UNIVERSITÀ DEL SALENTO

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PALERMOCITTÀUNIVERSITARIA

UNIVERSITÀ DEGLI STUDI DI NAPOLI FEDERICO II

Giornate studio sull'impiego dei Modelli Acquatici a fini scientifici
Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise 'G.Caporale'

IZSAM G.CAPORALE TERAMO

AISAL

15-16 ottobre 2018

ZEBRAFISH E ALTRI PESCI TELEOSTEI NELLA RICERCA BIOMEDICA

STAZIONE ZOOLOGICA ANTON DOHRN Napoli

Dipartimento Medicina Veterinaria Produzioni Animali

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UNIVERSITÀ DEGLI STUDI DI NAPOLI FEDERICO II

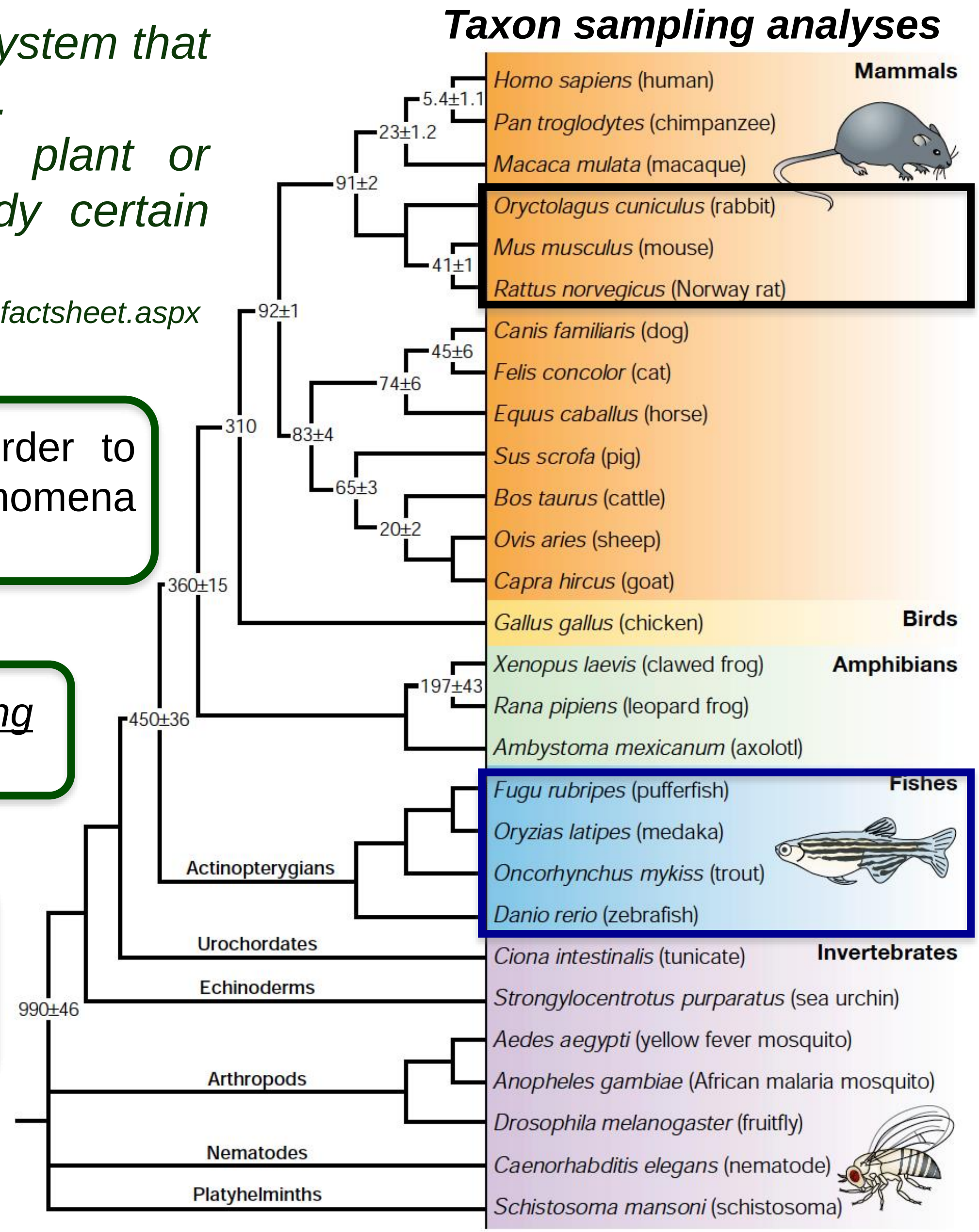
Model organisms : centerpieces of biomedical research

"In science, a model is a simplified system that is accessible and easily manipulated.
A model organism is an animal, plant or microbe that can be used to study certain biological processes."
https://www.nigms.nih.gov/Education/Pages/modelorg_factsheet.aspx

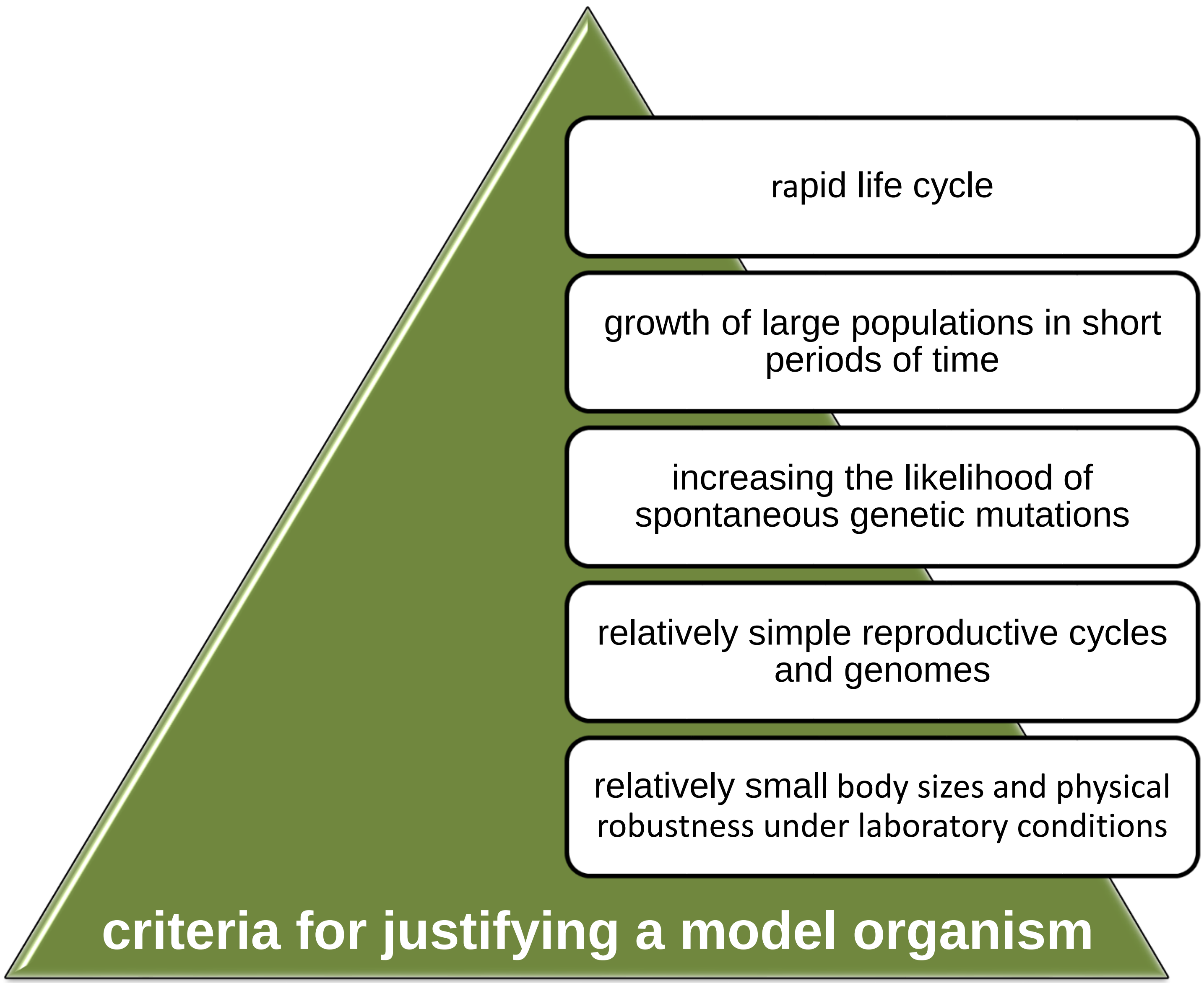
Developed as resource materials in order to study particular biological phenomena exhaustively or in great detail.

