^{-16} \)) (fig. 2A, supplementary table S4, Supplementary Material online), indicating a progressive nature of age-dependent expression changes.

In order to identify the biological processes that are most affected during aging of wild fish, we performed Gene Ontology (GO) overrepresentation analysis for the genes in the four quadrants of figure 2A. Results are reported in figure 2B and supplementary table S5, Supplementary Material online. Genes with monotonic downregulation showed an overrepresentation of categories related to cell cycle, mitotic nuclear division and synapse organization, and axonogenesis, indicating a progressive age-dependent decrease in mitotic activity (neurogenesis) and formation of neural connections (fig. 2B and C). Genes with monotonic upregulation showed overrepresentation of categories related to peptidyl lysine modifications, epigenetics, and autophagy (fig. 2B and C). Genes with a reversal in their regulation also showed overrepresentation of specific categories: genes initially downregulated for mRNA processing, RNA splicing, and protein folding, and genes initially upregulated for central nervous system (CNS) development (fig. 2B).

Wild and Captive Animals Differ in the Age-Dependent Regulation of Genes Related to DNA Repair and Neural Functions

We set to detect the effects of captive versus wild condition on gene expression. We performed a second experiment where we compared four 34–37-day-old captive male fish and four 39-day-old wild male fish processed in parallel to eliminate batch effects linked to the RNAseq process, and we also compared the result of our first RNAseq experiment with a publicly available RNAseq dataset of age-dependent gene expression comprising five time points that partially overlap with those studied in the wild (Baumgart et al. 2016) (fig. 3A and supplementary table S6, Supplementary Material online).

Since the genetic structure of these populations is not known, we first computed identity by descent by analyzing SNPs in the RNA sequence data using the package SNPRelate (Zheng et al. 2012). This analysis showed that
the wild population is genetically distinct from the captive MZM0410 strain (fig. 3B). In addition, the two captive samples sequenced in 2012 and 2019 were also genetically distinct, indicating a drift in this population. Analysis of kinship and diversity indicated that the captive population has on average a lower diversity (measured as the distance from the centroid) and a higher average kinship (fig. 3C).

To correct for the effect of genetic relatedness on gene expression, we used MACAU, an approach based on Poisson mixed models that is specifically designed to correct for the effects of genetic relatedness on gene expression by modeling the heritability of expression levels (Sun et al. 2017).

We first created a model where the condition is the independent variable and age and batch are treated as covariates. The analysis with MACAU resulted in 11,379 DEGs (false discover rate, FDR < 0.1) (supplementary table S7, Supplementary Material online) between wild and captive animals. As this number is very high and corresponds to roughly 40% of the genes in the genome, we compared these results with those obtained by applying DESeq2 only to the dataset of four wild and four captive fish processed in parallel. The number of DEGs with Padj < 0.05 was 8,115. Therefore, the number of DEGs we obtain with MACAU is not unreasonable and probably reflects the pervasive effect of the condition on gene expression. Moreover, we correlated the fold-changes measured with DESeq2 with the slopes calculated with MACAU on the complete dataset (fig. 3A, supplementary table S6, Supplementary Material online) and detected a high degree of correlation.

Generally applicable gene set enrichment Generally Applicable Gene-set (GAGE) (Luo et al. 2009) was applied in order to detect pathways whose expression is condition-dependent between wild and captive animals. In order to increase the stringency of the GAGE analysis, we performed GAGE analysis also on the DESeq2 results that we obtained by analyzing a single batch. We then retained as significant only the terms that were detected in both analyses. As the number of significantly overrepresented
terms (FDR < 0.1) is large, we used REVIGO (Supek et al. 2011) to cluster GO categories based on semantic similarities (fig. 3D). Genes upregulated in wild animals showed enrichment of categories related to mitochondrial biogenesis and energetics, RNA processing, and to mitosis. On the other hand, genes upregulated in captive animals were enriched in categories related to development, behavior, and function of the nervous system as well as ion transport (supplementary table S8, Supplementary Material online).

Among the top differentially regulated genes, we found WEE2, a protein kinase that phosphorylates inhibitory sites in CDK1 (Leise and Mueller 2002), FTL, the ferritin light chain, LAMTOR1, an anchor protein that creates an interface for the mTORC1 complex on late endosomes thereby regulating cell metabolism (Bar-Peled et al. 2012) and CSMD3, a gene coding for a postsynaptic protein highly expressed in the brain that regulates dendrite development (Mizukami et al. 2016) and neuronal maturation.
(Gutierrez et al. 2019). Differential expression of these genes was validated by quantitative PCR (fig. 3F).

We created two further models—one for captive fish and one for wild fish. In these models, age is the independent variable and batch is treated as a covariate. This analysis revealed that the effect of captive versus wild condition is larger than the effect of age and that the effect of batch is smaller than that of either of the two (fig. 4A).

Principal component analysis (PCA) was applied to visualize relationships between the origin of the sample and the effects of age on global gene expression. As expected, the first component (69.52% of variance) separated fish conditions (wild vs. captive); the second component (5.46% of variance) identified an effect of age on global gene expression that was similar in the two conditions (fig. 4B).

Notably, PC1 separated by condition also the samples of the new sequencing batch. However, the separation of these samples on PC1 was smaller than the samples of the 2012 and 2018 sequencing, indicating that batch effects contribute to sample separation, although to a lesser extent than captive- versus wild condition. This is consistent also with the distribution of effect sizes of condition, age, and batch as measured by MACAU and reported in figure 4A.

