Age-related decline of mitochondrial DNA copy number and function in the short-lived fish *Nothobranchius furzeri*

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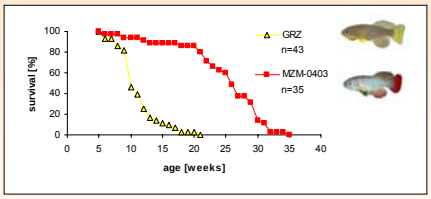
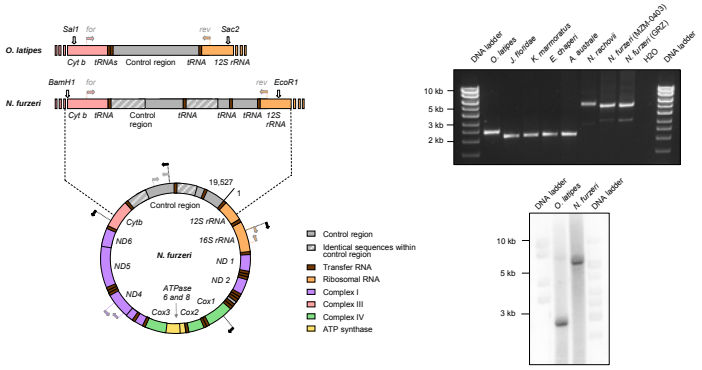
BACKGROUND

The turquoise killifish *Nothobranchius furzeri* has an extremely short lifespan and shows typical signs of ageing. Here we used *N. furzeri* to study whether ageing is associated with mitochondrial DNA (mtDNA) alterations and changes of mitochondrial function.

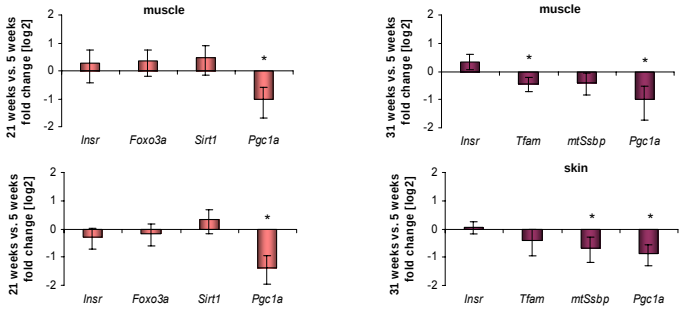
N. furzeri shows typical changes with age as reported for other fish species.
Several strains of *N. furzeri* differ in the maximum lifespan.



The mitogenome of *N. furzeri* harbours an extended control region

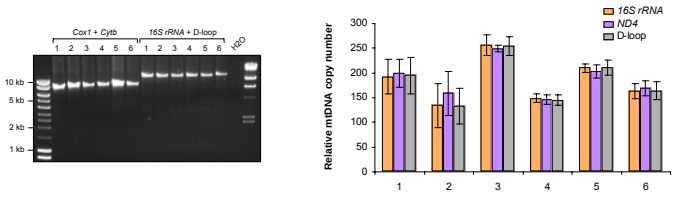


Expression of *Pgc-1α*, *Tfam* and *mtSsbp* declines with ageing



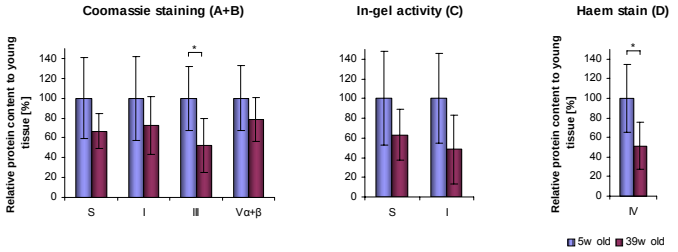
Changes of gene expression were determined in aged (n=9) versus young (n=11) muscle and skin samples using real-time PCR. The *Pgc-1a* gene, which encodes a transcriptional co-activator that increases mitochondrial biogenesis, was significantly down-regulated with age. Tfam and mtSsbp bind to mitochondrial DNA and in particular Tfam is considered to be a target of Pgc-1α.