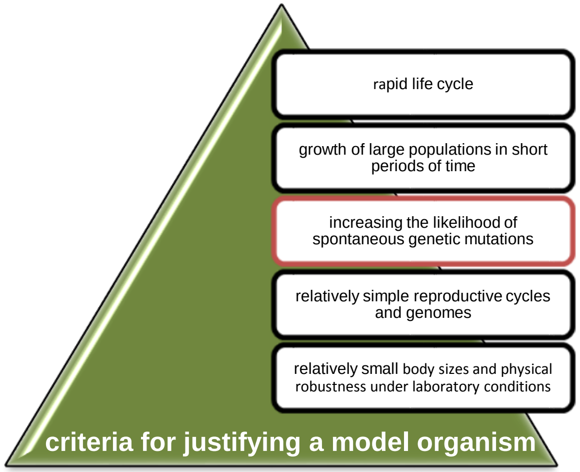
Translability to other organisms, *including humans*.

Contemporary model organisms tend to be species (or, more precisely, strains of these species) that are relatively simple and experimentally tractable.



Hedges, Nature reviews (2002)


rapid life cycle
growth of large populations in short periods of time
increasing the likelihood of spontaneous genetic mutations
relatively simple reproductive cycles and genomes
relatively small body sizes and physical robustness under laboratory conditions
criteria for justifying a model organism



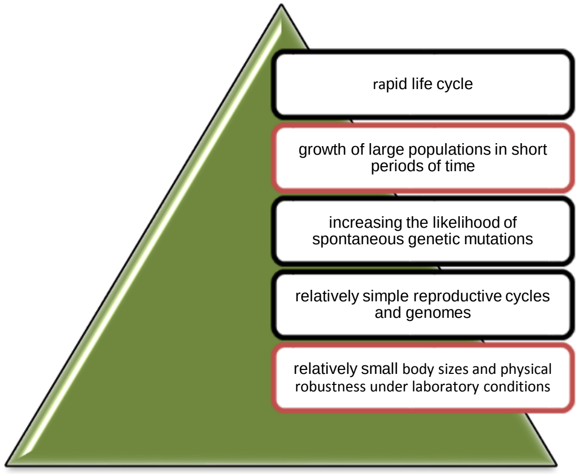
zebrafish as research model

1. Genetic similarity to humans

Zebrafish are vertebrates and therefore share a high degree of sequence and functional homology with mammals, including humans.

2. Impact of any genetic mutation or drug treatment is easy to see

Zebrafish embryos and larvae are completely transparent, meaning that it is possible to follow the impact of a genetic manipulation or pharmacological treatment using non-invasive imaging techniques. Less intrusive techniques minimise animal suffering.



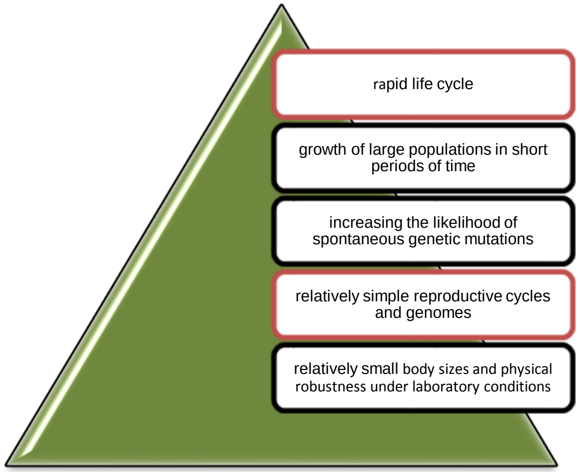
zebrafish as research model

3. Easier and cheaper to house and care for than rodents

Due to their small size and the relatively simple nature of their natural environment, it is easier to keep zebrafish in what appear to be more natural conditions than it is possible to simulate for mammals. This minimises housing stress and the impact such stress may have on the outcome of experiments.

4. Lots of offspring

Zebrafish have a much larger number of offspring in each generation than rodents. Rodents have 5-10 offspring per pairing, in comparison to the 200-300 obtained from fish.