In order to compare aging-related differences in RNA expression between the two conditions, we then plotted the effect of age (i.e., the weight \( \beta \) of age in the Poisson mixed model) in the captive animals on the X-axis and in the wild animals on the Y-axis. The DEGs showed preferentially the same direction of regulation in the two conditions and were concentrated in quadrants I and III with a highly significant positive correlation (fig. 4C, supplementary table S9, Supplementary Material online). Notably, virtually all points in quadrant I are located above the diagonal of that quadrant, indicating that the age-dependent rate of upregulation is larger in wild fish. A similar, but smaller, effect can be noticed in quadrant III, where most points are located below the diagonal.

These results suggest a deceleration of transcriptional aging in captive animals (as shown in the next paragraph).

We performed GO terms overrepresentation analysis for the genes in the four quadrants of figure 4C. Results are reported in figure 4D and supplementary table S9, Supplementary Material online. Genes downregulated in captivity, but upregulated in the wild during aging were enriched in terms related to DNA repair, cell cycle and synapese organization (figs. 4D, 5A, 5D–F, and 5I). Interestingly, the expression of genes related to DNA repair and non-homologous end joining was positively correlated with longevity in two different comparative RNAseq studies of mammals spanning a large spectrum of lifespans (Fushan et al. 2015; Lu et al. 2022). These same genes are frequently regulated in opposite directions in captive and wild animals (fig. 5B and C). We set to validate these differences in an independent biological sample using qPCR. Since it is not possible to directly compare the “biological” age in the two conditions, we decided to compare similar calendar ages, that is, 39 and 109 dph for the wild fish and 34–37 and 83–87 dph for the captive fish. Among the genes that are downregulated in captive animals but upregulated in wild animals, there were DNA2, a key enzyme involved in DNA replication and DNA repair in the nucleus and mitochondrion (Zheng et al. 2020), XRCC2 and RAD51, proteins involved in the homologous recombination DNA repair pathway (Suwaki et al. 2011), and PRIM1, a subunit of the DNA primase complex (Loeb and Monnat 2008). Divergent regulation of these genes was confirmed by qPCR (fig. 5D–F and supplementary table S10, Supplementary Material online).

Genes upregulated during aging in both wild and captive animals were most enriched in categories related to autophagy and response to reactive oxygen species (fig. 4D). Genes downregulated during aging in both conditions showed a highly significant enrichment for cytokinesis and mitotic nuclear division categories, chromatin remodeling, and nervous system development (figs. 4D and 5E and supplementary table S10, Supplementary Material online). As representative examples of concordant downregulation, we selected the genes ANLN, required for cytokinesis and essential for the structural integrity of the cleavage furrow (Kim et al. 2017), and KIF11, a motor protein required for establishing a bipolar spindle during mitosis (Johnson et al. 2014) (fig. 5F and G and supplementary table S10, Supplementary Material online).

An “Accelerated” Aging Profile in Wild Animals

In order to quantify the differences in aging profile in the two conditions, we defined as transcriptional biomarkers of aging those transcripts with either negative or positive monotonic dependency on age in either captive- or wild fish (fig. 6A–C and supplementary table S11, Supplementary Material online). These transcripts showed the same direction of regulation in either condition but their age-dependency varied according to the condition. When captive biomarkers are used as a reference, wild- and captive fish appear to have similar slopes, but different intercepts, so that the curves of wild fish are left-shifted. When wild biomarkers are used as a reference, however, the slopes of age-dependent changes were clearly less steep in the captive fish, particularly for the genes upregulated with age. As mentioned earlier, a larger rate for age-dependent changes in wild fish can also be noted in the scatterplot in figure 4C.

These results strengthen the suggestion that captive animals show a “decelerated” aging profile when compared with the captive animals.

Discussion

In this paper, we studied age-dependent gene expression in the brain of wild individuals of a N. furzeri population that was followed longitudinally over its natural lifespan (Vrtilek, Zak, Polacik et al. 2018). The time points analyzed correspond to adult stages, and it is of particular relevance that all fish in this study have reached asymptotic body
size prior to the first sampling, and hence no further growth was observed across sampling age points. This enabled us to separate the effects of aging from the effects of growth that are confounded in the captive fish populations.

The first result of this study is that only minor sex differences were detected in brain gene expression and the pattern of age-dependent regulation. This is rather surprising given the marked sexual dimorphism of N. furzeri (Cellerino et al. 2016), the fact that sex-dependent changes in gene expression during aging were described in model organisms and humans (Yuan et al. 2012; Wruck and Adjaye 2020; Zhao et al. 2020) and that N. furzeri males are subject to a higher mortality in the wild (Vrtilék, Zak, Polacik et al. 2018). However, the captive population of N. furzeri do not show sex differences in the aging component of their survival or in expression of age-dependent markers, suggesting that the intrinsic rate of aging is not sex-dependent in this species (Reichard et al. 2022). Instead, increased male mortality is related to sex-specific predation and male–male competition for reproductive opportunities (Reichard et al. 2014). It should also be noted that analysis of zebrafish brains also revealed only ~100 genes expressed in a sex-specific manner (Yuan et al. 2019), suggesting that transcriptional dimorphism may be less developed in teleost fishes.

