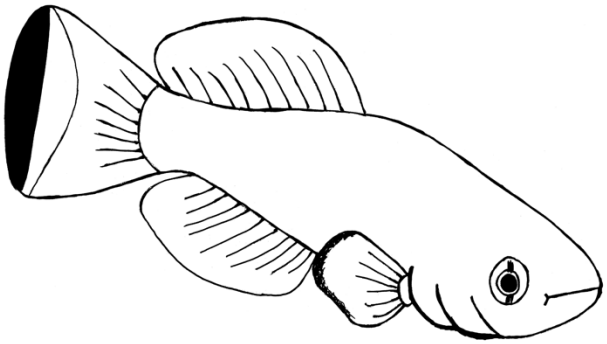
ABSTRACT BOOK



4th Nothobranchius Symposium, 3-4 June 2021

Brno, Czech Republic

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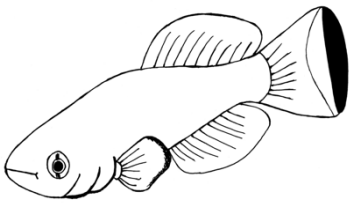
4th Nothobranchius Symposium, 3-4 June 2021



THURSDAY 3 June

9:00 - 9:15 Welcome and introduction (including technical introduction)

9:15 - 10:10 Plenary Lecture by **Jason Podrabsky**: Embryo development and diapause in annual killifish (and beyond) 40 min + 15 min discussion

*** Student talks are labelled in red ***Sponsor talks are in green

10:15 - 11:20 Ageing phenotype (3 talks for 15+5 min) Chair: CELLERINO

- Dissecting aging piece by piece: how proteasome inhibition contributes to the onset of brain aging phenotypes in the turquoise killifish. (Kelmer Sacramento E.)
- Analysis of methylation dynamics reveals a tissue-specific, age-dependent decline in 5 methylcytosine within the genome of the vertebrate aging model *Nothobranchius furzeri*. (Pusch O.)
- Sponsor talk - Tecniplast (5 min)
- Sustained AMPK-γ1 complex activity prevents age-related metabolic disorders and promotes longevity in killifish. (Ripa R.)

11:30 - 12:55 Brain aging and neuroscience (4 talks for 15+5 min) 3 student talks Chair: ENGLERT

- Aging is associated with a degeneration of locus coeruleus neurons, but not dopaminergic neurons, in the short-lived killifish *Nothobranchius furzeri*. (Bagnoli S.)
- The killifish visual system: an in vivo model to investigate age-related phenomena and rejuvenating strategies in the central nervous system. (Bergmans S.)
- Sponsor talk – VisualSonic (5 min)
- Improving neurorepair in the aged brain, what the killifish pallium can tell. (Van houcke J.)
- Single cell transcriptomics analyses unravel the heterogeneous progenitor and neuron landscape in the adult killifish (*N. furzeri*) brain. (Ayana R.)

12:55 - 14:00 LUNCH BREAK

14:00 - 15:25 Homeostasis and healthspan (4 talks for 15+5 min) 1 student talk Chair: ARCKENS

- Identification of protein aggregates with prion-like and phase separation properties in the aging vertebrate brain. (Harel I.)
- Anatomy and tissue homeostasis of the gut tube of the turquoise killifish. (Schöfer C.)
- Sponsor talk - Aquaneering (5 min)
- RNA-seq analysis of aging in *Nothobranchius furzeri*: effects of genetic background and captive vs. wild environment. (Mazzetto M.)
- Health span effects of metformin are potentially sex specific. (Martirosyan A.)

15:40 - 16:30 SPEED TALKS (for 16 posters, each 2 min) includes Microbiotest (5 min) and Loligo (5 min)
List of speed talks is provided on page 3 of this document, along with designated time plan for sponsor talks

16:35 - 18:00 POSTER SESSION (break-out rooms for 16 posters, and for 5 sponsors)

18:15 - 20:00 Break-out rooms (topical, personal)

FRIDAY 4 June

9:00 - 9:05 Introduction and technical issues

9:05 - 10:05 Biorhythms and regeneration Chair: VALENZANO

- A core circadian clock network in the turquoise killifish. (Lee S., Kim Y.)
- Enhancers and the uneven distribution of regenerative capacities in vertebrates. (Wang W.)
- Mechanisms of aging-induced decline in wound-healing. (Paatero I.)

10:15 - 10:45 Sex determination Chair: VALENZANO

- Cytogenomics of *Nothobranchius furzeri* and *N. kadleci*: sex chromosomes and repetitive DNA dynamics. (Sember A.)
- Beyond gdf6Y – elucidating the determination and development of sex in the annual turquoise killifish *Nothobranchius furzeri*. (Richter A.)

10:50 - 11:30 Tools and applications 1 student talk Chair: TERZIBASI

- Generating a transparent vertebrate model for in vivo applications in aging research. (Krug J.)
- In vitro fertilization, blood extraction from live animals and in-tank fish tracking in *N. furzeri*. (Dolfi L.)

11:40 - 12:20 Husbandry 1 student talk Chair: TERZIBASI

- Behind the scenes of successful aging research. (Hoppe B.)
- Are bloodworms optimal feed for laboratory *Nothobranchius furzeri*? (Žák J.)

12:20 - 13:30 LUNCH BREAK

13:30 - 14:50 Diapause and embryo development Chair: BEREZIKOV

- Natural course of embryo development in the wild populations of African *Nothobranchius* and American *Austrolebias* species. (Polačik M.).
- Plasticities within and across generations in *Austrolebias* annual killifish. (Van Dooren T.J.M.)
- Axis formation in annual killifish: Nodal coordinates morphogenesis in absence of Huluwa prepatterning. (Abitua P.B.)
- The genome of the bi-annual Rio Pearlfish (*Nematolebias whitei*) informs the genetic regulation of diapause and environmentally-cued hatching in extreme environments. (Thompson A.W.)

15:10 - 16:10 Behaviour 1 student talk Chair: PHILIPPE

- Body coloration, aggression and learning in *Nothobranchius guentheri.* (Demidova T.)
- Turquoise killifish on antidepressants: towards understanding the ecological risks of neurochemical pollution. (Thoré E.S.J.)
- *Nothobranchius furzeri* as an emerging model for mate choice: female choice revealed by animations. (Johnson B.D.)

16:10 - 16:40 Final remarks, Best student talk and poster – poll and results

16:50 - 19:00 General discussion, Break-out rooms available (topical, personal)

SPEED TALKS FOR POSTERS (10 student posters)

Starts 15:40. Each speed talk lasts 2 min, questions relegated to Poster session afterwards

Giannuzzi C. Multiomics longitudinal study of aging in *Nothobranchius furzeri*.

Ballhysa E. Deciphering the interplay between nucleic acid surveillance pathways, inflammation and vertebrate healthspan.

Borgonovo J. Organization of the catecholaminergic system in the short-lived fish *Nothobranchius furzeri*.

Reuter H. Analysis of the development and regeneration of the killifish heart.

Vrtílek M. Trade-off between the rate of embryonic development and adult growth plasticity in *N. furzeri*.

Hassan S. Differential expression of transcriptome and proteome in developing and diapause embryos of turquoise killifish.

Sponsor talk Microbiotest (5 min) Assumed 15:52 - 15:57

Sánchez W. N-Cadherin affects mitotic index and epithelial cell shape during early morphogenesis in killifish embryos.

Godinho Ferreira M. Lifespan and telomere length variation across wild-derived African killifish populations.

Součková K. Establishment of cell lines from embryos of *Nothobranchius* annual killifish.

Brogi L. *Nothobranchius furzeri* organotypic cultures: towards a model of ex vivo brain aging.

Wittorski A. An automated shuttle test for cognition evaluation in fishes.

Rivas N. Strength of reproductive isolation between *Austrolebias reicherti* and *A. charrua* varies depending on life expectancies.

Sponsor talk Loligo (5 min) Assumed 16:10 - 16:15

Blažek R. *Mycobacterium* infection and *N. furzeri* survival.

Vanhunsel S. The age factor in optic nerve regeneration: intrinsic and extrinsic barriers hinder successful functional recovery in killifish.

Zandecki C. Characterization of progenitor diversity in the aging brain with the use of a killifish transgenic toolbox.

Mariën V. A deep dive in the aged killifish brain: ameliorating functionality of newborn neurons.

Expected to finish at 16:30 (with approximately 10 min of extra reserved time)

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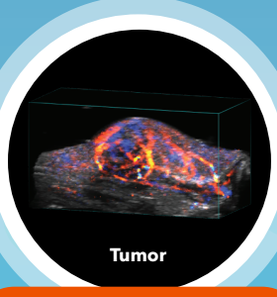

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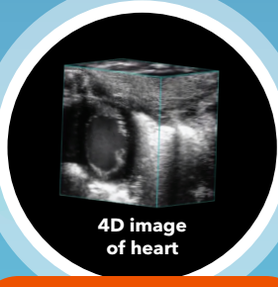

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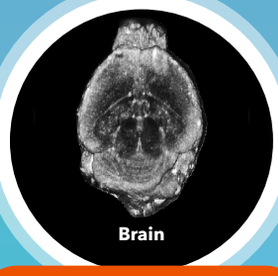
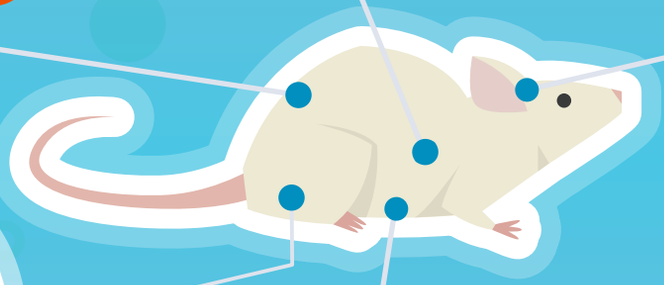


NEUROBIOLOGY
Visualize anatomy and functional processes including stroke and cancer, as well as delivery of drugs, stem cells or other agents



MOLECULAR IMAGING
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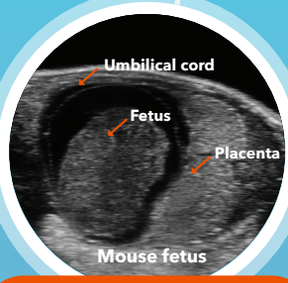