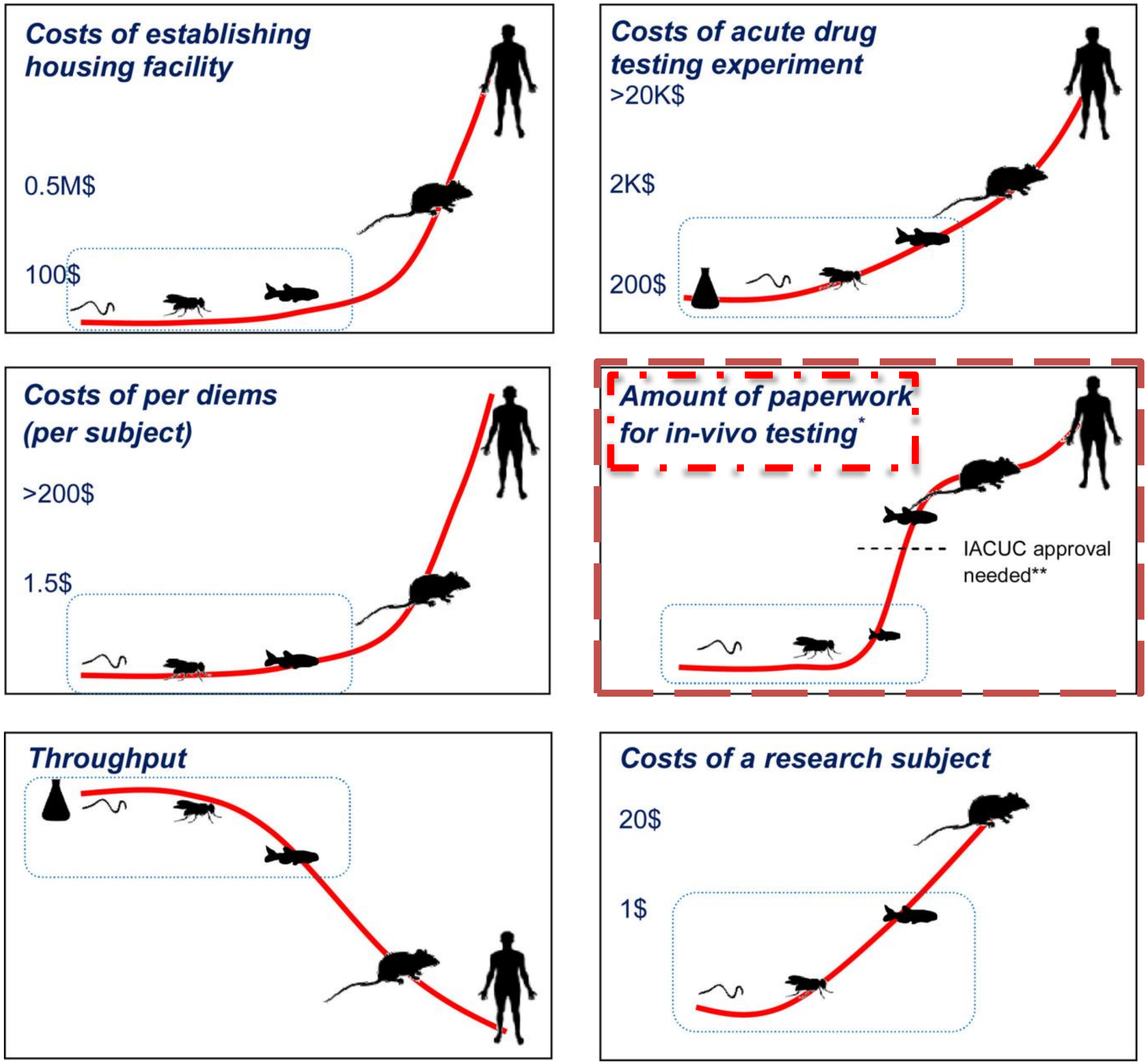


zebrafish as research model

5. Zebrafish offspring grows and develops very quickly

In the space of **24 hours**, a zebrafish cell can grow into a beating heart.
In comparison, it takes 8 and a half days for a mouse cell to grow into a beating heart.

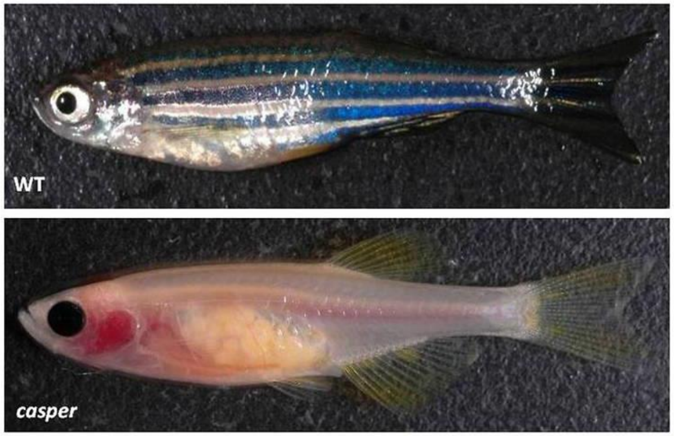
Costs of zebrafish behavioral and physiological research compared to other model organisms



Kalueff et al., Aquatic Toxicology (2016)

Danio rerio *(zebrafish)*

selected strains used in biomedical research



Strain	Details	Behavioral phenotypes
Strains		
AB	Commonly used 'high-performance' strain, developed by G. Streisinger	Active strain, sensitive to various experimental (genetic and pharmacological) manipulations
Casper	Mutant strain translucent throughout adulthood due to a lack of melanocytes and reflective cells	Display active locomotor phenotype and some differences to developmental drug treatment
Ekkwill (EKW)	Derived from Ekkwill breeders (FL)	Active strain, sensitive to various experimental manipulations
Nadia	Domesticated strain derived from a wild-caught zebrafish	More anxious zebrafish
Tubingen (Tu)	Short-fin wild type strain, commonly used in neurobehavioral tests. Utilized for genome sequencing project by Sanger Institute	Active, sensitive to various genetic and pharmacological manipulations
Wild Indian Karyotype (WIK)	Derived from wild-caught Indian zebrafish, used for genome mapping	Highly anxious zebrafish
Wild-caught	Zebrafish caught in the wild in India	Highly anxious zebrafish
Color variants		
Long-fin variant	Contain spontaneous mutation causing long fins (Figure 1c)	More anxious and sensitive to anxiogenic stimuli
Leopard color variant	Contain spontaneous mutation causing spotting in adult fish (Figure 1c)	More anxious and sensitive to anxiogenic stimuli
Mutants		
naddne^{3256}	An N-ethyl-N-nitrosourea-induced mutant used to study the rewarding effects of amphetamine	Fails to respond to amphetamine
jpy	Increased number of mitotic cells "Jumpy" fish exhibiting cocaine sensitivity	Fails to respond to cocaine

The zebrafish genome

http://www.sanger.ac.uk/science/data/zebrafish-genome-project

"reference genome" is based on Tubingen strain

NATURE | LETTER **OPEN**

日本語要約

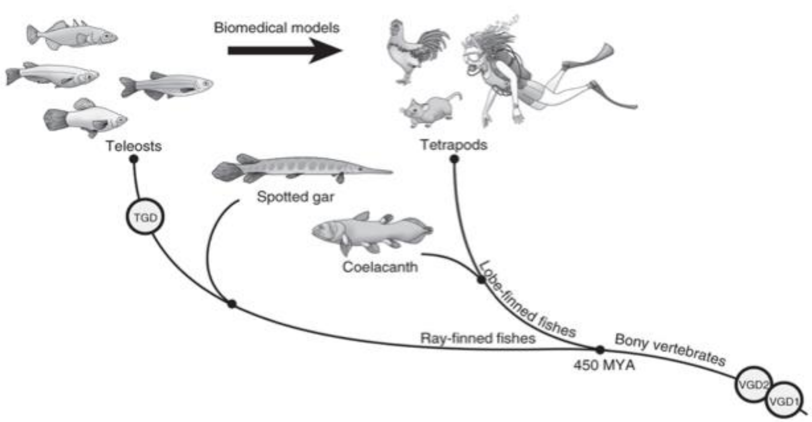
The zebrafish reference genome sequence and its relationship to the human genome

Kerstin Howe, Matthew D. Clark, Carlos F. Torroja, James Torrance, Camille Berthelot, Matthieu Muffato, John E. Collins, Sean Humphray, Karen McLaren, Lucy Matthews, Stuart McLaren, Ian Sealy, Mario Caccamo, Carol Churcher, Carol Scott, Jeffrey C. Barrett, Romke Koch, Gerd-Jörg Rauch, Simon White, William Chow, Britt Kilian, Leonor T. Quintais, José A. Guerra-Assunção, Yi Zhou, Yong Gu + *et al.*

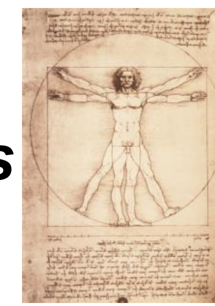
great genetic heterogenenity between strains> highly resistant to inbreeding

About 71% of the **20.479 protein-coding genes** of *H. sapiens* have orthologs in the zebrafish.
69% of the **26.206** protein coding zebrafish genes have human counterparts.