The majority of the changes that were observed in the wild population were progressive; that is, the...
direction of the regulation was consistent in the two comparisons 39 versus 73 dph and 73 versus 108 dph. This indicates that condition-dependent mortality and the associated selection do not have a large impact on the data we obtained. Age-dependent downregulation affects primarily genes related to neurogenesis and synaptogenesis. Depression of adult neurogenesis is a typical age-related trait in vertebrates (Peckce et al. 2008; Ben Abdallah et al. 2010; Tozzini et al. 2012; Edelmann et al. 2013), and downregulation of synaptic proteins is a typical feature of human brain aging (Lu et al. 2004; Somel et al. 2010; Berchtold...
We show that these aspects of aging are conserved in the wild population. Upregulated genes showed overrepresentation of a number of different categories. Two particularly interesting terms were lysosome and epigenetic regulation of gene expression and histone modifications (supplementary table S5, Supplementary Material online). Upregulation of genes coding for lysosomal proteins is among the most conserved transcriptional signatures of aging across species (Zahn et al. 2006; de Magalhaes et al. 2009; Aramillo Irizar et al. 2018). Epigenetic regulation was described in captive N. furzeri. In particular, upregulation of repressive histone marks such as H3K27 trimethylation and downregulation of activating histone marks was detected (Baumgart et al. 2014; Cencioni et al. 2019).

Despite these conserved age-related patterns, baseline differences in expression between wild and captive populations were observed for a large number of genes.

The origin of this difference is likely manifold. Differences in nutrition almost certainly play an important role. First, captive animals are fed exclusively with Chironomus larvae. This diet does not correspond to the dietary protein target of wild fish (and is instead richer in lipids) and leads to lower body condition, overfeeding, and male liver enlargement (Zak et al. 2022). Second, wild animals have regular access to food over daytime rather than intermittent feeding once or twice a day. These two factors result in much faster growth of wild fish that, unlike captive animals, reach asymptotic size within 5 weeks. In wild fish, density-dependent effects may slow growth when resources become limited (Vrtilek et al. 2019), but this is not the case in our study population (A41). Indeed, the study population was specifically selected because it does not suffer from density-related restrictions, and this is further confirmed by the continuous presence of food in the gut throughout the
day (Zak et al. 2019). A second highly relevant aspect of differences between wild and captive fish is thermal stress. Wild animals are subject to diel oscillations in temperature that reach almost 20 °C (Zak and Reichard 2020). In South American killifish, such oscillations are known to induce upregulation of heat shock proteins expression (Podrabsky and Somero 2004), suggesting that also in wild N. furzeri, cyclical thermal shock likely induces stress-response pathways. Finally, activity levels also differ between wild and captive animals even though it is difficult to predict how they can impact gene expression.

Genes upregulated in wild fish were particularly enriched in clusters of terms that are related to two main functions: mitochondrial respiration and translation and cell cycle/DNA replication. Mitochondrial respiration is a key regulator of aging. Partial inhibition of mitochondrial respiration is life extending in nematode worms, fruit flies and N. furzeri (Dillin et al. 2002; Copeland et al. 2009; Baumgart et al. 2016). Nuclear genes coding for proteins of the respiratory chain are under positive selection in multiple clades associated with the evolution of exceptional lifespan (Sahm et al. 2019), expression of complex I components negatively correlates with lifespan in interspecific comparison of mammals (Mota-Martorell et al. 2020) and rate of production of reactive oxygen species (ROS) by the complex I of the respiratory chain is inversely correlated with lifespan (Munro et al. 2013). The transcriptional coordination of mitochondrial translation and synthesis of the respiratory complex is termed mitonuclear balance and is a key regulator of aging across metazoans (Houtkooper et al. 2013; Baumgart et al. 2016). Therefore, wild and captive fish differed in the expression of the key genetic pathway for the regulation of aging. These differences may be a consequence of the activation of stress-response pathways in wild fish. Differences in the expression of cell cycle and DNA replication genes are paradoxical because wild animals have ceased growing. One possible explanation is activation of DNA repair pathways. A second possibility may be related to the circadian oscillation in the activity of adult neuronal stem cells of teleosts (Akle et al. 2017). The diel temperature oscillation may entrain the circadian rhythm leading to higher daytime mitotic activity in wild animals.

Genes upregulated in captive animals were enriched for GO terms related to neuronal communication and synaptic function. This result is surprising since exposure to a more complex environment is expected to induce synaptogenesis (van Praag et al. 2000). However, several factors may explain this seemingly paradoxical effect. In the first place, the brain of captive animals grows considerably during the examined period while captive animals have reached asymptotic size. Second, the stress levels of wild animals may curb synaptogenesis.