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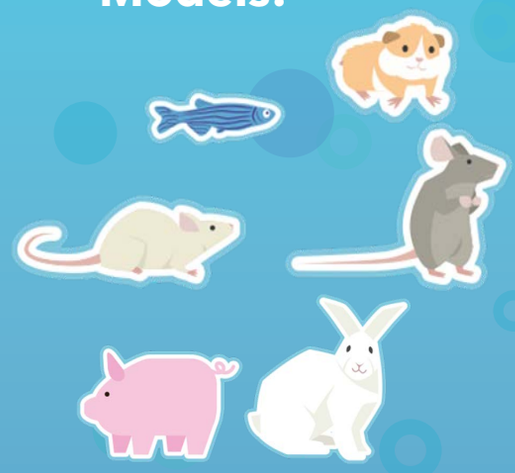

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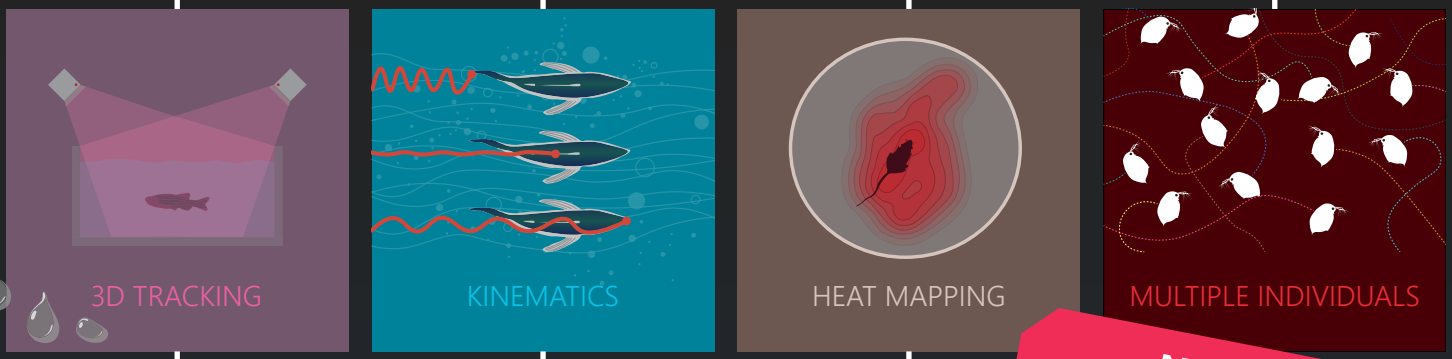

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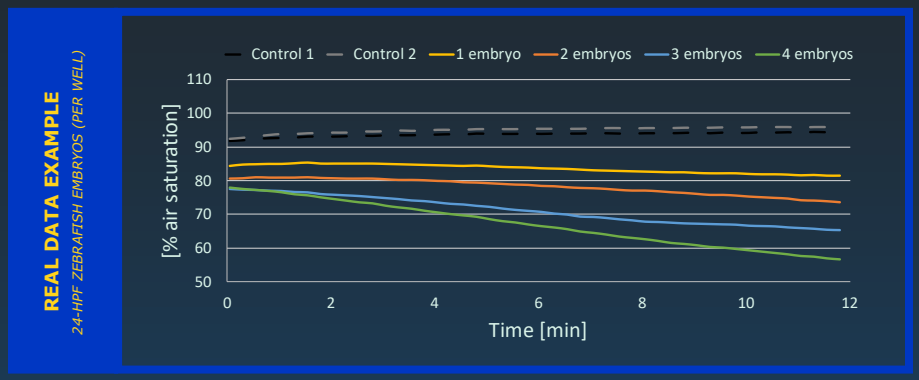

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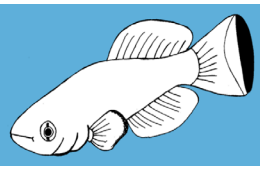

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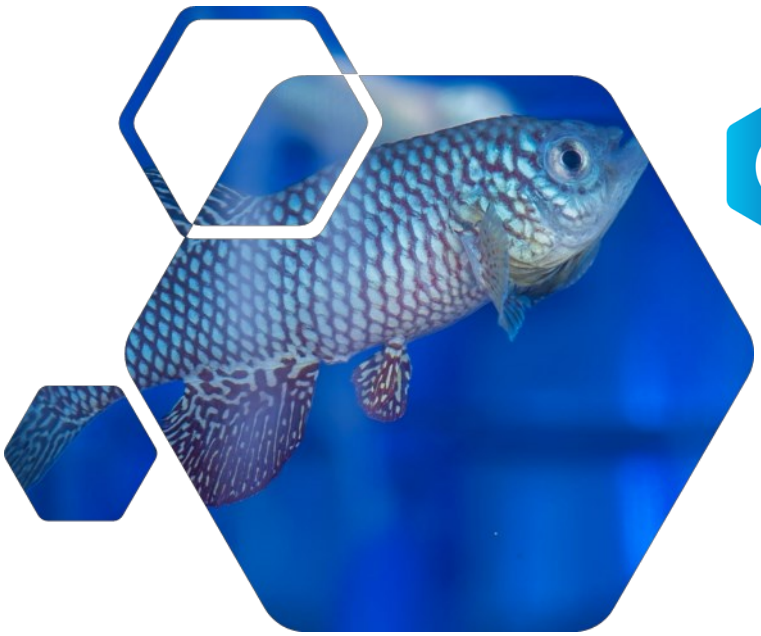

MICROBIOTESTS

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- √ SYNCHRONIZED AND STABLE HATCHING SUCCESS
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PLENARY LECTURE

Embryo development and diapause in annual killifish (and beyond)

PODRABSKY J.E.

Portland State University, Oregon, USA

*Austrofundulus limnae*us, native to northern Venezuela, is a well-established model for the study of dormancy, development, and stress tolerance in annual killifishes. Embryos of *A. limnaeus* can arrest in all three stages of diapause (diapause I, II, and III) possible in annual killifishes in response to environmental cues such as temperature, light, and presence of adult fishes. Evidence from transcriptomic surveys and pharmacological inhibition studies indicate that entrance into diapause II is regulated by vitamin D signaling in a temperature-dependent manner. Exposure to exogenous vitamin D can also reactivate development in diapausing embryos. Changes in DNA methylation in response to exogenous vitamin D suggest a critical role for chromatin structure in the exit of embryos from diapause II. Differential methylation studies suggest that insulin-like growth factor signaling and some key developmental transcription factors are activated in response to vitamin D exposure. Regulation of diapause II is similar to that observed in nematode dauer dormancy which suggests that metabolic dormancy may be controlled by a highly conserved mechanism across distantly related Phyla. The ecological significance of these laboratory-based studies remains unclear. The possible interplay between environmental cues experienced by the embryonic and maternal life stages have yet to be explored, but are likely to add critical environmental context to these mechanistic studies.

(PLENARY LECTURE)

POSTERS

Deciphering the interplay between nucleic acid surveillance pathways, inflammation and vertebrate healthspan

BALLHYSA E., RIPA R., ANTEBI A.

Max Planck Institute for Biology of Ageing, Cologne, Germany

The nucleic acid surveillance pathway cGAS/STING recognizes misplaced dsDNA and leads to the activation of a widespread inflammatory response. It has been found that cGAS plays a major role in a variety of cellular processes, such as senescence, DNA damage response, neoplasia and inflammation. Both the inputs and the outputs of the pathway are known to manifest during normative ageing, however, there is no direct investigation of the pathway's contribution to organismal physiology during ageing. cGAS has been shown to have variable sensitivity between different species, however, its enzymatic and DNA binding domains are highly conserved among all vertebrates. This conservation provides the opportunity to study the pathway in the context of ageing using the shortest-lived vertebrate that can survive in captivity, the killifish *Nothobranchius furzeri*. In this study, we use CRISPR mutants to investigate if and how does the cGAS/STING pathway effect healthspan and lifespan.

(POSTER PRESENTATION)

Mycobacterium infection and *N. furzeri* survival

BLAŽEK R. (1,2), BYSTRÝ V. (3), DYKOVÁ I. (2), POLAČIK M. (1), REICHARD M. (1,2,4), SOUČKOVÁ K. (3), SLABÝ O. (3, 5), VRTÍLEK M. (1), ŽÁK J. (1, 6)

(1) Institute of Vertebrate Biology, Czech Academy of Sciences, Brno, Czech Republic; (2) Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic; (3) Central European Institute of Technology, Masaryk University, Brno, Czech Republic; (4) Department of Ecology and Vertebrate Zoology, University of Lodz, Lodz, Poland; (5) Department of Comprehensive Cancer Care, Masaryk Memorial Cancer Institute, Brno, Czech Republic; (6) Department of Zoology, Faculty of Science, Charles University, Brno, Czech Republic

One of the main challenges of *Nothobranchius* husbandry is to keep fish healthy. Feeding with live or frozen food represents continuous risk of introduction of various pathogens. Non-tuberculous mycobacteria are common bacterial pathogens of *Nothobranchius* fishes causing systemic infection. The disease is not curable currently, so potential routes for its transmission and embracing the pathology of mycobacteriosis is of imminent concern. Egg sterilization obviously presents the key tool for preventing transgenerational transmission of mycobacteria attached to the egg surface. We tested efficiency of different egg treatments and early life environment on fish viability and the frequency of belly-sliding. In addition, we monitored pathology of freshly deceased individuals to identify their most likely cause of death. We found out that juvenile fish originating from sterilized eggs developed mycobacterial symptoms at much lower frequency. *Mycobacterium* infection affecting swim bladder has been confirmed as the primary cause of juvenile belly sliding and related fish mortality. Apart from belly-sliding, mycobacteriosis remains visually undetected until the very last stage of the disease when fish become moribund (do not feed, are less active, often lose coloration and subsequently die within a few days). This poses a problem because any *Mycobacterium*-infected fish is a source of infection to other tankmates as mycobacteria accumulate in excretory system of cranial part of the kidney. Additional symptoms included exophthalmos, fish spinning uncoordinatedly, extremely swollen belly and whitish lesions on the mouth. Individual susceptibility to the infection likely plays an important role as the occurrence of the symptoms and mortality was not uniform across all the fish within a tank.