Genome duplication of bony fish

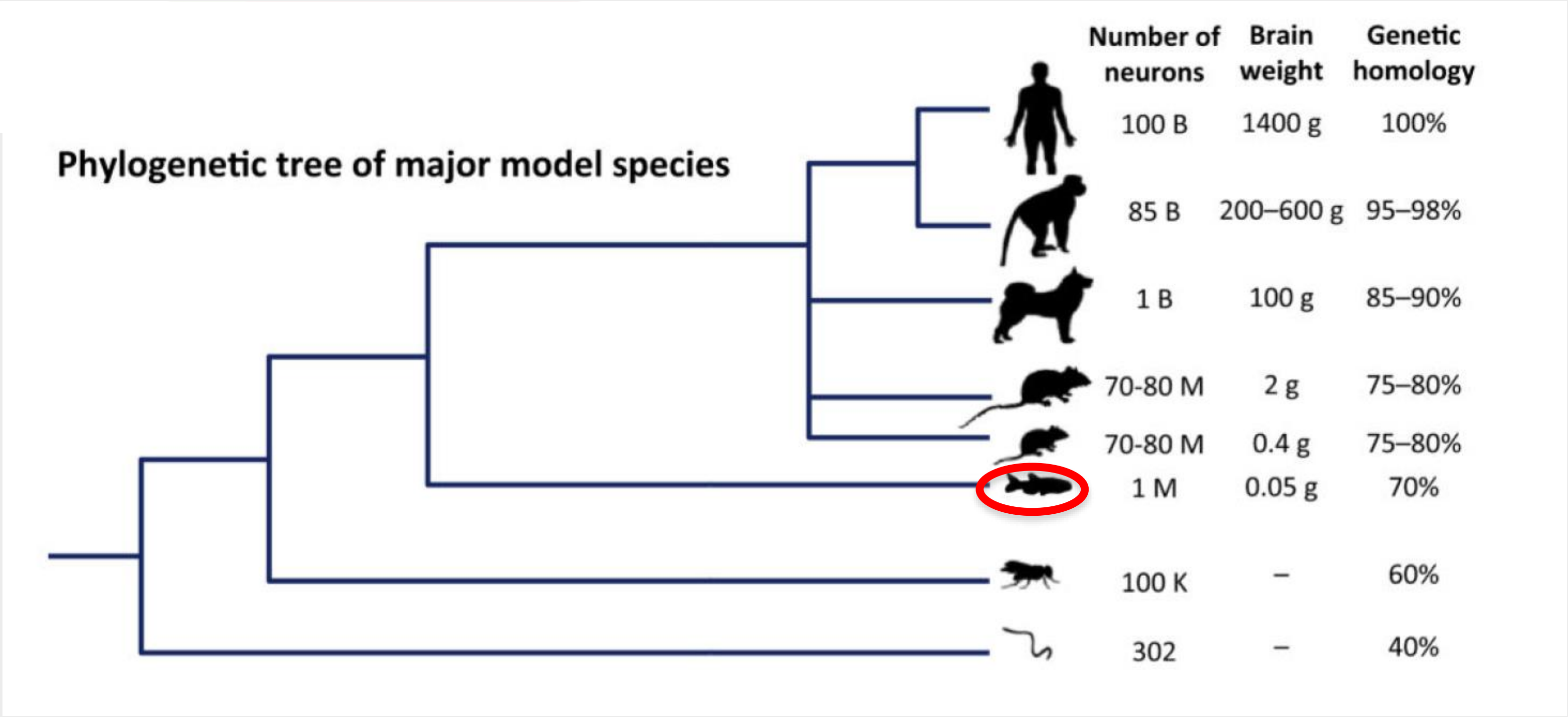


vs



4.556 **RNA genes**, among which miRNAs with the key role in the regulation of protein coding-genes. miRNAs are largely conserved.

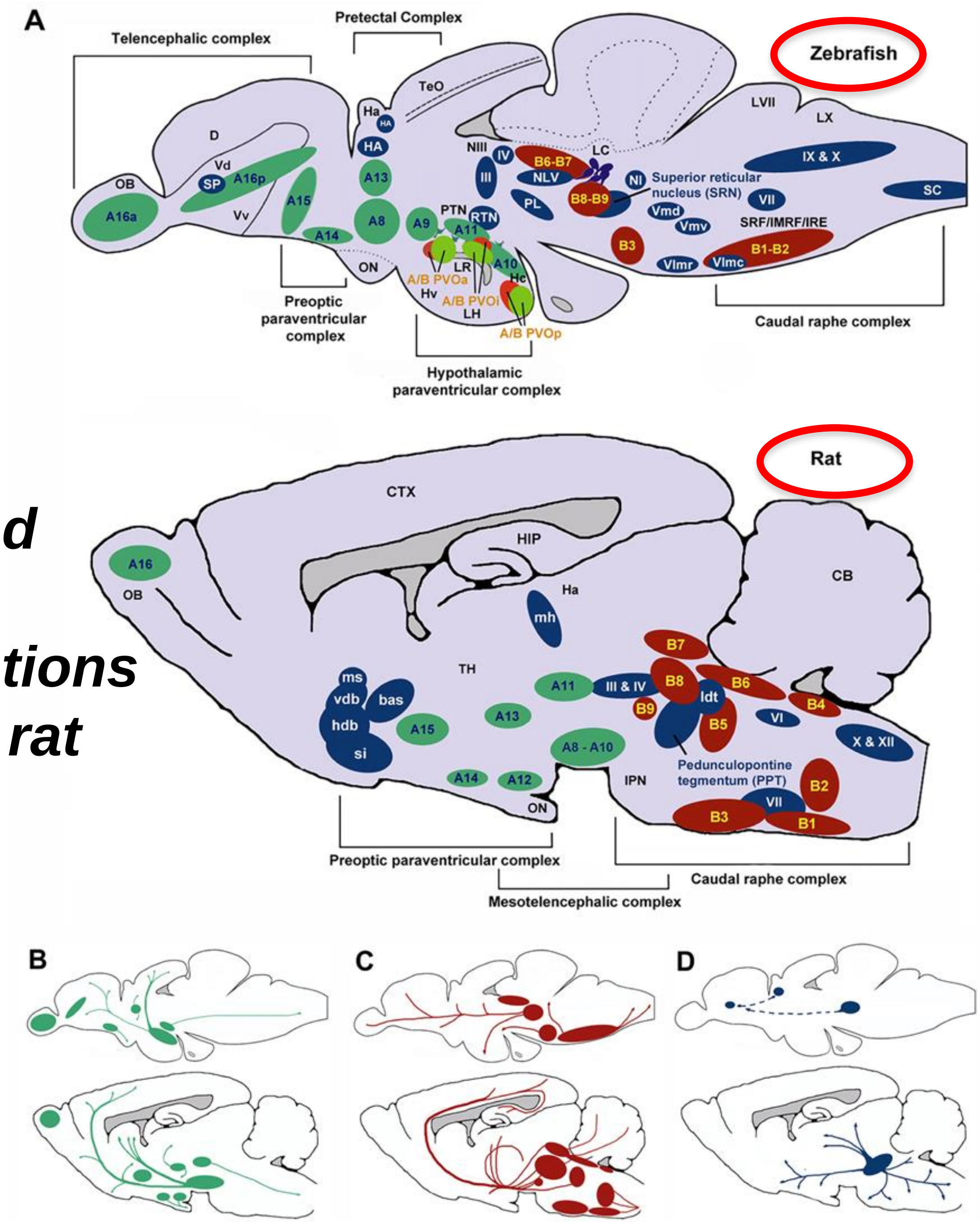
Zebrafish an *alternative* model for neuroscientific studies