When age-dependent regulation was compared between wild and captive animals, more than 85% of the significant cases showed a consistent direction of regulation indicating a high degree of conservation between aging in the wild and in captive conditions. Considering that wild fish did not grow between the time points sampled, this result shows that most gene expression changes are not related to age-dependent deceleration of growth observed in captive animals. Downregulated genes showed over-representation of a large number of GO terms (supplementary table S9, Supplementary Material online) with the most significant enrichment for regulation of the mitotic cell cycle, protein folding, and chromatin but also neuronal development and formation of neuronal connection. This signal is clearly related to a reduction of neurogenesis with the formation of new connections by newborn neurons. Upregulated genes showed over-representation of a large number of GO categories (supplementary table S9, Supplementary Material online) with the most significant enrichment for terms related to translation, response to oxidative stress, and autophagy. The relationship of these processes with aging is well established (López-Otín et al. 2013; Steffen and Dillin 2016). Of interest is the upregulation of the Polycomb repressive complex, as its activity increases with age in N. furzeri (Baumgart et al. 2014; Cencioni et al. 2019) and sites targeted by Polycomb show age-dependent methylation in mammals (e.g., Minteer et al. 2022). Genes with opposite regulations also showed over-representation of specific GO terms and provide some interesting insights. Genes upregulated in captivity but downregulated in the wild show the most significant enrichment for genes coding for synaptic proteins. It should be noted that these GO terms also show significant differences in baseline conditions. This result suggests that brain connections develop differently in captive and wild environments. Genes upregulated in wild and downregulated in captivity show the most significant enrichment for DNA replication. This result is surprising and shows a disjunction in the regulation of cell-cycle-related genes and may be related to the heterochrony of somatic growth between wild and captive fish. Captive fish show continuous deceleration of growth during adult life, and this is reflected in a reduction of mitotic activity of adult neuronal precursors (Tozzini et al. 2012). Wild fish of the population examined, on the other hand, completed their growth during the first 5 weeks and could show the effects of aging isolated from somatic growth. The heterochrony of ontogeny and the more rapid development of wild fish are also reflected in the time-dependent age expression assessed by using monotonous genes as biomarkers. When captive-fish biomarkers were assessed in wild fish, their curves were left-shifted indicating that comparable expression levels were reached earlier in the wild. Wild-fish biomarkers, on the other hand, reflected genes whose expression was age-dependent in the absence of somatic growth. In this case, both starting values and slopes differed. The two combined observations indicate more rapid transcriptomic aging in wild fish. Interestingly, slower demographic aging rates were recently demonstrated for captive populations of turtles when compared with wild turtles (da Silva et al. 2022), indicating...
that other vertebrate taxa also respond to more favorable conditions by reducing the rate of senescence.

A particularly interesting result identified the contrasts in the DNA repair/DNA recombination pathway. This pathway shows age-dependent upregulation in wild fish but is downregulated with age in captive fish. The robust implication of this pathway in the evolution of longevity originates from several independent lines of evidence. Positive selection in genes coding for proteins related to DNA repair and homologous recombination was associated with evolutionary longevity in rockfishes (Kolora et al. 2021), cetaceans (Tollis et al. 2019), and long-living Galapagos tortoise (Quesada et al. 2019). Most relevant for the present study is a recent comparison of 45 killifishes of varying life history detected pervasive relaxation of positive selection on genes coding for DNA repair proteins in annual (short-lived) species (Cui et al. 2020). Complementary evidence to sequence analysis is provided by studies of expression profiling. One study correlated gene expression levels with longevity in a phylogenetically broad collection of 33 species of terrestrial mammals and identified DNA repair pathways to be overrepresented among genes whose expression is positively correlated with size-corrected lifespan (Fushan et al. 2015). This result was replicated and extended in a larger study focused on 6 tissues of 26 species of Rodentia and Eulipotyphla (Lu et al. 2022). These genes have been identified in the gray whale (Toren et al. 2020).

At the single gene level, genes coding for proteins of the double-strand repair pathway were originally for their ability to complement DNA damage induced by X-ray (XRCCs). The gene XRCC1 is under positive selection in rockfishes (Kolora et al. 2021), the gene XRCC6 is under positive selection in the giant tortoise (Quesada et al. 2019), and expression of the genes XRCC5 and XRCC6 is positively correlated with lifespan in two mammalian datasets (Fushan et al. 2015; Lu et al. 2022). These genes have opposite regulation in captive and wild N. furzeri, and we confirmed the opposite age-dependent regulation of XRCC2 also by qPCR.

At a functional level, the activity of double-strand repair pathways declines with age in mice (Vaidya et al. 2014). On the other hand, long-living mammals show the higher basal activity of the double strand break (DSB) repair pathway (Tian et al. 2019). Interestingly, a longitudinal study of gene expression in N. furzeri revealed that the slope of age-dependent downregulation in the expression of genes in the DNA repair pathway is associated with a shorter lifespan (Kelmer Sacramento et al. 2020). On the other hand, longitudinal studies in long-living bats revealed age-dependent upregulation of the same pathway (Huang et al. 2019). It is notable that the age-dependent regulation of this pathway is different between wild and captive fish, suggesting that the wild conditions entail stimuli able to activate this pathway.

From the evolutionary perspective, gene regulatory mechanisms were conserved between wild and captive fish, suggesting the existence of conserved regulatory trajectories of gene expression employed across environmental conditions. Those trajectories were modified by developmental dynamics. Wild fish displayed rapid growth and reached growth asymptote before the first sampling. Irrespective of growth cessation, their temporal changes in gene expression were then in captive fish, and we propose that wild fish displayed accelerated aging. The pace of aging is known to respond to environmental conditions and contrasting populations of single species often display population-characteristic aging patterns (marsupials: [Austad 1993], snakes: [Bronikowski 2008], invertebrates: [Tatar et al. 1997; Dudycha and Tessier 1999], guppy: [Reznick et al. 2004], killifish: [Terzibasi et al. 2008; Blazek et al. 2017]). To recognize how mutation load (Willemsen et al. 2020) or epigenetic modification of gene expression affects this variation in gene expression dynamic is important for understanding the evolution of aging at a microevolutionary scale, and annual killifish appear ideally suited to contribute to resolving this question.

In conclusion, our study reveals that, despite baseline levels of brain gene expression varied between samples from wild and captive populations, the direction of regulation is mostly consistent. Intersexual differences in brain gene expression were minimal and in part related to sex determination. However, some pathways were differentially regulated in the two conditions, notably pathways related to DNA repair mechanisms, and this could result from heterochrony in the ontogeny.