(POSTER PRESENTATION)

Organization of the catecholaminergic system in the short-lived fish *Nothobranchius furzeri*

Borgonovo J. (1,2,3), Ahumada-Galleguillos P. (1,2), Oñate-Ponce A. (1,2,3), Allende-Castro C. (1,2,3), Henny P. (4), Concha M.L. (1,2,3)

(1) Program of Integrative Biology, Institute of Biomedical Sciences, Faculty of Medicine, Universidad de Chile, Santiago, Chile; (2) Biomedical Neuroscience Institute, Santiago, Chile; (3) Center for Geroscience, Brain Health and Metabolism, Santiago, Chile; (4) Department of Anatomy and Interdisciplinary Center of Neurosciences, School of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile

The catecholaminergic (CAergic) system plays a regulatory role in many cerebral functions and is implicated in a number of human diseases. Dysfunction in dopaminergic neurotransmission significantly contribute to Parkinson´s Disease (PD) and psychiatric disorders. In addition, the CAergic system is affected in the course of physiological aging, revealing that the deterioration of the system is a cross-cutting issue of normal and pathological aging. In this study, we provided a systematic description of the neuroanatomical distribution of catecholaminergic neurons in the brain of adult *Nothobranchius furzeri* based on tyrosine hydroxylase (TH) immunoreactivity. In the telencephalon, numerous TH+ neurons were observed in the olfactory bulbs and the ventral telencephalic areas, arranged as strips extending through the rostrocaudal axis. We found the largest TH+ groups in the diencephalon at the preoptic region level, the ventral thalamus, the pretectal region, the posterior tuberculum, and the caudal hypothalamus. In the dorsal mesencephalic tegmentum, we identified a particular catecholaminergic group. The rostral rhombencephalon housed TH+ cells in the locus coeruleus and the medulla oblongata, distributing in a region dorsal to the inferior reticular formation, the vagal lobe, and the area postrema. Finally, scattered TH+ neurons were present in the ventral spinal cord and the retina. From a comparative perspective, the overall organization of catecholaminergic neurons is consistent with the general pattern reported for other teleosts. However, *N. furzeri* shows some particular features, including the presence of catecholaminergic cells in the midbrain. This work provides a detailed neuroanatomical map of the catecholaminergic system of *N. furzeri*, a powerful aging model, also contributing to the phylogenetic understanding of one of the most ancient neurochemical systems. Funding: FONDAP 15150012, P09-015-F, PIA ACT192015.

(Poster presentation)

***Nothobranchius furzeri* organotypic cultures: towards a model of *ex vivo* brain aging**

Brogi L. (1), Bagnoli S. (1), Terzibasi Tozzini E. (2), Cellerino A. (1,3)

(1) Scuola Normale Superiore, Pisa, Italy; (2) Stazione Zoologica Anton Dohrn, Napoli, Italy; (3) Leibniz institute on Aging, Jena, Germany

Organotypic culture of brain slices is an ex-vivo technique used to investigate long-term neuronal survival. Organotypic cultures maintain a three-dimensional organization and mimic the *in vivo* development of cells and synapses. The absence of the blood-brain barrier allows direct access of small molecules to the culture. Also, organotypic cultures allow to study the effects of age on brain in isolation without the influence of the systemic milieu. The ex-vivo model has been widely used in rodents for conducting molecular, pharmacological and physiological studies. To our knowledge, no long-term culture system for fish brains is established. The short-lived annual fish *Nothobranchius furzeri* shows extremely short life span and accelerated expression of age markers and a long-term culture system would enable the study of brain aging ex-vivo. We thus established organotypic cultures from brain slices of *N. furzeri*. The brains were extracted from MZCS-222 fish of 5, 12, 30 weeks after hatching from which we cut 500 µm slices of various brain regions. The brain slices were incubated on porous membranes in an ad-hoc medium for at least of 5 weeks. Slices were incubated with EdU for the first three days to label newborn cells. One week after Edu treatment, we observed neurogenesis in all slices indicating that adult neurogenesis is retained ex-vivo even in slices from old fish. as well as *in vivo*. In addition, we specifically tested the viability of noradrenergic neurons labelled with TH and we observed that these neurons persist for at least five weeks *in vitro*. Our future aims are to prolong the culture period to test whether brain aging markers become expressed *in vitro* and finally test drugs and nutraceutical compound.

(Poster presentation)

Multiomics longitudinal study of aging in *Nothobranchius furzeri*

GIANNUZZI C. (1,2), BAUMGART M. (2), NERI F. (2), ORI A. (2), CELLERINO A. (1,2)

(1) Scuola Normale Superiore, Pisa, Italy; (2) Leibniz institute on Aging, Jena, Germany

Aging has been found to be associated with changes in DNA methylation, in gene expression, with progressive loss of protein homeostasis, decoupling between mRNA and proteins, and alterations in composition of gut microbiota. Moreover, expression of RNAs and microbiome composition can predict individual lifespan in *N. furzeri*. However, a holistic view of these different layers of molecular changes, which are interconnected during aging, is missing. In this work, we propose an integrated analysis of multi-omics datasets to provide a comprehensive profile of the heterogeneous aging process. To this end, we initiated a longitudinal study inspired by our previous approach (Baumgart et al., 2016) in which we will analyse at 10 and 20 weeks of age methylome, transcriptome, proteome from fin biopsies and faecal microbiome in 170 fish to correlate all these variable one with another and with individual lifespan. In a preparation phase, we performed a cross-sectional study of fin aging and compared age-dependent changes in transcripts and proteins expression with those occurring in the brain to identify changes that are coordinated between the two tissues. We also observed that in the fin a progressive reduction in the global correlation between proteins and mRNA occurs during aging, as we previously found in the brain (Kelmer et al., 2020). We have then established a protocol for DNA methylation analysis, carried out here for the first time in *N. furzeri*. All the differentially methylated regions (DMRs) during aging show the same direction in brain and fin. Similarly to what had already described in mammals, we have then built in *N. furzeri* an "epigenetic clock", able to predict with high precision the chronological age. The ultimate goal of our study will be to identify which among mRNA, proteins, DMRs and bacterial species is more predictive for individual lifespan, construct a multi-layer network, identify its main hubs and drive candidates for experimental validation.

(POSTER PRESENTATION)

Lifespan and telomere length variation across wild-derived African killifish populations

GODINHO FERREIRA M. (1,2), GIANNETTI K. (2), FERREIRA T. (2), MAOUCHE A. (1), VRTÍLEK M. (3), POLAČIK M. (3), BLAŽEK R. (3), REICHARD M. (3,4)

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Telomeres and telomerase prevent the continuous erosion of chromosome-ends caused by lifelong cell division. Shortened telomeres are associated with age-related pathologies. While short telomere length is positively correlated with increased lethality at the individual level, short telomeres are associated with long (and not short) lifespans in comparisons across species. Here, we tested this conundrum between individual and evolutionary patterns in telomere length using African annual killifish. We analysed lifespan and telomere length in a set of captive strains derived from well-defined wild populations of *Nothobranchius furzeri* and its sister species, *N. kadleci*, from sites along a strong gradient of aridity which ultimately determines maximum natural lifespan. Overall, males lived shorter than females, and males had shorter telomeres. Aridity in the site of strain origin was negatively associated with male lifespans in controlled laboratory conditions, but positively with telomere length. In addition, fish that grew larger during juvenile period possessed shorter telomeres. This demonstrates that individual condition and population-level selection indeed modulate relationship between telomere length and lifespan in opposite directions, validating existence of those inverse trends within a single taxon. Unlike mean telomere length, heterogeneity of telomere length (CV) and, therefore the shortest telomeres, was not associated with other distribution parameters, suggesting that the shortest telomeres are controlled by regulatory pathways other than those that determine average telomere length. The substantial variation in telomere length between strains from different environments identifies killifish as powerful system in understanding adaptive value of telomere length.

(POSTER PRESENTATION)

Differential expression of transcriptome and proteome in developing and diapause embryos of turquoise killifish

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Diapause is a programmed suspension of development to survive sub-optimal environments. This dormant phase does not seem to be associated with aging and preserves the subsequent lifespan and fertility of killifish. Using RNA-seq and mass spectrometry, we started to analyses genome regulation in embryos that have been in Diapause II for 2 weeks compared to developing embryos at the same age. We could quantify the expression of 15,870 transcripts and 3236 proteins. Of these, 8693 transcripts and 1138 proteins significantly differed in expression during diapause by at least 1.5-fold. We detected only a weak positive correlation between the transcript and corresponding protein levels (R = 0.35), suggesting the importance of post-transcriptional layers of regulation during diapause. Integrated transcriptome and proteome analyses revealed diverse functional enrichments amongst genes that are induced or repressed during diapause. Notably, genes encoding ribosomal proteins and translation-initiation factors showed opposite regulation: they were induced at the transcript level as reported before (Hu et al. 2020); However, typically repressed at the protein level. This finding indicates that proteins involved in translation may be down-regulated during diapause by translational repression and/or protein degradation. Together, this preliminary analysis illustrates how genome regulation is re-programmed transcriptionally and post-transcriptionally to enable a distinct dormant state whilst maintaining proteins important to re-initiate development when possible. Hu, Chi-Kuo et al. 2020. "Vertebrate Diapause Preserves Organisms Long Term through Polycomb Complex Members." Science 367(6480): 870–74.

(POSTER PRESENTATION)

Analysis of the development and regeneration of the killifish heart

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Organ failure and degenerative diseases have a major impact on human health as we age. It is therefore of great interest to understand molecular mechanisms underlying regeneration processes to potentially improve regenerative strategies in humans. The aim of this project is to analyse how age impacts the regenerative capacity of the fish heart, using *Nothobranchius furzeri* as a model. First, we investigated the regeneration capacity of the adult killifish heart upon cryoinjury in comparison to the zebrafish heart. In contrast to zebrafish, killifish still had a prominent scar at 30 days post injury, even in the young age cohort. Interestingly, regeneration capacities have been reported upon resection of the killifish heart (Wang et al., Science, 2020). This resection model is characterized by a reduced scar response in contrast to the cryoinjury model that mimics myocardial infarction. In order to have the ability to study any age group, even much younger fish, we aimed to generate a non-invasive model to study heart regeneration in killifish. To this end we generated a transgenic line expressing Nitroreductase and EGFP under the control of the heart specific myl7 promoter. For this we are using a transparent killifish line that we have recently developed. The new line will also allow us to investigate heart development in *N. furzeri*. First live imaging experiments have shown that reporter expression can be detected first in two separate heart fields. These two fields merge within few hours and elongate to form a tube that finally bends and balloons to form the chambered embryonic heart. Future experiments with this line will allow us to follow heart regeneration processes *in vivo*.