adapted by Stewart et al., Trends Neurosci., 2014

Conserved neurotransmitters pathways

***Dopaminergic*, *serotonergic*, and *cholinergic* neuronal populations in zebrafish and rat**

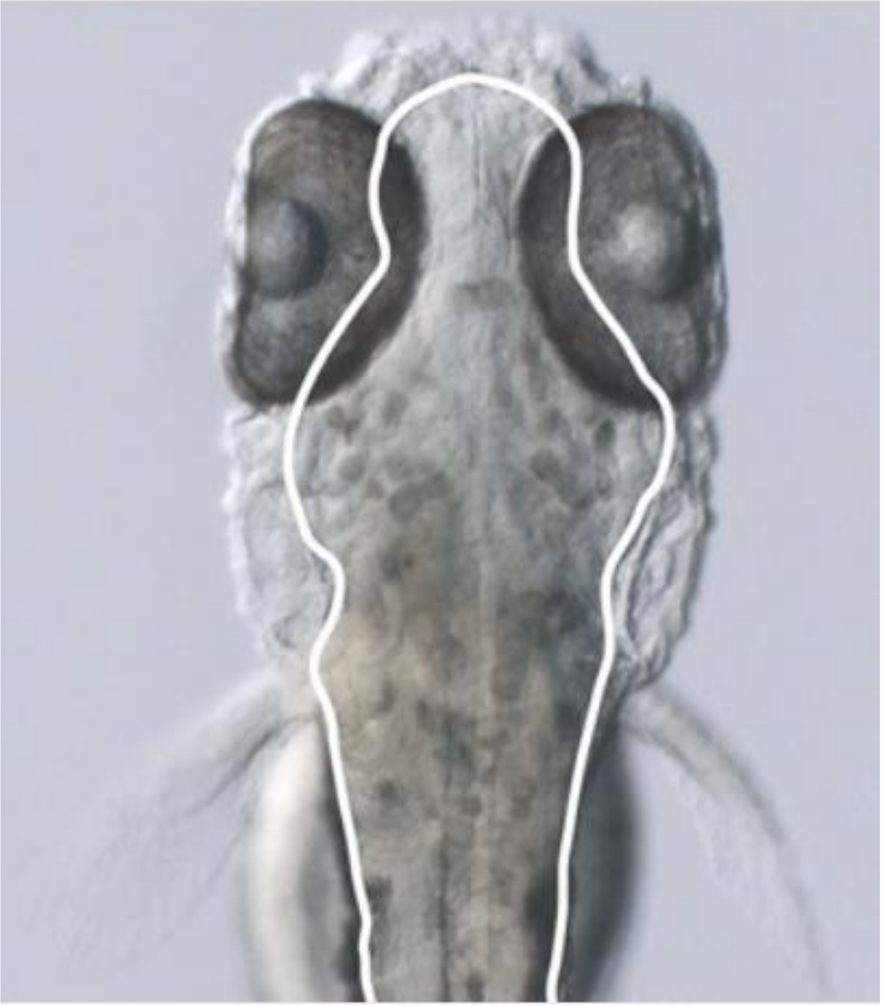


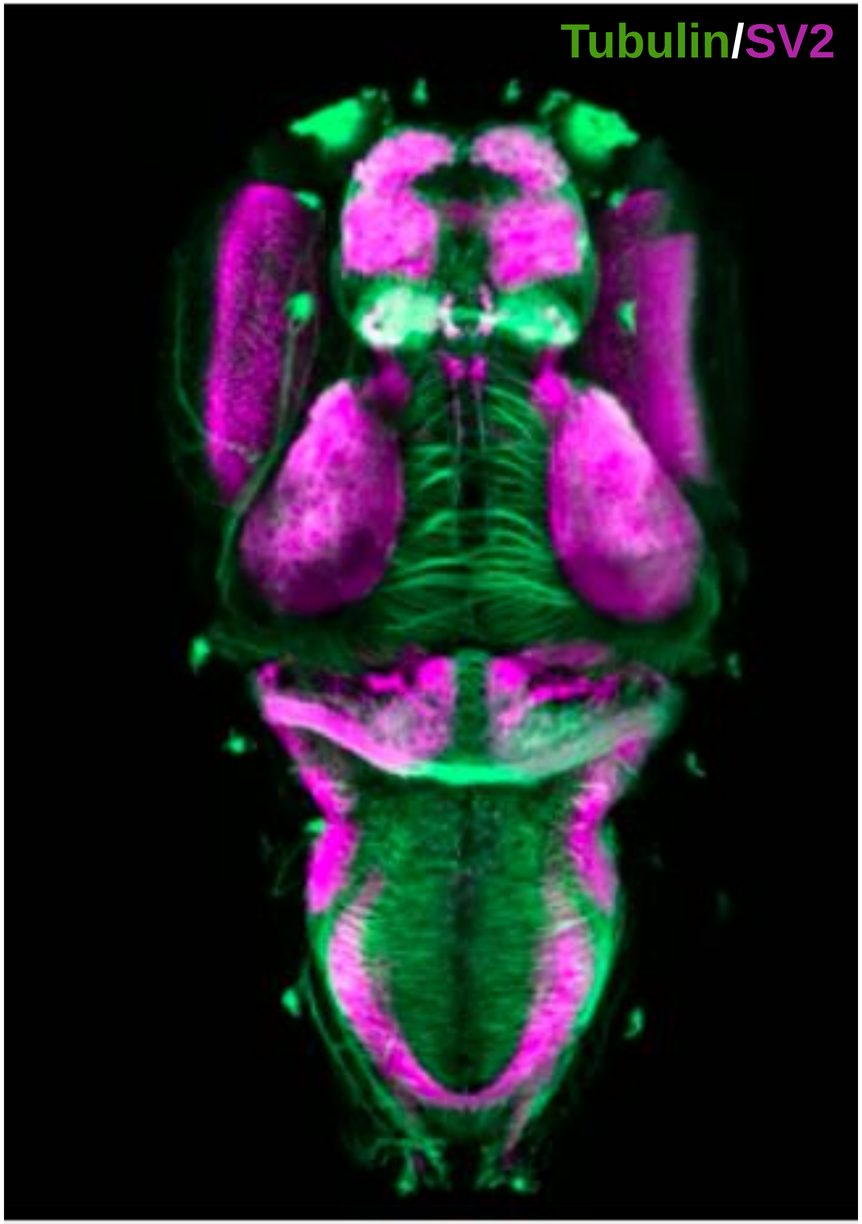
Parker et al., Front. Neural Circuits (2013)