Experimental Procedures
Sample Collection
Wild fish were collected by seine nets in natural ponds and immediately dissected. The collection and dissection took place between 7:00 and 9:00 h, that is, 1–3 h after sunrise. Upon dissection, the brain and other organs were stored in RNAlater at 4 °C and frozen at −80 °C after arrival in the laboratory (within 1–2 weeks after collection). Fish for dissections were chosen randomly from a set of captured fish. Their body size was measured. The age of the fish was estimated from the time of pool flooding and was confirmed by reading the number of daily rings on otoliths (Vrtilek, Zak, Polacík et al. 2018). Tissue from captive fish was collected as described (Baumgart et al. 2014).

Fish Husbandry and Sampling
Fish were housed in a recirculating system (Aqua Schwartz, GmbH, Göttingen, Germany) and were singly housed in 2.8 l tanks with a divider. Water temperature was maintained at 26 °C and conductivity at 600 µS/cm. Animals were subject to a 12:12 light/dark cycle and fed exclusively with Chironomus larvae.

Animals were always sacrificed fasted in the morning, and the entire brain was extracted and immediately frozen on dry ice.
Ethical Statements
All work with animals was carried out in accordance with relevant guidelines and regulations. Sample collection of wild animals complied with legal regulations of Mozambique (collection licence: ADNAP-170/7.10/16) and research procedures were approved by the ethical committee of the Institute of Vertebrate Biology, in accordance with legal regulations of the Czech Republic.

Captive fish were bred and kept in FLI’s fish facility under licence number J-003798 (Veterinär- und Lebensmittelüberwachungsamt, Thuringia, Germany). Sacrifice and organ harvesting were performed according to §4(3) of the German Animal Welfare Act. No experimental procedure on live animals was carried out.

RNA Extraction
RNA was extracted as described (Baumgart et al. 2014) with a modified Trizol protocol, using Qiazol lysis reagent (Qiagen). In brief, samples were homogenized and lysed in QIAzol. After 5 min at room temperature, Chloroform was added and samples were mixed. After 3 min at room temperature, samples were centrifuged at 4 °C in a tabletop centrifuge. After phase separation, the upper aqueous phase was withdrawn and mixed with 1.1 volumes of isopropl alcohol, 0.16 volumes 2M, pH 4.2 Sodium acetate, and 1 µl (10 µg) of GlycoBlue. Samples were precipitated by centrifugation for 30 min at 4 °C, washed twice with 80% ethanol, and air-dried for 5 min. RNA was dissolved in nuclease-free water.

RNA Sequencing
Sequencing of RNA samples was performed using Illumina’s next-generation sequencing methodology. In detail, total RNA was quantified, and quality-checked using Agilent 2100 Bioanalyzer Instrument (Agilent RNA 6000 Nano). Libraries were prepared from 500 ng of input material (total RNA): GSE183039—TruSeq Stranded mRNA kit (Illumina) following the manufacturer’s instructions with subsequent quantification and quality check, using Agilent 2100 Bioanalyzer Instrument (DNA 7500 kit). Libraries were pooled and sequenced in 4 lanes of the HiSeq 2500 System running in 51 cycle/single-end/high output mode. Sequence information was converted to FASTQ format using bcl2fastq v1.8.4. GSE183037—NEBNext Ultra II Directional RNA Library Prep Kit was prepared in combination with a NEBNext Poly(A) mRNA Magnetic Isolation Module (both New England Biolabs) following the manufacturer’s instructions. Libraries were subsequently quantified and quality-checked using Agilent 2100 Bioanalyzer Instrument (DNA 7500 kit). Libraries were pooled and sequenced in 2 lanes of the HiSeq 2500 System running in 51 cycle/single-end/high output mode. Sequence information was converted to FASTQ format using bcl2fastq v2.20.0.422.

Per sample, the reads were mapped to the N. furzeri genome (NFINgb—N. furzeri Genome Browser: https://nfingb.leibniz-fli.de/data/raw/notho4/Nfu_20150522.hardmasked_genome.fa.gz) and respective annotation (NFINgb: https://nfingb.leibniz-fli.de/data/raw/notho4/Nfu_20150522.genes_20150922.gff3.gz) using tophat2 v2.1 (parameters: --no-convert-bam --no-coverage-search -x 1 -g 1). Reads per gene were counted using featureCounts v1.5 (GSE183039) or v1.6.5 (GSE183037) (parameters: -s 2). Reads counts were introduced into the statistical environment R in order to calculate (reads per million mappable reads) and RPKMs (reads per kilobase and million mappable reads). For the calculation of RPKMs, lengths of transcripts were taken from featureCounts output.

The statistics relative to the different samples are reported in supplementary table S12, Supplementary Material online.

Variant Analysis
Variant calling was performed on the aligned BAM files using bcftools (Li 2011) and then used for genotype calling and identity-by-descent (IBD) analysis using the SNPRelate package (Zheng et al. 2012). For the first analysis, linkage equilibrium through LD-based SNP pruning was applied to the variants, and then genotypes for each SNP were obtained. For the second one, kinship values among the samples were measured using the Maximum Likelihood Estimation method.