No evidence for mtDNA deletions



Using DNA from muscle tissue of six old fish for long-range and real-time PCR showed no signs of large-scale mtDNA deletions.

Aged muscle displays decreased content of OXPHOS complexes III and IV

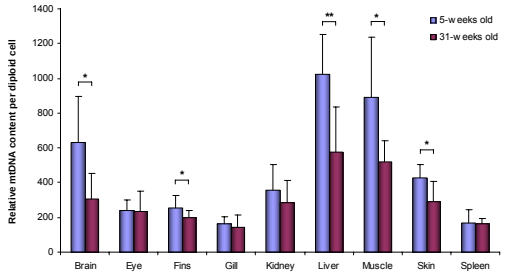


Respiratory chain complexes of skeletal muscle homogenates were separated by blue native gel electrophoresis and content or activity of complexes were densitometrically quantified.

Mitochondrial DNA abundance is decreased with aging

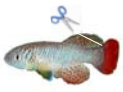
The mtDNA copy number was determined in different tissues using quantitative PCR with *N. furzeri*-specific primers for the *16S rRNA* gene (mitochondrial) and the *Cdkn2a/b* gene locus (nuclear).

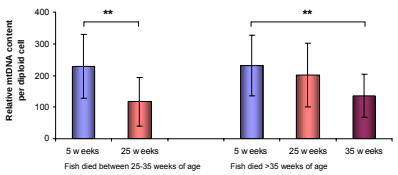
Mitochondrial DNA copy number also significantly decreased with age in the dorsal fin of the same individual.



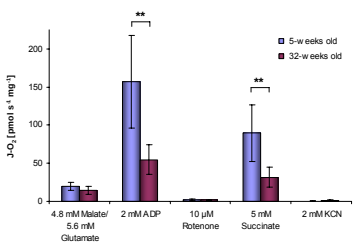
Fin clip	Age	No.
1.	5 weeks	9
2.	25 weeks	9
Dead: < 35 weeks		

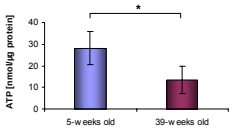
Fin clip	Age	No.
1.	5 weeks	11
2.	25 weeks	11
3.	35 weeks	11
Dead: > 35 weeks		





Mitochondrial bioenergetics is impaired in aged muscle tissue





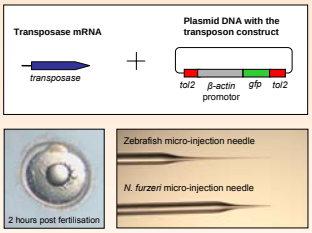
Oxygen consumption was measured from isolated mitochondria of young and aged skeletal muscle tissue to determine mitochondrial respiration. ATP content of muscle homogenates were quantified using HPLC analysis.

CONCLUSION & OUTLOOK

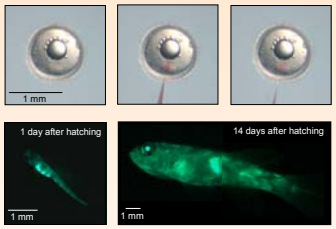
- The mtDNA copy number is significantly reduced in a number of tissues of aged fish
- Gene expression of *Pgc-1a* (and *Tfam* and *mtSsbp*) is decreased with age in skeletal muscle and skin
- Mitochondrial function as determined by the content of respiratory chain complexes, respiration rate, and ATP content is reduced in aged muscle

What next?

- Effect of improved mitochondrial function on ageing and lifespan
- Manipulation of gene expression in *N. furzeri*, *e. g.* over-expression of *Pgc1a* to increase overall mtDNA copy number



We have chosen the *Tol2* transposon system to establish transgenesis in *N. furzeri*.



After injecting transposase mRNA and a transposon construct into the 1-cell stage, GFP-positive fish show a typical mosaic expression pattern.