Brain subdivisions

Zebrafish brain at 4 days post fertilization

Dorsal views





larval zebrafish brain has a size of ***<0.5 mm^3***

www.zebrafishbrain.org

brain and development

First neurons form after **7.5 hours post fertilisation** (hpf)

At hatching **10,000** neurons

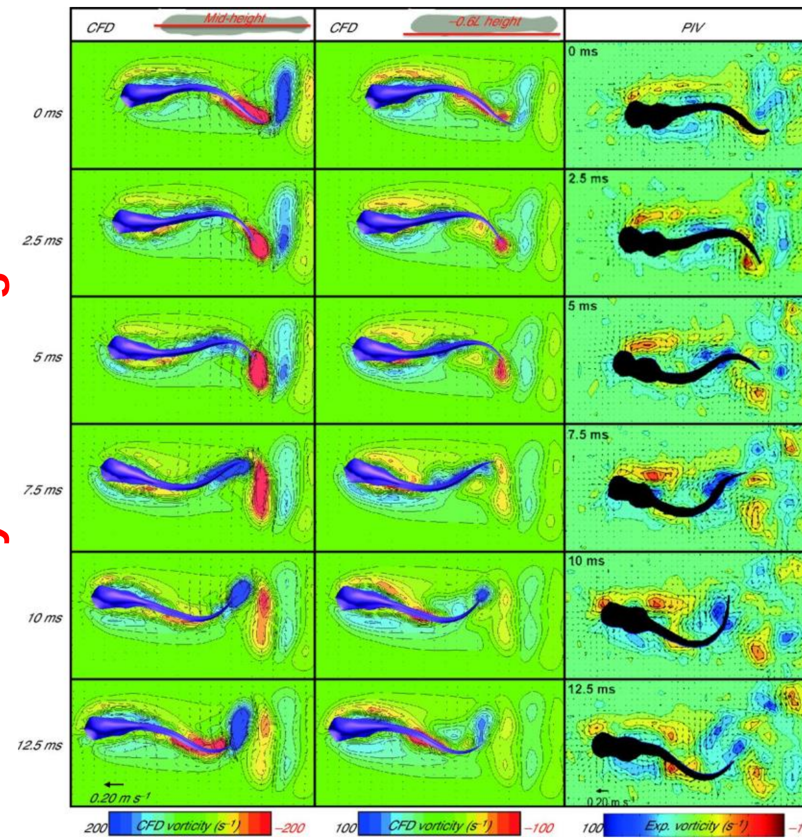
~100,000 number of neurons in larval brain of zebrafish (168 hpf) *whole-brain maps of stimulus and behavior-dependent neural activity*

Whole-brain activity mapping onto a zebrafish brain atlas

Owen Randlett[1], Caroline L Wee[2], Eva A Naumann[1,8], Onyeka Nnaemeka[1], David Schoppik[1,8], James E Fitzgerald[3], Ruben Portugues[1,8], Alix M B Lacoste[1], Clemens Riegler[1,4], Florian Engert[1,9] & Alexander F Schier[1,3,5–7,9]

Anatomical platform of neural activity in 6dpf larvae which have a well defined behaviours pattern (hunting, sleep, etc.)