Differential Analysis
To eliminate low-expressed genes, we filtered out all genes whose raw expression was not ≥1 count in all samples, excluding 5,220 genes out of the 23,545 protein-coding genes annotated in the N. furzeri genome. Differentially expressed transcripts in wild animals were obtained with the DESeq2® package (Love et al. 2014). To compare wild and captive animals, we used the MACAU software (Sun et al. 2017) to correct genetic relatedness among captive animals. MACAU models one gene at a time and assumes that the reads are distributed according to a Poisson distribution. If $N_i$ is the total number of reads in a sample and $\lambda_i$ is the fraction of $N_i$ mapping to the gene of interest, the distribution of reads is defined by:

$$y_i = P_0(N_i|\lambda_i)$$

MACAU further assumes that $\lambda_i$ can be modeled as follows:

$$\log(\lambda_i) = w_i\alpha + x_i\beta + g_i + e_i$$

where $w_i\alpha$ is the linear combination of covariates, $x_i$ is the predictor variable (age or wild/captive in our case), and $\beta$ is its weight. The last two terms model the contribution of
MACAU further assumes that both terms are defined by a multivariate normal distribution, in the case of $g$: this contribution is defined by the covariance matrix $K$ (i.e., a normalized IBD matrix) and the heritability of gene expression $h$ that we set to 0.4.

$$\bar{g} = (g_1, \ldots, g_n) \sim \text{MVN}(0, \sigma^2 h^2 K)$$

$$e = (e_1, \ldots, e_n) \sim \text{MVN}(0, \sigma^2 (1-h)^2 I_{n \times n})$$

where $I_{n \times n}$ is the identity matrix of dimension $n$.

We have used the MACAU analysis twice. In the first analysis, we set age as covariate and condition as a predictor variable to identify genes differentially expressed in wild animals. In the second analysis, we analyzed the effects of age as a predictor variable on the two conditions separately and then plotted, for each DEG, $\beta_{\text{wild}}$ versus $\beta_{\text{captive}}$.

**Enrichment Analysis**

Gene ontology was performed for each tissue with the WebGestalt online tool using GO process; enriched categories for each quadrant were filtered for FDR $< 0.05$.

Generally Applicable Gene-set/pathway enrichment (GAGE) was performed using the gage() package; enriched categories were filtered for $q$-value $< 0.05$ and used for visualization with the Revigo software. Entrez IDs were used as identifiers for the analysis, from the human orthologues gene symbols: these were obtained with the biomaRt() package.

**Principal Component Analysis**

Visualization of the data (for both transcriptome) was performed with PCA: DEGs for different feature selection were obtained and selected for the analysis. Principal components of the samples were obtained through the prcomp() function and then plotted, after computing centroids as means of the replicates for each group of samples.

**Aging Biomarkers Isolation**

Aging biomarkers were computed as genes with linear expression trajectories during time: sequential pairwise comparisons across time points were obtained with the DESeq2() package (Love et al. 2014) and used to filter genes with fold-changes $>0$ (for positive biomarkers) or $<0$ (for negative biomarkers) in all comparisons. The $P$-values of all pairwise comparisons were then combined using meta-analysis, and the final $P$-value was adjusted to FDR using the p.adjust() function. Significant biomarkers were isolated for FDR $< 0.05$. To plot the expression trajectories over time in captive and wild animals, count data were cleaned for technical covariates (specifically for RIN coefficient) using the cleaningY() function from the Jaffelab GitHub repository (Collado-Torres et al. 2017), then were converted to z-score (centering each value on the mean and scaling on the standard deviation), and finally used to plot trajectories using the ggplot2 package (Wickham 2016).

**Experimental Validation**

Experimental validation was done by RT-qPCR from wild and captive RNA, using the primers listed in supplementary table S11, Supplementary Material online. In brief, 500 ng per sample was reverse transcribed with SuperScript IV Reverse Transcriptase (Invitrogen). Quantification was performed by use of Quantinova SYBR Green (Qiagen) with CFX384 real-time PCR system (Biorad). For the validation of condition-dependent genes, we used a total of 16 samples (8 wild at 39 dph and 8 captive at 5 wph), using TATA Binding Protein (TBP) for normalization. For the validation of age-dependent genes, we used a total of 16 samples, comprising 8 wild (4 at 39 dph, and 4 at 108 dph), and 8 captive fish (4 at 5 wph and 4 at 12 wph). For the visualization, we used each point, which represents a replicate, whereas the middle segment represents the median of the distribution. A $T$-test was used for statistics.

**Supplementary material**

Supplementary data are available at Molecular Biology and Evolution online.

**Acknowledgments**

Funding for M.R. came from Czech Science Foundation (19-01781S). We thank Barbora Rolečková for RNA extraction. We greatly appreciate the excellent and skilful technical assistance of Ivonne Goerlich (FLI, Jena).

**Author’s Contribution**

M.M.: performed analysis, prepared figures, and wrote the paper. C.C.: validation and data acquisition. M.G.: performed RNA sequencing, and data analysis. E.F.: performed the MACAU analysis and the permutation test. M.B.: initiated and supervised the study, and revised the paper. M.R.: designed the study, collected and processed the samples, and revised the paper. A.C.: designed and coordinated the study, and wrote the paper.

**Data Deposition**

Data from this study were deposited in gene expression omnibus, accession numbers GSE183037, GSE183039 and GSE52462.

**Conflict of interest statement.** The authors declare no competing financial interests.

**References**

Aging Atlas Consortium. 2021. Aging Atlas: a multi-omics database for aging biology. Nucleic Acids Res. 49:D825–D830.

Akle V, Stankiewicz AJ, Kharchenko V, Yu L, Kharchenko PV, Zhdanova IV. 2017. Circadian kinetics of cell cycle progression in adult neurogenic niches of a diurnal vertebrate. J Neurosci. 37:1900–1909.
Aramillo Irizar P, Schauble S, Esder D, Groth M, Frahm C, Priebes B, Baumgart M, Hartmann N, Marthandan S, Menzel U, et al. 2018. Transcripthonic alterations during aging reflect the shift from cancer to degenerative diseases in the elderly. Nat Commun. 9:327.