(POSTER PRESENTATION)

Strength of reproductive isolation between *Austrolebias reicherti* and *A. charrua* varies depending on life expectancies

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Sexual selection has been proposed as an important force influencing speciation as it affects the origin and maintenance of reproductive isolation between divergent populations. At present, it is considered that its strength can vary according to the ecological conditions and life history traits. Therefore, the isolation degree between closely related species can vary in time and space. *Austrolebias reicherti* and *A. charrua* are sister species that have recently diverged. They have parapatric distributions along the southeastern lowlands of Laguna Merín, in the eastern Uruguay, with an overlapping area in the lower basin of the Río Cebollatí, where they share a hybrid zone. Sexual selection strongly modulates their reproductive dynamic through mate choice and competition. However, the temporary ponds they inhabit suffer abrupt changes along the season, and it has been reported that females decrease choosiness by the end of the season, when life expectancies are reduced. In this preliminary study, we assessed if sexual selection and hybridization are influenced by the reduction in life expectancies. We tested the influence of water depth on individuals' choosiness. To do this, we kept 2 females and 2 males in tanks with different water depth during 6 weeks. Control tanks (n=6) had constant water depth, while treatment tanks (n=6) suffered weekly reductions of water, simulating a drying pond. Our results show that, in treatment tanks, individuals perform as many heterospecific courtships as homospecific ones, while in control tanks, individuals perform more homospecific courtships than heterospecific courtships. These results suggest that reproductive isolation between *A. reicherti* and *A. charrua* can vary in response to the reduction in life expectancies that these species experience at the end of the season.

(POSTER PRESENTATION)

N-Cadherin affects mitotic index and epithelial cell shape during early morphogenesis in killifish embryos

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We are interested in how shape emerges in the developing embryo focusing in the dynamic functionality associated with Cadherins molecules. In this study we analyse the expression and functionality of classical N-Cadherin along the embryonic-extra-embryonic cell interface during early morphogenesis in killifish embryo. We reveal that N-cadherin transcripts are maternally provided and protein express dynamically covering embryonic deep cells layer (DCL) as well as extra-embryonic structures such as epithelial enveloping cell layer (EVL) and yolk syncytial layer (YSL). Loss of function analysis demonstrate that mitotic cell division is affected. Using time-lapse confocal microscopy we noticed that the splitting of genomic DNA during mitosis is compromised in DN-Ncad derived embryos. Although multinucleate EVL cells are a common feature in this fish species, EVL derived from DN-Ncad embryos are bigger in size and showed differences in topological distribution of polygonal cell shapes along the epithelia. Thus, N-cadherin seems to play a role during mitosis and the cellular and molecular signalling involved in this functionality will be addressed soon. Furthermore, as E- and N-Cadherin are co-expressed within same cell types understanding the functional crosstalk between will be a necessary step to better understand the complexities of morphogenesis. Funding: This research was supported by the ANID (Chile) projects FONDECYT 1190806, PIA/ACT192015, Millennium Institute P09-015-F, FONDEQUIP EQM130051 and FONDAP 15150012 to M.L.C and Climat-AmSUD CLI2020004, FONDECYT 11170761 to G.R.

(POSTER PRESENTATION)

Establishment of cell lines from embryos of *Nothobranchius* annual killifish

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In this study, we established and characterized ten cell lines derived from single DII or DIII stage embryos of annual killifish *Nothobranchius furzeri* (NF MZM 0410) and *N. kadleci* (NK MZCS 430). Cell lines displayed fibroblast-like or epithelial-like cell morphology and remained uniform even after subcultivation for more than 60 passages (over 40 passages for lines with lower proliferation rate). Since the establishment of primary cultures, all cell lines were maintained in Leibovitz L-15 medium supplemented with 20% fetal bovine serum, glutamax and non-essential amino acids, with optimal growth temperature of 29°C (no need for CO2 supply). Aiming to track possible structural or numerical karyotype changes, we employed fluorescence in situ hybridization (FISH) with telomeric (TTAGGG)n, 5S and 18S rDNA, and centromere-specific satellite DNA probes on metaphase cells. We compared the obtained results with patterns observed on standard (non-cultured) metaphase spreads prepared from fin tissue of individuals belonging to the same *N. furzeri* and *N. kadleci* strain, respectively. The established cell lines from *Nothobranchius* embryos could become a useful *in vitro* model for conducting research in various fields. This work was supported by grant project GACR 19-20873S. Keywords: cell line, *Nothobranchius furzeri* MZM 0410, *Nothobranchius kadleci* NK MZCS 430, embryo, fish cells, FISH

(Poster presentation)

A deep dive in the aged killifish brain: ameliorating functionality of newborn neurons

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The worldwide prevalence of neurodegenerative diseases will continue to rise due to an increasing aging population. Despite intensive research, there are still no effective treatments available that can cure or even slow down their progression. To recover from brain pathology, a high level of neuroplasticity through de novo neurogenesis, cell migration, differentiation, and circuit integration is necessary. To study these processes throughout the lifespan, we make use of the short-lived African turquoise killifish (GRZ-AD). By injuring the Dm region of the telencephalon, we can uncover the mechanisms that drive recovery in young and aged killifish and hence investigate the impact of aging on the regeneration process. We recently discovered that aged fish are still able to regenerate neurons, but this capacity is much reduced compared to young fish. Next, we need to know if and to what extent the smaller population of newborn neurons in aged killifish still reaches a functional state and hence if they can expand their neurites, receive relevant synaptic inputs and become responsive to stimuli. Preliminary Western blot results already show that aged killifish are unable to restore their number of synapses (SV2), and regenerate dendrites (MAP2) by 23 days post-injury the way young fish can, pointing towards an intrinsic inability of the neurons to reach adequate maturation levels. To investigate this *in vivo*, we apply retroviral vector injections into the telencephalic ventricle, to label the stem cells and their progeny. This way, we can track the fluorescently labelled newborn neurons, and study their maturation, integration, and functionality with high-end microscopy, including calcium imaging. In the end, we hope to find strategies that will promote adequate maturation and integration of newborn neurons in aged killifish. In the long term, this research holds the potential of contributing to new therapies to treat neurodegenerative diseases in human patients.

(Poster presentation)

The age factor in optic nerve regeneration: intrinsic and extrinsic barriers hinder successful functional recovery in killifish

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Worldwide, a growing number of elderly is suffering from age-associated neuropathies. Accordingly, many research efforts currently focus on triggering repair in the damaged or diseased brain. Yet, this remains challenging, especially in an aged environment. As a regeneration-competent teleost fish, the fast-aging African turquoise killifish is ideally suited to unravel the impact of aging on CNS repair. Adopting the optic nerve crush (ONC) model in four killifish age groups, and employing techniques such as (immuno)histochemistry, *in vivo* tracing methods, gene expression studies and behavioural tests, we unveiled that nerve regrowth is impaired in aged killifish subjected to ONC. Depending on the age, repair of axonal damage is affected in different phases of the regenerative process. While middle-aged fish show a delay in nerve regeneration, do not seem to fully reform their synapses in the tectum and only partially recover vision, old and very old fish do not complete tectal reinnervation or restore vision at all. Further investigations into the intrinsic and extrinsic factors affecting the regenerative capacity revealed that the intrinsic response of the retinal ganglion cells themselves alters with age, resulting in an insufficient outgrowth potential. Additionally, the reactivation of both micro- and macroglial cells upon injury seems to be prolonged/enhanced, thereby establishing a non-optimal extrinsic environment and resulting in glial scar formation at the lesion site. Altogether, our results point towards an age-dependent decline in optic nerve regeneration in the killifish. Interestingly, while the repair process following nerve injury highly resembles that of zebrafish at young age, the killifish's ability to regenerate is more mammalian-like at old age. These findings urge further investigations into the cellular and molecular mechanisms underlying this impairment, thereby contributing to the search for effective neuroregenerative therapies.

(POSTER PRESENTATION)

Trade-off between the rate of embryonic development and adult growth plasticity in *N. furzeri*

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Metabolic processes are intertwined and share common regulatory pathways. They are also dynamic over time. Embryonic diapause of annual killifish offers an opportunity to test trade-off between metabolic rate at temporally distant life periods. We asked whether direct development in *N. furzeri* embryos, and activation of particular metabolic pathways, constrain their post-hatching growth plasticity. We tested this hypothesis by experimentally manipulating feeding regime of *N. furzeri* males. We reared two groups of embryos - with "escape" (i.e. direct-developing) and "diapause" (entering diapause II) trajectories. We then tested the effect of those alternative embryonic trajectories on capacity for compensatory growth. Using two feeding regimes - high and low (feeding twice or once a day), and their temporal change, we compared growth rates across 4 treatment groups (low-low, low-high, high-low and high-high ration), replicated across escape/diapause embryo trajectory. We predicted reduced capacity for compensatory growth in the low-high treatment in killifish from escape embryos compared to fish from the group with diapause. The ultimate goal is to use transcriptomics to test the extent of pleiotropic effects of the insulin-like growth factor 1 (IGF-1) pathway, and hence the consequences of embryonic diapause, on adult growth plasticity in *N. furzeri*.