on freely swimming fish

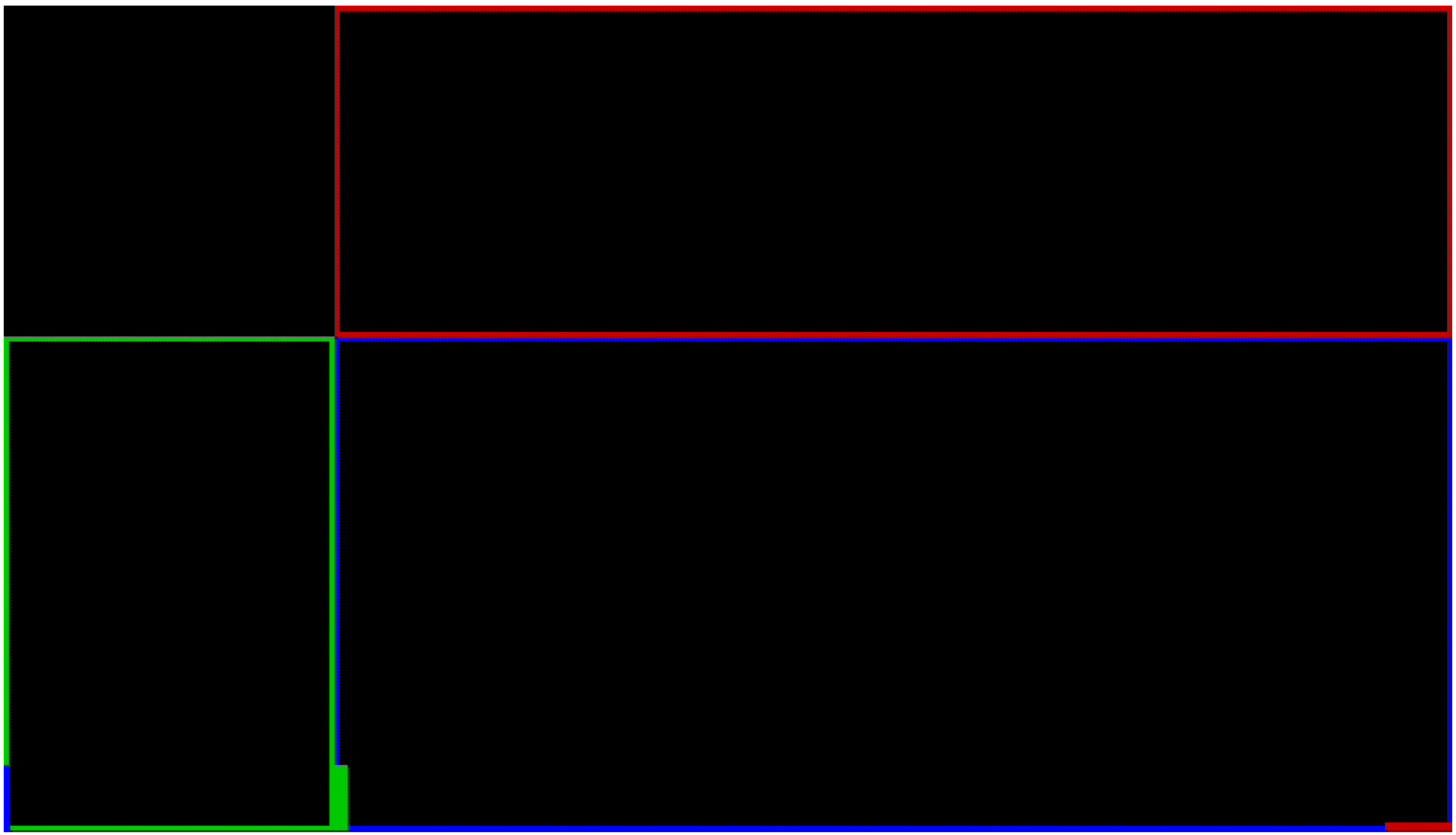


whole-brain maps of stimulus- and behavior-dependent neural activity

on freely swimming fish

higher (**green**) and lower (**magenta**) pERK levels
upon stimulus

exposure to noxious heat (37 ° C)

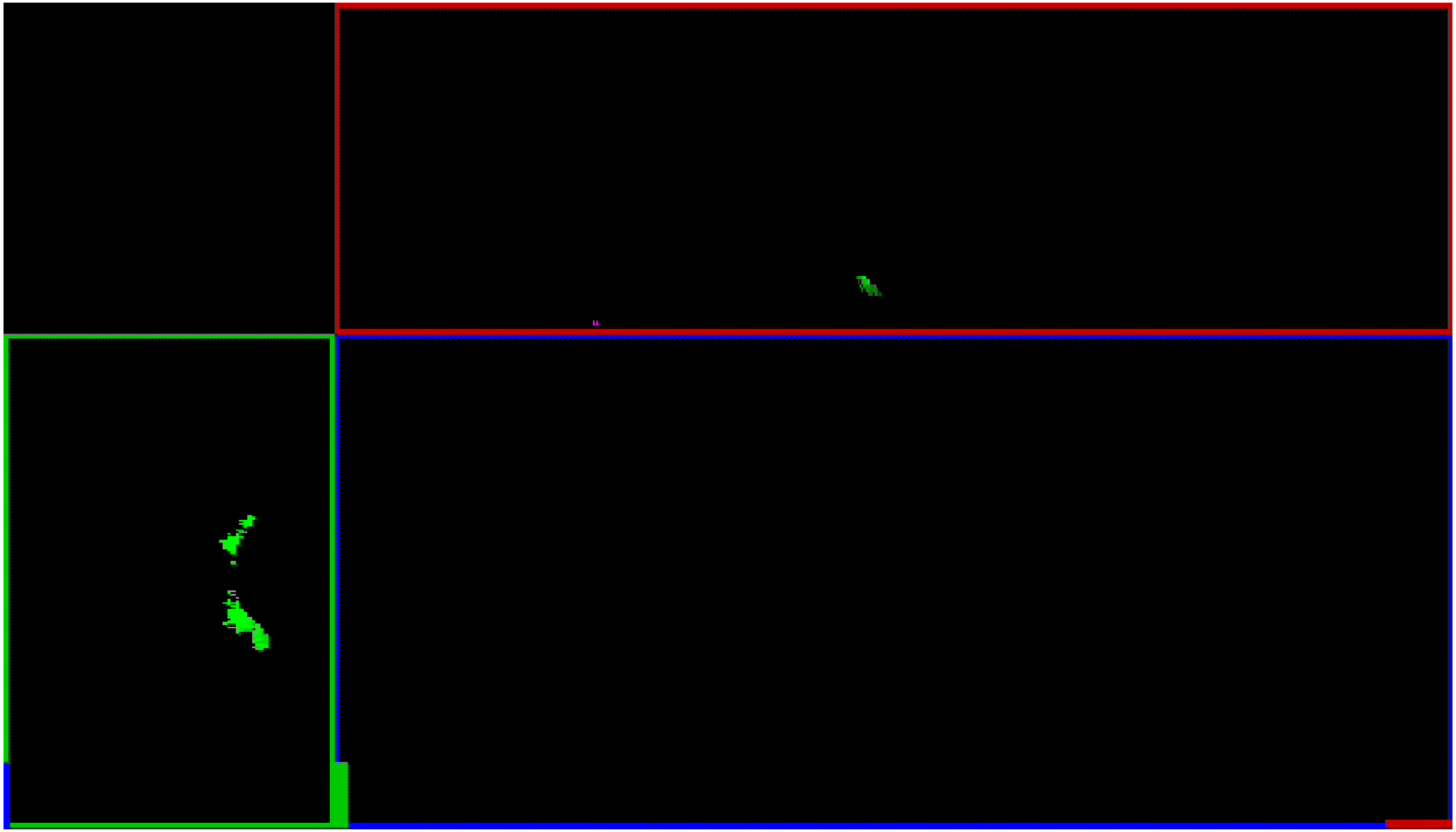


Randlett et al., Nat. Methods (2015)