Asstad SN. 1993. Retarded senescence in an insular population of Virginia opossums (Didelphis-virginiana). J Zool. 229:695–708.

Bar-Peled L, Schweitzer LD, Zoncu R, Sabatini DM. 2012. Regulator is a GEF for the rag GTPases that signal amino acid levels to mTORC1. Cell 150:1196–1208.

Baravalle R, Di Nardo G, Bandino A, Barone I, Catalano S, Ando S, Gilardi G. 2017. Impact of R264C and R264H polymorphisms in human aromatase function. J Steroid Biochem Mol Biol. 167:23–32.

Baumgart M, Groth M, Priebes S, Savino A, Testa G, Dixa R, Ripa R, Spallotta F, Gaetano C, Ori M, et al. 2014. RNA-seq of the aging brain in the short-lived fish *N. furzeri*—conserved pathways and novel genes associated with neurogeresis. Aging Cell 13:965–974.

Baumgart M, Priebes S, Groth M, Hartmann N, Menzel U, Pandolfini L, Koch P, Felder M, Ristow M, Engler C, et al. 2016. Longitudinal RNA-seq analysis of vertebrate aging identifies mitochondrial complex I as a small-molecule-sensitive modifier of lifespan. Cell Syst. 2:122–132.

Ben Abdallah NM, Slomianka L, Vyssotski AL, Lipp HP. 2010. Early age-related changes in adult hippocampal neurogenesis in C57 mice. Neurobiol Aging. 31:151–161.

Berchtold NC, Coleman PD, Cribbs DH, Rogers J, Gillen DL, Cotman CW. 2013. Synaptic genes are extensively downregulated across multiple brain regions in normal human aging and Alzheimer’s disease. Neurobiol Aging. 34:1653–1661.

Blazer R, Polack M, Kacer P, Cellerino A, Rezucha R, Methling C, Tomasek O, Syslova K, Terzubasi Tossini E, Albrecht T, et al. 2017. Repeated interspecific divergence in life span and aging of African annual fishes along an aridity gradient. Evolution 71: 386–402.

Bronikowski AM. 2008. The evolution of aging phenotypes in snakes: a review and synthesis with new data. Age 30:169–176.

Cellerino A, Valenzano DR, Reichard M. 2016. From the bush to the bench: the annual *Nothobranchius* fishes as a new model system in biology. Biol Rev Camb Philos Soc. 91:511–533.

Cencioni C, Heid J, Krepelova A, Rasa SMM, Kuenne C, Guenther S, Baumgart M, Cellerino A, Neri F, Spallotta F, et al. 2019. Aging triggers H3K27 trimethylation hoarding in the chromatin of *Nothobranchius furzeri* skeletal muscle. Cells 8:1169.

Charrau P, Johnston RA, Stahl DR, Lea A, Snyder-Mackler N, Smith DW, vonHoldt BM, Cole SW, Tung J, Wayne RK. 2016. Pervasive effects of aging on gene expression in wild wolves. Mol Biol Evol. 33:1967–1978.

Colonna E, Torres L, Nellagar A, Frazee AC, Wilks C, Love ML, Langmead B, irizarry RA, Leek JT, Jaffe AE. 2017. Flexible expressed region analysis for RNA-seq with derfinder. Nucleic Acids Res. 45:e9.

Copeland JM, Cho J, Lo T, Hur JH, Bahadorani S, Arabyan T, Rabie J, Soh J, Walker DW. 2009. Extension of *Drosophila* life span by RNAi of the mitochondrial respiratory chain. Curr Biol. 19: 1591–1598.

Corbin CJ, Graham-Lorence S, McPhaul M, Mason JI, Mendelson CR, Simpson ER. 1988. Isolation of a full-length cDNA insert encoding human aromatase system cytochrome P-450 and its expression in nonsteroidogenic cells. Proc Natl Acad Sci U S A. 85: 8948–8952.

Cui R, Medeiros T, Willemsen D, Iasi LNM, Collier GE, Graef M, Reichard M, Valenzano DR. 2020. Relaxed selection limits lifespan by increasing mutation load. Cell 180:1272–1279.

da Silva R, Conde DA, Baudisch A, Colcher F. 2022. Slow and negligible senescence among testudines challenges evolutionary theories of senescence. Science 376:1466–1470.

de Magalhaes JP, Curado J, Church GM. 2009. Meta-analysis of age-related gene expression profiles identifies common signatures of aging. Bioinformatics 25:875–881.

Di Cicco E, Tossini ET, Rossi G, Cellerino A. 2011. The short-lived annual fish *Nothobranchius furzeri* shows a typical teleost aging process reinforced by high incidence of age-dependent neoplasias. Exp Gerontol. 46:249–256.

Dillin A, Hsu AL, Arantes-Oliveira N, Lehrer-Graiwer J, Hsin H, Fraser AG, Kamath RS, Ahringer J, Kenyon C. 2002. Rates of behavior and aging specified by mitochondrial function during development. Science 298:2398–2401.

Dudycha JL, Tessier AJ. 1999. Natural genetic variation of life span, reproduction, and juvenile growth in daphnia. Evolution 53:1744–1756.