(POSTER PRESENTATION)

An automated shuttle test for cognition evaluation in fishes

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Over the last 30 years, assessing learning and cognition in model organisms has shown its ecological relevance. In many biological fields such as neuroscience, ecotoxicology, evolutionary physiology or behavioural ecology, the evaluation of an organism's aptitude to process new signals (learning) and its ability to recall an appropriate behaviour is a powerful tool. In the active avoidance test, the animal learns to avoid a negative stimulus using appropriate locomotor response triggered by conditioned signal. To this aim, scientists have struggled to build reliable and reproductive set-up. Reproductive intensity of both adverse and conditioned stimuli is of major importance to ensure the appropriate response and to allow comparison between treatments and/or replicates. In this protocol, we modified a set-up from "old school" shuttle box observations adapted for *Nothobranchius furzeri* by implementing computer analyses and automated activation of the stimulus. With the help of Ethovison XT video tracking technology, we could perform a test of one hour without any stimulus variation or fish disturbance. The adapted informatic hardware set-up (IO-Box) tracks the fish and then execute the appropriate stimulus regarding the last fish action/position or the time left before the end of the test. Preliminary tests have shown that *N. furzeri* reacts consistently to a red light used as conditioned stimulus. Depending on its personality, the fish would either stop swimming and avoid the coming adverse stimulus (intense bubbling) or stop swimming without escaping. The learning ability can then be assessed based on the performance index (P.I.), which is a floating average of 10 consecutive trials. This automated system for preference/avoidance measurements will help to improve the reproducibility while assessing the effect of aging on learning and cognitive processes in the model fish species *N. furzeri*.

(POSTER PRESENTATION)

Characterization of progenitor diversity in the aging brain with the use of a killifish transgenic toolbox

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Even though the aging brain has limited regenerative capacities, evidence of human adult neurogenesis incited research into endogenous stem cells as sources of new neurons. However, tools are still missing to study the potential of adult neural stem cells and the molecular mechanisms steering neurogenesis in an aging context. In recent years, the African turquoise killifish has been successfully established as an innovative aging model with great potential because of its short lifespan, many aging hallmarks and an ever-existing neural stem cell pool. One of the main limiting factors to using killifish to study postnatal neurogenesis and neurodegeneration is the paucity of broadly applicable transgenic lines. Therefore, we are developing an extensive genetic toolbox to target the visualization, isolation and tracing of the glial and non-glial stem cells. Using single cell RNA sequencing, we were able to identify different potential progenitor subtypes present in the killifish telencephalon, more specifically, non-glial progenitors and four distinct radial glia subtypes. However, the spatial and functional relation between these putative progenitor subtypes remains elusive. Using cell-type specific reporter lines, we will identify the relationships between the different putative neurogenic and gliogenic stem cells of the killifish telencephalon, and identify the age-dependent decline of their neurogenic potential. In addition, we molecularly compare the progenitor cells in a young and aged environment using a combination of cell type-specific Cre recombinase driver and Switch reporter lines. Altogether, this will create an influx of knowledge regarding adult neural stem cell potential and pave the way to the identification of new potential druggable pathways to boost neurogenesis.

(POSTER PRESENTATION)

ORAL CONTRIBUTIONS

Axis formation in annual killifish: Nodal coordinates morphogenesis in absence of Huluwa prepatterning

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Axis formation in fish and amphibians is initiated by a prepattern of maternal gene products in the blastula. The embryogenesis of annual killifish challenges prepatterning models because blastomeres disperse and then re-aggregate to form the germ layers and body axes. This dispersion-aggregation process prompts the question how axis determinants such as Huluwa and germ layer inducers such as Nodal function in annual killifish. Here we show in *Nothobranchius furzeri* that huluwa, the factor thought to break symmetry by stabilizing β-catenin, is a non-functional pseudogene. Nuclear β-catenin is not selectively stabilized on one side of the blastula but accumulates in cells forming the incipient aggregate. Inhibition of Nodal signalling blocks aggregation and disrupts coordinated cell migration, establishing a novel role for this signalling pathway. These results reveal a surprising departure from classic mechanisms of axis formation: canonical Huluwa-mediated prepatterning is dispensable and Nodal coordinates morphogenesis.

(ORAL PRESENTATION)

Single cell transcriptomics analyses unravel the heterogeneous progenitor and neuron landscape in the adult killifish (*N. furzeri*) brain

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The African turquoise killifish is an extremely short-lived vertebrate displaying striking similarities to human with respect to age-related neurodegeneration. Killifish also possess ample neuro-regenerative capacity but, different from other teleosts, this capacity is impaired with age. This age-dependent loss of CNS regenerative ability holds high-potential clinical opportunities, which cannot be elucidated in mammalian models. So far, the contribution of stem and progenitor cell types to successful neurorepair is not fully understood. We performed single-cell sequencing of the young adult killifish telencephalon to identify the heterogeneous progenitor landscape that shapes neuro-regenesis. We found unique non-glial progenitors or NGPs (11%) and glial (13%) subtypes, and 59% of all cells to be of neuronal type. We could establish an array of cellular markers for 17 cell types, including the atypical NGPs, (im)mature neurons, microglia and radial glia (quiescent and proliferative; RG). We next divided the cells into broad classes of progenitor (PC) and neuronal (NC) nature and performed iterative sub-clustering. PC-based clustering revealed 4 independent and unique RG types, including an astroglia population that could be distinguished by transcription factors and intermediate stages. Pseudotime analysis of PC-based clusters determined the source cell of neuro- and gliogenesis to be a stem-like RG subtype, followed by NGPs. We identified the NGP as a highly proliferative mediator cell cluster, connecting different RG progenitor cell types. NC-based cell clustering classified mature neurons into 5 classes (excitatory or inhibitory) and ascertained an intermediate-progenitor-like cell to be the start point of the neural lineage post NGP. This comprehensive adult killifish-specific dataset can now pave the way for detailed investigations on neuro-regenerative medicine and design of novel therapies against neurodegenerative diseases.

(ORAL PRESENTATION)

Aging is associated with a degeneration of locus coeruleus neurons, but not dopaminergic neurons, in the short-lived killifish *Nothobranchius furzeri*

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Parkinson's disease (PD) is one of the most common forms of age-related neurodegenerative diseases. It is characterized by phosphorylation and aggregation of the protein α-Synuclein and ensuing neuronal death progressing from the noradrenergic locus coeruleus to midbrain dopaminergic neurons of the substantia nigra, generally identified by a reduction of Tyrosine Hydroxylase staining. Genetically modified rodents are commonly used as models of the disease. However, the most common form of PD is not caused by genetic mutations and a spontaneous model with late onset would provide a better model for the human disease. In 2019, Matsui and colleagues reported in Cell Reports a spontaneous age-dependent degeneration of dopaminergic neurons located in the hypothalamic posterior tuberculum (teleost homolog to the mammalian substantia nigra) and an even greater neurodegeneration of the noradrenergic neurons in the locus coeruleus in the short-lived killifish *Nothobranchius furzeri*, closely resembling the degeneration pattern typical of PD. This degeneration was moreover prevented by ablation of α-Synuclein. Given the great possible relevance of a spontaneous model for PD, we set to confirm their results by whole-brain clarification to enable 3D nuclei reconstruction and quantification of total cell numbers between young and old animals. We observed an age dependent neurodegeneration limited to the locus coeruleus nuclei and not involving the posterior tuberculum, and we were also not able to detect a reduction of global Tyrosine Hydroxylase expression not at protein nor at transcript level. In addition, we observed the presence of phospho-Synuclein staining in locus coeruleus cell bodies detectable already at a young age and increasing during ageing. We thus propose *N. furzeri* as an idiopathic model of early stages of PD but not of later stages involving the degeneration of dopaminergic neurons.

(ORAL PRESENTATION)

The killifish visual system: an *in vivo* model to investigate age-related phenomena and rejuvenating strategies in the central nervous system

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The increased life expectancy in modern society is driving a tremendous increment in the number of elderly, and in consequence, the prevalence of age-related neurodegenerative disorders, such as Alzheimer's and Parkinson's disease, or glaucoma. Unfortunately, healthy brain aging remains challenging despite intensive research efforts trying to disclose aging processes and more specifically their involvement in functional decline, whether or not caused by pathological neurodegeneration. One hallmark of aging, which is often correlated with neurodegenerative manifestations, is visual decline. As vision is described as the most valuable sense among people and age-related functional decline within the visual pathway severely impacts life quality, we performed a comprehensive characterization of the aged killifish visual system, comprising of the retina, optic projections and optic tectum. Immunohistological (IHC), biochemical and molecular analyses revealed that several aging hallmarks manifest within the aged killifish visual system, eventually resulting in a reduced visual functionality. Besides an increase in senescence-associated markers detected via histological staining for senescence-associated beta-galactosidase and qPCR for p21 and p27, a declined neurogenic potential was detected via IHC staining for proliferating cells, as well as stem cells. Next to the appearance of reactive gliosis and inflammaging, which manifests in an increased number of microglia and an altered cytokine profile, we found evidence for the occurrence of neurodegenerative events in the old killifish retina. More specifically, we demonstrated a decline in the number of dopaminergic amacrine cells in the retina, similar as reported in Parkinson's disease patients. Our findings launch the visual system of the fast-aging killifish as a valuable model for target validation and drug discovery of rejuvenating or neuroprotective therapies for healthy brain aging.

(ORAL PRESENTATION)

Body coloration, aggression and learning in *Nothobranchius guentheri*

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Interactions between *Nothobranchius* males are generally aggressive and establish linear hierarchy in captivity. Pace-of-life syndrome hypothesis predicted that more aggressive and bolder individuals would be faster explorers of new environment. We predicted that more aggressive individuals would be more exploratory in novel environment. Color patterns are important as honest signals of individual quality and status. In the process of agonistic interactions, color patterns are commonly used to display threat. We have studied a correlation between aggression, color intensity and body size in males of *Nothobranchius guentheri*. The difference in behavioural type between individuals can drive the difference in learning ability. We hypothesize that the more aggressive and bold individuals would learn faster, but less flexible in reversal learning. The experiments were carried out on males of the laboratory line *N. guentheri*. Aggression was studied in mirror test. Boldness was calculated as time of freezing after weight drop into water. Exploration was studied in a test with the exit of fish from shelter to open field. Learning ability was studied in colour discrimination test. The criterion for learning were considered 5 consecutive or more than 70% correct choices. After reaching the criterion reversal learning test was performed. After the tests the fish were photographed under anesthesia and the colour intensity and body size were measured. Aggression and boldness were repeatable among individuals and significantly correlated with colour intensity. More aggressive fish were larger and darker than less aggressive. We didn't find correlation between exploratory behaviour and aggression. Our hypothesis of correlation between aggression and learning ability was also not confirmed. In conclusion, more aggressive males of *N. guentheri* were darker and bigger, but not more explorative or smarter than less aggressive.