Edelmann K, Glashauser L, Sprungala S, Heil B, Fritschle M, Ninkovic J, Godinho L, Chapouton P. 2013. Increased radial glia quiescence, decreased reactivation upon injury and unaltered neuroblast behavior underlie decreased neurogenesis in the aging zebrafish telencephalon. J Comp Neurol. 521:3099–3115.

Fushan AA, Turanov AA, Lee SG, Kim EB, Lobanov AV, Yim SH, Buffenstein R, Lee SR, Chang KT, Rhee H, et al. 2015. Gene expression defines new changes in mammalian lifespan. Aging Cell 14:352–365.

Gutierrez MA, Dwyer BE, Franco SJ. 2019. Csmd2 is a synaptic transmembrane protein that interacts with PSD-95 and is required for neuronal maturation. eNeuro 6:ENEURO.0434-18.2019.

Hartmann N, Reichwald K, Lechel A, Graf M, Kirschner J, Dorn A, Terzubasi E, Wellner J, Platzer M, Rudolph KL, et al. 2009. Telomeres shorten while Tert expression increases during aging of the short-lived fish *Nothobranchius furzeri*. Mech Ageing Dev. 130:290–296.

Hartmann N, Reichwald K, Wittig I, Drose S, Schmeiser S, Luck C, Hahn C, Graf M, Gaußmann U, Terzubasi E, et al. 2011. Mitochondrial DNA copy number and function decrease with age in the short-lived fish *Nothobranchius furzeri*. Aging Cell 10:828–831.

Houtkooper RH, Mouchiroud L, Ryu D, Moullan N, Katsumba E, Knott G, Williams RW, Auwerx J. 2013. Mitonuclear protein imbalance as a conserved longevity mechanism. Nature 497:451–457.

Hu CK, Brunet A. 2018. The African turquoise killifish: a research organism to study vertebrate aging and diapause. Aging Cell 17:e12757.

Huang Z, Whelan CV, Foley NM, Jedd B, Touzalin F, Petit EJ, Puechmaijlle SJ, Teeling EC. 2019. Longitudinal comparative transcriptomics reveals unique mechanisms underlying extended healthspan in bats. Nat Ecol Evol. 3:1110–1120.

Johnson K, Moriarity C, Tania N, Orntman A, DiPietrantonio K, Edens B, Eisenman J, Ok D, Krikorian S, Barragan J, et al. 2014. Ki67 dependent cell cycle progression in radial glial cells is required for proper neurogenesis in the zebrafish neural tube. Dev Biol. 387:73–92.

Kelmer Sacramento E, Kirkpatrick JM, Mazzetto M, Baumgart M, Bartalone A, Di Sanzo S, Cervino C, Sanguanini M, Papaevgeniou N, Lefaki M, et al. 2020. Reduced proteasome activity in the aging brain results in ribosome stoichiometric loss linked to aggregation. Mol Syst Biol. 16:e00956.

Kim H, Johnson JM, Lera RF, Brahma S, Burkard ME. 2017. Anillin phosphorylation controls timely membrane association and successful cytokinesis. PLoS Genet. 13:e1006511.

Kolora SRR, Owens GL, Vazquez JM, Stubbs A, Chatla K, Jainese C, Seeto K, McCrea M, Sandel MW, Viana JA, et al. 2021. Origins and evolution of extreme life span in Pacific Ocean rockfishes. Science 374:842–847.

Leise W 3rd, Mueller PR. 2002. Multiple Cdk1 inhibitory kinases regulate the cell cycle during development. Dev Biol. 249:156–173.

Li H. 2011. A statistical framework for SNP calling, mutation discovery, association mapping and population genetic parameter estimation from sequencing data. Bioinformatics 27:2987–2993.

Loeb LA, Monnat RJ Jr. 2008. DNA polymerases and human disease. Annu Rev Genet. 42:501–533.

Love MI, Huber W, Anders S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15:550.

López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. 2013. The hallmarks of aging. Cell 153:1194–1217.

Lu T, Pan Y, Kao SY, Li C, Kohna I, Chan J, Yankner BA. 2004. Gene regulation and DNA damage in the aging human brain. Nature 429:883–891.
profiling of aging in human muscle reveals a common aging signature. PLoS Genet. 2:e115.

Zak J, Reichard M. 2020. Fluctuating temperatures extend median lifespan, improve reproduction and reduce growth in turquoise killifish. Exp Gerontol. 140:111073.

Zak J, Roy K, Dykova I, Mraz J, Reichard M. 2022. Starter feed for carnivorous species as a practical replacement of bloodworms for a vertebrate model organism in ageing, the turquoise killifish Nothobranchius furzeri. J Fish Biol. 100:894–908.

Zak J, Vrtílek M, Reichard M. 2019. Diel schedules of locomotor, reproductive and feeding activity in wild populations of African annual killifish. Biol J Linn Soc. 128:435–450.

Zhao N, Ren Y, Yamazaki Y, Qiao W, Li F, Felton LM, Mahmoudiandehkordi S, Kueider-Paisley A, Sonoustoun B, Arnold M, et al. 2020. Alzheimer’s risk factors age, APOE genotype, and sex drive distinct molecular pathways. Neuron 106:727–742 e726.

Zheng X, Levine D, Shen J, Gogarten SM, Laurie C, Weir BS. 2012. A high-performance computing toolset for relatedness and principal component analysis of SNP data. Bioinformatics 28:3326–3328.

Zheng L, Meng Y, Campbell JL, Shen B. 2020. Multiple roles of DNA2 nuclease/helicase in DNA metabolism, genome stability and human diseases. Nucleic Acids Res. 48:16–35.