(ORAL PRESENTATION)

In vitro fertilization, blood extraction from live animals and in-tank fish tracking in *N. furzeri*

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Over the last decade, the short-lived killifish *Nothobranchius furzeri* has emerged as an excellent model organism for the study of vertebrate physiology and aging. Several unique physiological features make *N. furzeri* an attractive system, including diapause, rapid growth, and short lifespan, which have led to its progressive spread worldwide. Though many protocols and techniques have been established in this model, the knowledge gap between *N. furzeri* and other fish models, however, still remains. To further promote the widespread of *N. furzeri*, we developed three general techniques that could aid in a large variety of studies. First, we developed a protocol for *in vitro* fertilization. We optimized several parameters to preserve sperm in liquid nitrogen for months, and potentially years. Upon revival, the sperm are able to fertilize eggs with a success rate of 20-40%, and generate viable and fertile fish. This technique is very useful for long term preservation of specific genotypes or transgenic lines, and limits the risks of strain loss or inbreeding. Second, we worked out a technique of blood extraction from a living fish. This slightly invasive procedure allows the removal of 1-7 µl of blood from the live animal, with little damage to the tissues. Blood can be used for molecular and histological analyses or for the evaluation of parameters such as glycemic index or triglyceride levels. Importantly, repeated extractions over time can be done on the same fish, allowing longitudinal studies. Third, we established live tracking of single fish directly inside the tank where they are housed. The setup is inexpensive, adaptable, and scalable for any number of tanks. With the proper software, tracking data can be readily analysed. In preliminary experiments, fish showed a rough increase of activity up to 14 weeks, followed afterwards by progressive decline.

(ORAL PRESENTATION)

Identification of protein aggregates with prion-like and phase separation properties in the aging vertebrate brain

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Protein aggregation is a hallmark of neurodegenerative diseases, and these aggregates can sometimes spread in a prion-like manner. But whether protein aggregates form in the brain during normal aging, and whether such aggregates have prion-like seeding properties, remains unknown. Here we use quantitative proteomics in the African killifish to identify protein aggregates that accumulate in the old vertebrate brain. Interestingly, proteins that aggregate during brain aging are enriched for prion-like domains and include RNA binding proteins, including DDX5 – a RNA helicase. We validate that DDX5 forms cytoplasmic aggregates in the brains of old killifish and aged mice. Surprisingly, DDX5 aggregates act as bona fide prions that propagate across many generations in yeast. DDX5 aggregate seeding occurs in a protein-autonomous manner *in vitro*, with phase-separation into a droplet-like structure that over time become solid. Mutations that affect DDX5 prion-like properties in cells also impair the ability of DDX5 to phase-separate. Thus, protein aggregates with prion-like properties can form during normal aging, and their phase-separation properties could contribute to the age-dependency of cognitive decline.

(ORAL PRESENTATION)

Behind the scenes of successful aging research

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Nothobranchius has become a standard model organism in aging research. Housing and successful breeding still can be very challenging. Besides a good breeding plan also an efficient health monitoring system is essential for providing healthy fish to the researchers. During the last years we, at the FLI have developed a high quality health monitoring strategy, including broad regular health monitoring and Score Sheets for daily health checks. An up to date recording of relevant clinical findings allows us to keep an overview about possible hygiene outbreaks and to react fast. Especially, mycobacteria and microsporidian species seem to have a significant impact on *Nothobranchius* health. For this reason effective hygiene and disinfection plans are essential to keep the pathogen load on a low level. As one of the largest *Nothobranchius* units with a capacity of over 5000 fish we want to share our knowledge also with other facility managers and researchers in the field, to turn the attention behind the scene of successful aging research.

(ORAL PRESENTATION)

Dissecting aging piece by piece: how proteasome inhibition contributes to the onset of brain aging phenotypes in the turquoise killifish

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Aging and neurodegenerative diseases share key molecular hallmarks, including proteasomal and mitochondrial dysfunction. However, the causal relationship between these events remains elusive. Recent works from our group and others demonstrated that reduced activity of the proteasome, an essential protein degradation machinery, is an early event during brain aging. Here, we aim to dissect the molecular events triggered by proteasome inhibition *in vivo* by taking advantage of the short-lived turquoise killifish model. We performed intraperitoneal injections of bortezomib, a specific proteasome inhibitor, once a week for a month in adult animals. We confirmed a partial reduction of proteasome activity (approx. 50%) in the brains of treated fish. Next, we applied unbiased proteome and transcriptome analysis to interrogate whether bortezomib treatment can induce known brain aging signatures. We found a significant overlap of proteins affected in these conditions, corroborating a causal link between proteasome inhibition and the disruption of age-affected molecular networks. This integrated analysis allowed us to pinpoint specific responses of the proteostasis network and identify a subset of proteins whose abundance depends on proteasome activity. Specifically, we found that proteasome inhibition impacts mitochondria homeostasis. We observed a global decrease in mitochondrial proteins, and like brain aging, bortezomib led to a reduced ratio of mtDNA / genomic DNA. While we did not find evidence for reduced mitochondria biogenesis, bortezomib affected the expression levels of master regulators of mitochondria fusion and fission, suggesting altered dynamics. These data suggest that mitochondria dysfunction might emerge during brain aging due to reduced proteasome activity. We believe that understanding this type of causal relationship between age-related impairments can reveal therapeutical targets to sustain neuronal survival throughout a lifetime.

(ORAL PRESENTATION)

***Nothobranchius furzeri* as an emerging model for mate choice: female choice revealed by animations**

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The African turquoise killifish (*Nothobranchius furzeri*) is the shortest-lived vertebrate research model. It is also sexually dimorphic, making it suitable for studying sexual selection. We take advantage of a natural tail colour polymorphism in males and investigate female responses to computer animations of males that differ in this phenotype. Our findings indicate that GRZ (Gonarezhou) females prefer animated males with traits specific to their strain (a yellow tail with a black band) compared to males exhibiting traits from another strain of the same species (a red tail). When females were simultaneously shown animations of both males, they spent significantly more time on the side of the tank where the yellow-tailed animation was visible, and significantly more time interacting with the yellow-tailed animation. Given these repeatable responses and the availability of genomic resources, *N. furzeri* represents an excellent, untapped model for studying the genetic basis of preferences and reproductive behaviours.

(ORAL PRESENTATION)

Generating a transparent vertebrate model for *in vivo* applications in aging research

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Body pigmentation is a major limitation for *in vivo* imaging. A possibility to circumvent this obstacle is the use of e.g. zebrafish larvae or adult fish from pigmentation mutants. Zebrafish is widely used to study development, genetics and regeneration, however, its life expectancy of up to five years does not make it a convenient model for aging research. To study aging and aging-related diseases, the turquoise killifish *Nothobranchius furzeri* has been established as a suitable model organism. *N. furzeri* possesses different pigment cell types, namely melanophores, iridophores and xanthophores, preventing live imaging of inner organs. We successfully employed CRISPR/Cas9-mediated inactivation of key genes involved in pigmentation in order to generate a transparent *N. furzeri* line. We induced the simultaneous mutation of three target genes by a single microinjection of different single-guide RNAs into *N. furzeri* embryos. In fish of the F0 generation, we observed a mosaic loss of body pigmentation. Additionally, some animals already displayed an almost complete loss of pigmentation, suggesting very efficient Cas9 activity. Fish homozygous for mutations in all three genes have a transparent phenotype, allowing a view on inner organs. Despite the lack of body pigmentation, transparent *N. furzeri* show normal breeding behaviour and produce viable offspring. The transparent *N. furzeri* line, which we named klara, can serve as a tool for various analyses. At present, we use this line for the *in vivo* analysis of senescence. For this purpose, we integrated a reporter construct via a CRISPR/Cas9-mediated knock-in into the p21 locus of klara. With this construct, senescent cells can be labelled and in addition also be ablated via the NTR/Mtz system to further elucidate the effect of senescent cells on life- and healthspan.

(ORAL PRESENTATION)

Health span effects of metformin are potentially sex specific

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Metformin is among the most frequently prescribed drugs and a first-line medication to treat type 2 diabetes. Clinical trials are underway to test metformin in old humans as a longevity-extending drug. Previously, we found that metformin fails to prolong the lifespan of old *C. elegans*, contrary to its effect in young animals. We linked this difference to exacerbation of aging-associated mitochondrial dysfunction by metformin and failure of metabolic adaptation in old cells (Espada et al., 2019). Currently, we are testing if age-specific metformin effects can be seen *in vivo* in higher metazoans by using *N. furzeri* as a model. By liver proteomics analysis of metformin-exposed young fish, we observed that the same molecular responses (changes in peroxisome content, lipid turnover responses, and ribosomal content) are triggered by metformin in young fish as in young worms, demonstrating high conservation of the metformin longevity response. Interestingly, the molecular effects of metformin appeared to be different in young males and females, and this difference was exacerbated during aging. Gene ontology overrepresentation analysis revealed strong differences between the sexes in the induction of pathways implicated in lipid turnover and carbohydrate metabolism, which appear to play a pivotal role in organismal adaptations to metformin. We are currently performing brain proteomics to investigate sex- and age-specific responses to metformin in this organ as well. We also test if the observed sex-specific proteome shifts correspond to distinct histological and cellular changes in the brain and liver of metformin-exposed fish. In parallel, we use C. elegans genetics and reporter tests to investigate and validate the putative conserved mechanism of sex-specific metformin effects.

(ORAL PRESENTATION)

RNA-seq analysis of aging in *Nothobranchius furzeri*: effects of genetic background and captive vs. wild environment

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Age-dependent regulation of gene expression in *Nothobranchius furzeri* has been thoroughly characterized (Baumgart, 2016). Yet, the effects of genetic background and environmental variables remain unknown. On the one hand, *N. furzeri* is characterized by the presence of multiple genetically-differentiated geographic strains, which originate from different areas of Mozambique and Zimbabwe and show large heritable differences in captive lifespan (Terzibasi et al., 2008), on the other hand, *N. furzeri* populations kept in captivity showed differences in growth rate and demography from natural populations monitored in the wild (Vrtilek et al., 2018). Taking advantage of a RNA-seq dataset with a complex experimental design comprising 4 tissues, up to 5 time points, 3 different strains (two captives and one wild) and 5 biological replicates per time point we compared age-dependent gene expression in: - two captive strains of *N. furzeri* (MZM-0410 and GRZ, Chefu lineage) that differ greatly in their lifespan; - the two captive strains mentioned above with a wild *N. furzeri* population (Chefu lineage). We observed a gene expression signature that is common across tissues and separates the two strains already from the embryonic (mid-somitogenesis) stage. We also noticed an "anticipated aging" of GRZ transcriptome, i.e. young GRZ samples showed a transcriptomic signature typical of older MZM-0410. Finally, we observed both a common signature between captive and wild aging and a specific signature of aging in the wild. In addition, wild animals show an "accelerated aging" feature in that the rate of change in gene expression is higher.

(ORAL PRESENTATION)

Mechanisms of aging-induced decline in wound-healing

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Aging results in decline in wound-healing in wide range of species, including humans. Although this change is natural, it generates large costs for society and significant burden to elderly patients. The underlying mechanisms of aging-related decline in wound-healing are, however, poorly understood and hence effective treatments are lacking. Traditional biomedical research models, such as rodents, are poorly suited for elucidation the underlying mechanisms due to their relatively long lifespan. Therefore, we utilize short-lived *Nothobranchius furzeri* as a model to identify mechanisms underlying poor wound-healing in aged individuals. We aim to identify potentially deleterious alterations in wound-healing in aged individuals and to confirm these functionally by using pharmacological tools. Although our study is still underway, we have already made some novel observations on mechanisms underlying aging-induced decline in wound-healing. The ultimate goal is to confirm these findings in human samples and find pharmacological agents, which would enhance wound-healing in aged individuals.

(ORAL PRESENTATION)

Natural course of embryo development in the wild populations of African *Nothobranchius* and American *Austrolebias* species

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Embryonic development of annual killifish has been thoroughly studied under laboratory conditions. Data on the natural course of development were missing, their availability hampered mainly by the lack of a practical egg sampling method. Embryos of annual killifish survive for months encased in dry pool substrate. They face drastic seasonal changes in environmental conditions. The development in their natural habitats presents a fundamental baseline for interpretation of laboratory research merging developmental, ecological and evolutionary perspectives. We recently developed a method for embryo collection in the field and sampled egg banks of the African *Nothobranchius* spp. in Mozambique and South American *Austrolebias* spp. in Uruguay. The sampling campaigns included repeated sampling of egg banks over the natural seasonal cycle. Additionally, we performed field experiments to test selected hypotheses on the killifish embryo development. Overall, we found a high degree developmental synchrony within populations and robust evidence for extrinsic control over the embryonic development in both the African and American groups. Our findings from the field offer a new perspective on the role of intrinsic (bet-hedging) component in the embryonic development of annual killifish, frequently recorded in laboratory-based studies. Apparently, the natural environment provides annual killifish embryos with cues reliable enough (or developmental constraints) to result in a canalization of their development across an egg bank.

(ORAL PRESENTATION)

Beyond gdf6Y – elucidating the determination and development of sex in the annual turquoise killifish *Nothobranchius furzeri*

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Nothobranchius furzeri lives in seasonal freshwater ponds. To survive in the limited environment, the species evolved remarkable traits. Besides an optionally arrested embryonic development, rapid growth and early sexual maturation, *N. furzeri* possesses an XX/XY sex determination system, which ensures an equal distribution of both sexes. Sequence comparisons of the sex-determining regions of different *N. furzeri* strains revealed the TGF-β family member Gdf6 as a candidate for the master sex determinant in this species. The CRISPR/Cas9-mediated knockout of the Y-chromosomal gdf6 copy (gdf6Y) leads to complete male to female sex reversal in XY animals. Among the offspring of those phenofemales, embryos with two Y-chromosomes were found. Those did not hatch and showed malformations, similar to GDF6 mutants in other species. Interestingly, the ubiquitous expression of gdf6Y leads to a detrimental phenotype as well, suggesting an evolutionary divergence of gdf6 and gdf6Y with still partial overlapping functions. In this regard, we currently investigate the functional differences of gdf6 and gdf6Y as well as potential causes for those differences considering both regulatory and functional aspects. To analyse the role of gdf6Y in sex determination in *N. furzeri*, its spatiotemporal expression in testes was analysed using RNAscope. Furthermore, we analysed RNA-Seq data of males and females at different stages to shed further light onto the timing of sexual development in the turquoise killifish. Currently, we investigate potential downstream genes of gdf6Y, which were identified upon RNA-Seq. Therefore, we use the previously established gdf6Y knockout line, RNAscope and CRISPR/Cas9-mediated knockouts of those genes.

(ORAL PRESENTATION)

Sustained AMPK-γ1 complex activity prevents age-related metabolic disorders and promotes longevity in killifish

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During aging, adipose tissue undergoes dramatic changes in mass, distribution, cellular composition, secretory profiles leading to multiorgan dysfunctions, type II diabetes, and cardiovascular diseases. Understanding how age impacts adipocytes function could help to prevent age-related metabolic disorders, improve life quality, and extend lifespan. Here, we used turquoise killifish as a model to explore the underlying mechanism of aging in the adipose tissue. We show that killifish recapitulate many aspects of human adipose tissue aging. Transcriptome analyses indicate that aging drives a program of chronic fasting response (CFR) resulting in a persistent downregulation of numerous metabolic pathways, such as glycolysis, TCA cycle, oxidative phosphorylation, and ribosome biogenesis. This associates with an uncontrolled release of free fatty acids (FFAs) into the bloodstream that ultimately accumulate in non-adipose tissues. Mechanistically, CFR represses the expression of PRKAG1, the regulatory subunit Y1 of the AMP-activated protein kinase (AMPK) complex. We demonstrate that fish lacking the AMPK-γ1 subunit exhibit uncontrolled release of FFAs into the plasma, ectopic lipid accumulation, and signs of insulin resistance early in life. Conversely, sustained AMPK-γ1 subunit expression through adulthood is sufficient to significantly rescue the age-associated CFR, preserve lipid and glucose homeostasis, and extend lifespan. Overall, these data reveal that the specific promotion of the activity of AMPK-γ1 complex counters the metabolic impairment induced by aging and increase lifespan in vertebrate.

(ORAL PRESENTATION)

Anatomy and tissue homeostasis of the gut tube of the turquoise killifish

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We studied the morphology of the gut tube and the regenerative potential of intestinal stem cells throughout the life span of *N. furzeri*. The adult gut tube is devoid of a distinct stomach and runs as a straight expandable tube form the oesophagus towards the left ventral side of the body cavity where the common bile duct enters. Subsequently, the gut forms a loop bending towards the right side followed by an upward rotation showing considerable individual variability at this position. From the dorsal midline the gut tube runs in a straight line towards the anus. The colon can clearly be discriminated form the small intestine by a conspicuous incision which is accompanied by smooth muscle of the *Tunica muscularis* suggesting a valve function at this intestinal junction. The gut represents as straight tube in freshly hatched fish and develops the adult anatomy until week 3. In later age steps there is a tendency for reduction of the loop, in particular of the forward bend. Yet, no significant reduction in gut length and weight occurs relative to total fish in later life. The mucosal lining is characterized by crescent-shaped folds which become more complex towards the posterior parts due to formation of sub-folds leading to a branched appearance. Typical markers of intestinal functions show a rather homogenous expression throughout the length of the small intestine with no distinct functional zonation. We used double pulse labelling with nucleotide precursors to identify intestinal stem cells. The majority of stem cells were identified at the basal parts of the small intestine located between folds and are present in stem cell clusters. Throughout life those clusters become reduced in stem cell numbers and the clusters lie further apart. However, we did not observe reduced division potential of stem cells in aged fish. Post-mortem fish show intact intestinal lining suggesting that intestinal stem cell division potential is not rate limiting for *N. furzeri* life span.

(ORAL PRESENTATION)

Cytogenomics of *Nothobranchius furzeri* and *N. kadleci*: sex chromosomes and repetitive DNA dynamics

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African annual killifishes of the genus *Nothobranchius* (Teleostei: Nothobranchiidae) display unique adaptations to ephemeral water pools in African savannahs and available cytogenetic and genomic data suggest fast rate of karyotype and sex chromosome evolution in their small allopatric populations. Here, we analysed the repetitive DNA landscape and sex chromosome differentiation in turquoise killifish, *N. furzeri* (three populations including GRZ strain) and its sister species *N. kadleci* (two populations). Besides a combination of standard and molecular cytogenetic (i.e. fluorescence in situ hybridization-based) protocols and immunostaining of synaptonemal complexes, we mapped sex chromosome-specific bacterial artificial chromosome (BAC) clones from *N. furzeri* genome library and performed low-coverage genome sequencing with subsequent RepeatExplorer analysis to identify abundant repetitive DNA sequences in genomes of both species and to map their chromosomal distribution. We revealed shared XY sex chromosome system varying in degree of differentiation among populations of both species. Combination of XY-specific hybridization patterns allowed us to propose sequence of events leading to stepwise XY differentiation. RepeatExplorer uncovered most abundant repeats to be shared by both species and we mapped three of them chromosomally. By complementing data from earlier study, we showed that two repeats are centromere-specific and the third one is scattered throughout the genomes but remarkably accumulated on Y chromosome. Subsequent mapping to chromosomes of other *Nothobranchius* species showed, besides various types of polymorphisms, fast turnover of two centromeric repeats but the conserved presence of the third repeat, varying in abundance and distribution among all *Nothobranchius* genomes under study. Our data collectively imply important contribution of repetitive DNA to sex chromosome evolution, karyotype dynamics and species divergence in *Nothobranchius* killifishes.

(ORAL PRESENTATION)

A core circadian clock network in the turquoise killifish

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Circadian rhythm is altered during aging, although the underlying molecular mechanisms remain largely unknown. Here, we used the turquoise killifish as a short-lived vertebrate model to examine the effects of aging on the major circadian network comprising the four mammalian clock protein homologs, Bmal1, Clockb, Cry1b, and Per3, which are highly conserved in the killifish with 50%–85% amino acid sequence identity to their human counterparts. The amplitude of circadian rhythm was smaller in old fish (14 weeks) than in young fish (6 weeks). In old fish brain, the Bmal1 protein level was significantly downregulated. However, the Bmal1 interaction with Clockb and chromatin binding of Bmal1 to its downstream target promoters were retained. Furthermore, Bmal1 was relatively well maintained in the pineal gland compared with other regions of the old fish brain. The results suggest that the circadian clock system in the killifish becomes spatially confined to the pineal gland upon aging.

(ORAL PRESENTATION)

The genome of the bi-annual Rio Pearlfish (*Nematolebias whitei*) informs the genetic regulation of diapause and environmentally-cued hatching in extreme environments

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We sequenced a chromosome level genome of the bi-annual Rio Pearlfish, *Nematolebias whitei*. Rio Pearlfish are native to seasonal pools in the coastal plains near Rio de Janeiro, Brazil, where they complete two life cycles per year. Our model species represents an independent origin of annualism, different from other sequenced killifish models like *Nothobranchius* and *Austrofundulus* making this species a powerful research organism to study convergent changes associated with dormancy and senescence in vertebrates. When the habitat floods, annual killifishes terminate their third diapause (DIII), hatch, and begin a new lifecycle. In Pearlfish, DIII is also tightly linked with expression of a complex family of hatching enzymes (HEs), and our analysis of killifish HEs, including those of Pearlfish, reveals a complex evolutionary history of HE genes. While HEs have been identified in many animal species, much less is known about how hatching is regulated from a developmental or genetic standpoint or how environmental cues are integrated with gene regulatory networks to control this fundamental developmental timepoint. The Rio Pearlfish possesses an expanded repertoire of HE genes compared to other killifish species and offers an opportunity to examine the genetic developmental basis for hatching control in light of gene duplication and environmental signals. We also identify the location of the hatching gland in this species, a critical first step in understanding hatching control in killifishes. Moving forward, studies of Rio Pearlfish will build on the resources of other killifish genomes to illuminate the mechanisms of the convergent evolution of rapid senescence and developmental phenotypes in extreme environments as well as the diversity of vertebrate hatching strategies. Additionally, we use the Rio Pearlfish to create Killi-Kits, an educational outreach toolkit for Eco-Evo-Devo.

Turquoise killifish on antidepressants: towards understanding the ecological risks of neurochemical pollution

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Ongoing pollution of aquatic ecosystems with neurochemical compounds warrants an improved understanding of its ecological consequences. Neurochemicals typically alter wildlife behaviour, yet most studies to date only assessed such effects after short-term exposure in a limited timeframe of the organism's lifetime. The recent establishment of *Nothobranchius furzeri* as a fish model for ecotoxicological studies allows to study how chronic exposure to antidepressants affects life-history and behaviour in fish. Through several studies, we showed that exposure to low doses of fluoxetine, the active ingredient of Prozac, impairs feeding behaviour, locomotor activity, risk-taking behaviour and growth in *N. furzeri*. Interestingly, fish also got more social and attempted to mate more often, which doubled their egg production compared to non-exposed fish. We furthermore showed that natural daily patterns in fish behaviour may confound the results of ecotoxicological testing and even disappear upon exposure to antidepressants. Because such effects could have far-reaching fitness consequences for fish around the globe, standardised toxicity methods with *N. furzeri* are currently developed based on an improved understanding of its behavioural baseline to further assess the ecological risks of neurochemical pollution.

(ORAL PRESENTATION)

Plasticities within and across generations in *Austrolebias* annual killifish

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Results are presented from an experiment where developing embryos of the Argentinian pearl killifish *Austrolebias bellottii* underwent three crossed environmental treatments. Embryos were incubated in water that had either housed fish or not. They were subjected to different temperatures during incubation. In addition, the eggs were laid by parents that either experienced short- or long daylength regimes before and at egg laying. Effects of the three environmental treatments on stage-specific survival and developmental rates are tested and compared. We found an acceleration of all types of events with temperature. A constant diversified bet-hedging distribution of diapause stages was not observed across all temperatures. There were some effects of parental light regime and signals of adults in the water on survival, and a deceleration of late development in the parental short day light regime. This last finding is contrary to the idea that the timings of annual killifish development are adaptations to hatch a second time within a wet season. It is consistent with the idea that fish multigenerational reaction norms are adapted to the normal annual seasonal cycle.

(ORAL PRESENTATION)

Improving neurorepair in the aged brain, what the killifish pallium can tell

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The aging brain displays progressive limited regenerative abilities. Recovery after neuron loss is extremely restricted. Many research models fall short in recapitulating mammalian aging hallmarks or have an impractically long lifespan. We established a brain injury model in the killifish (*N. furzeri*), a regeneration-competent vertebrate that ages extremely fast. Stab-wound injury of the aged killifish pallium unveiled an impaired and incomplete regeneration response when compared to young individuals. We observed glial scarring in the aged pallium, while in young fish, brain repair occurred in a seamless manner. The injury elicited reactive proliferation of the ventricular progenitor cells. This was however not supported by the radial glia, but by the non-glial progenitors (NGPs). Proliferation of the NGPs was significantly lower in aged killifish, resulting in less newborn neurons and thus reduced repair. While the NGPs became exhausted with age, the radial glia became more proliferative, suggesting that aged fish use this as a strategy to replenish the existing stem cell pool. ScRNA seq confirmed that most radial glia in the adult killifish telencephalon are quiescent, yet become more proliferative with old age. In addition, we discovered that the killifish telencephalon harbors four different types of radial glia, representing a progenitor subtype, ependymoglia and two types of astroglia. Differential proteomics uncovered a high senescent cell burden in the aged killifish telencephalon. We hypothesized that the high abundance of senescent cells creates a non-permissive environment for neurorepair. We tested the impact of the removal of the senescent cells via senolytic drugs on the neuroregeneration success. First preliminary results show that this strategy improves neurorepair in the aged pallium. Summarized, our work reveals the power of the aged killifish brain to create new knowledge towards novel therapies for age-related neurodegenerative disease.

(ORAL PRESENTATION)

Enhancers and the uneven distribution of regenerative capacities in vertebrates

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Species such as bony fishes display extensive regenerative capacities, while others such as mammals regenerate poorly. The mechanisms underlying the broad disparity of regenerative capacities in animals remains elusive. Here we report on a comparative epigenomic and transcriptomic approach which identified an evolutionarily conserved regeneration response program (RRP) in vertebrates. By defining the cis-regulomes and transcriptomes of early stages of regeneration in the distantly related zebrafish *Danio rerio* and the African killifish *Nothobranchius furzeri*, we were able to discriminate between species-specific and evolutionarily conserved genomic responses to amputation. Importantly, functional testing by systematic transgenic reporter assays of the conserved inhibin beta A (inhba) regeneration responsive enhancer (RRE) from zebrafish, killifish, and humans identified species-specific variations. Furthermore, deletion of the killifish inhba RRE significantly perturbed caudal fin regeneration and completely abrogated cardiac regeneration. We also show that inhba RRE activity requires the presence of predicted binding motifs for the Activator Protein 1 (AP-1) complex. Interestingly, AP-1 binding motifs can be identified in the conserved and non-conserved teleost RREs reported in this study, indicating that AP-1 may be required for both injury and regeneration responses. We propose that changes in RREs driven by natural selection are likely a crucial source of loss of regenerative capacities in vertebrates, including humans.

(ORAL PRESENTATION)

Analysis of methylation dynamics reveals a tissue-specific, age-dependent decline in 5 methylcytosine within the genome of the vertebrate aging model *Nothobranchius furzeri*

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Erosion of the epigenetic DNA methylation landscape is a widely recognized hallmark of aging. Recent progress in epigenomics has led to the development of prediction systems that enable accurate age estimation from high-throughput DNA methylation data in humans and mice. In vertebrates, methylation of cytosine at the C5 position of CpG dinucleotides is executed by DNA methyltransferases (DNMTs) while the process of active demethylation is highly dependent on the activity of the ten-eleven translocation methylcytosine dioxygenase (TET) family of enzymes. Here, we report the identification of the key players constituting the DNA methylation machinery in the short-lived teleost aging model *Nothobranchius furzeri* and provide a detailed spatio-temporal expression profile of the methylation-associated enzymes from the onset of embryogenesis to late adulthood, mapping the entire killifish life cycle. Data mining of the published *N. furzeri* genome produced five dnmt gene family orthologues corresponding to the mammalian DNMTs (DNMT1, 2, 3A and 3B). A related search for the DNMT1 recruitment factor UHRF1 and TET family members resulted in the identification of *N. furzeri* uhrf1, tet1, tet2 and tet3. Phylogenetic analysis revealed high cross-species similarity on the amino acid level of all individual dnmts, tets and uhrf1, emphasizing a high degree of functional conservation. In adult *N. furzeri*, DNA methylation regulating enzymes showed a ubiquitous tissue distribution. Specifically, we observed a significant age-dependent downregulation of dnmts, and to some extent uhrf1, which strongly correlated with a significant decrease of global DNA methylation levels in the aging killifish liver and muscle. The age-dependent DNA methylation profile and spatio-temporal expression characteristics of the DNA methylation machinery reported here may serve as a useful starting point for the development of an epigenetic aging clock in the new vertebrate model system *N. furzeri*.

(ORAL PRESENTATION)

Are bloodworms optimal feed for laboratory *Nothobranchius furzeri*?

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The diet of laboratory fish is one of the major environmental variables which should be controlled in experimental studies. The most frequently provided diet to the laboratory *Nothobranchius furzeri* are chironomid larvae – the live feed with variable nutritional content, availability and feed associated with risk of disease introduction. On the other hand, bloodworms provide satisfactory growth and reproductive function. Recently, a few dry feeds were applied in *Nothobranchius* husbandry with comparable performance to fish fed by bloodworms. The good growth and reproduction in captivity diet does not necessarily mean healthy fish. Comparisons with wild counterparts are valuable for assessment of healthy performance. Here we present the most recent results of fish performance fed by bloodworms and practical dry feeds and these results are contrasted to available parameters from wild fish. Our results show that several somatic parameters are biased in the bloodworm group in comparison to wild fish and this is not the case in some dry feed groups. The un-natural somatic performance of fish fed by bloodworms should be taken into account when laboratory results are interpreted. On the other hand, the vigorous acceptance of dry feeds by *N. furzeri* is promising for future development and use of the standardized laboratory diet.

(ORAL PRESENTATION)