in vivo analysis of neuronal activity

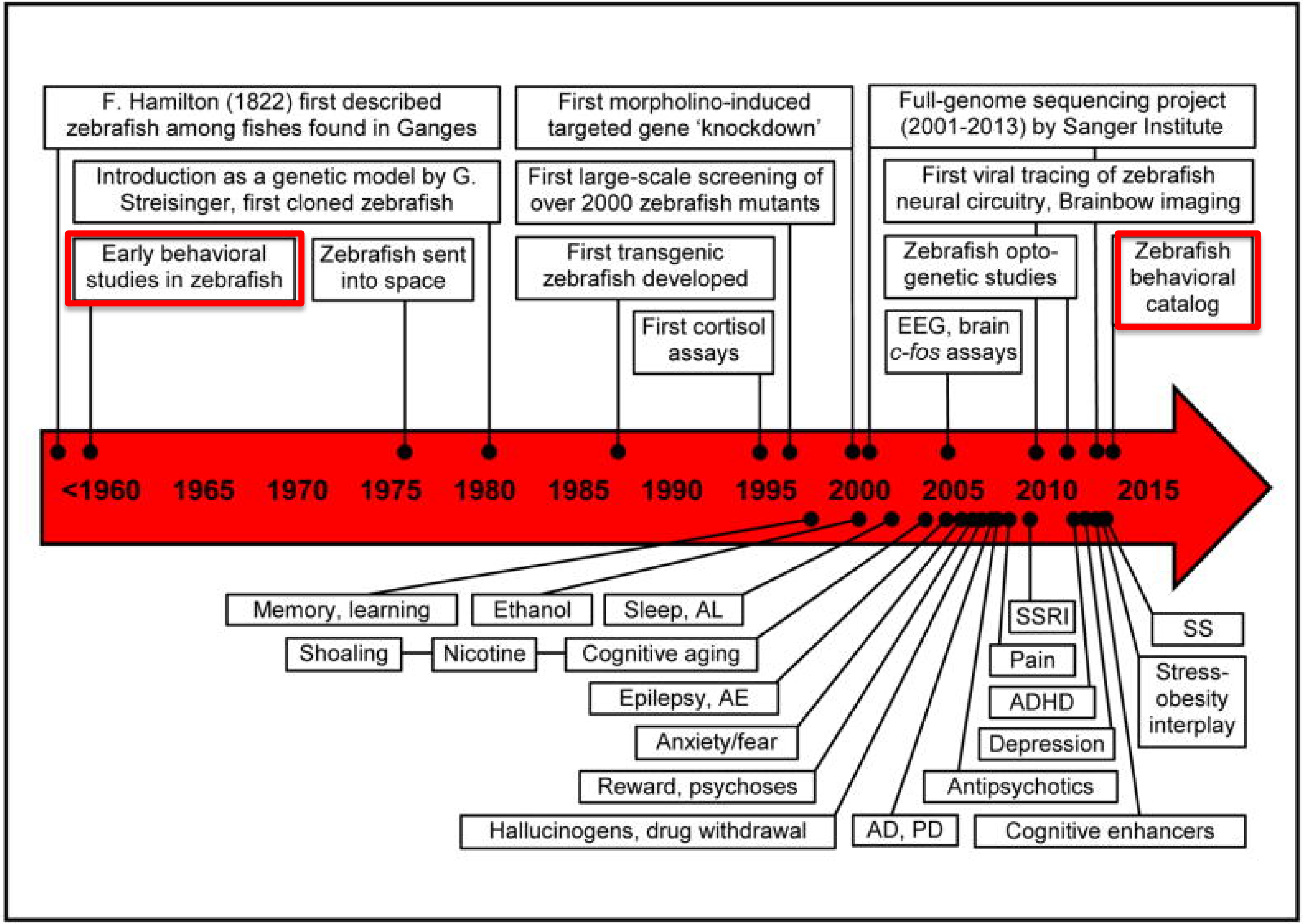
whole-brain maps of stimulus- and behavior-dependent neural activity
on freely swimming fish

exposure to the anaesthetics MS-222



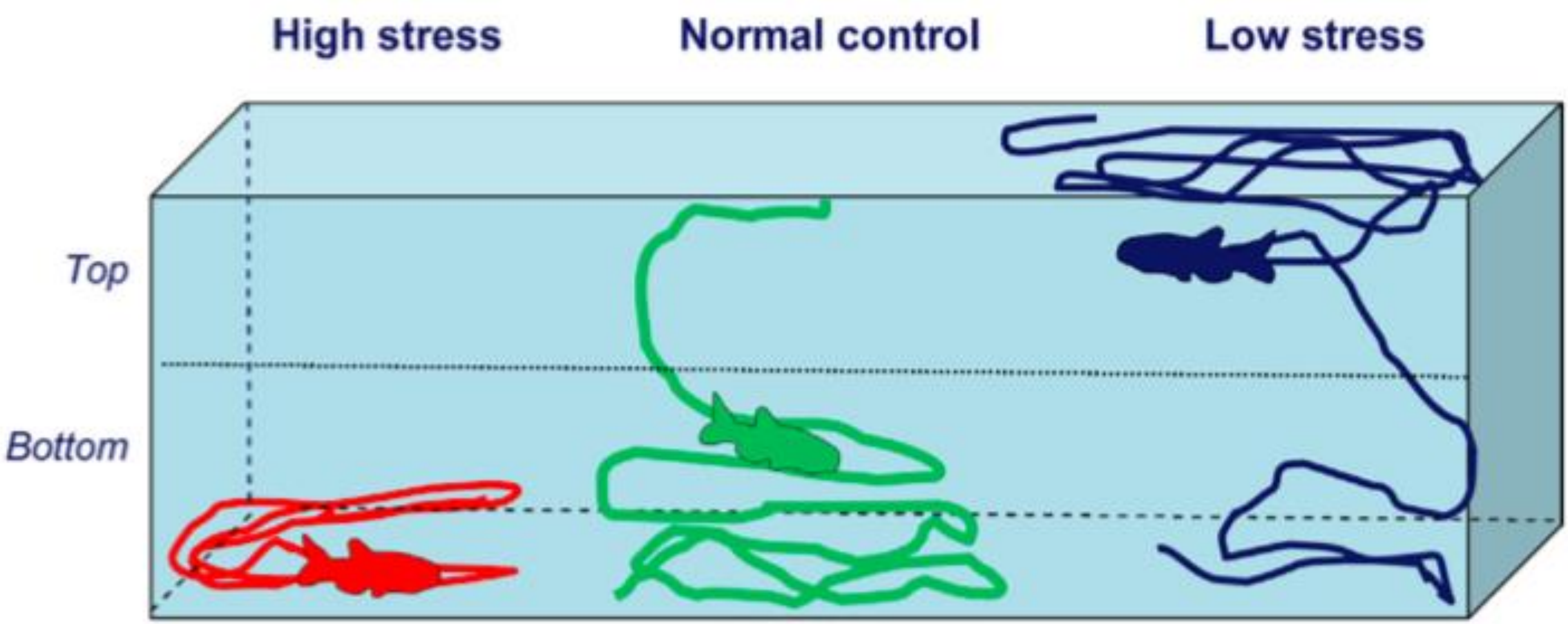
Randlett et al., Nat. Methods (2015)

history of zebrafish models in neuroscience research



Kalueff et al., Trends Pharmacol Sci. (2014)

behavior of zebrafish



ZEBRAFISH
Volume 10, Number 1, 2013

DOI: 10.1089/zeb.2012.0861

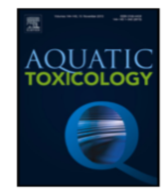
Towards a Comprehensive Catalog of Zebrafish Behavior 1.0 and Beyond

Allan V. Kalueff,[1,2] Michael Gebhardt,[1,2] Adam Michael Stewart,[1–3], Jonathan M. Cachat,[1,2] Mallorie Brimmer,[1]
Jonathan S. Chawla,[1] Cassandra Craddock,[1] Evan J. Kyzar,[1] Andrew Roth,[1] Samuel Landsman,[1]
Siddharth Gaikwad,[1] Kyle Robinson,[1,2] Erik Baatrup,[4] Keith Tierney,[5] Angela Shamchuk,[5] William Norton,[6]
Noam Miller,[7] Teresa Nicolson,[8] Oliver Braubach,[9] Charles P. Gilman,[10] Julian Pittman,[11]
Denis B. Rosemberg,[12] Robert Gerlai,[13] David Echevarria,[14] Elisabeth Lamb,[14] Stephan C.F. Neuhauss,[15]
Wei Weng,[16] Laure Bally-Cuif,[17] Henning Schneider,[18]
and the Zebrafish Neuroscience Research Consortium (ZNRC)[2]

Contents lists available at ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aquatox



Zebrafish neurobehavioral phenomics for aquatic neuropharmacology and toxicology research

Allan V. Kalueff[a,b,c,d,j,*], David J. Echevarria[b,e], Sumit Homechaudhuri[f], Adam Michael Stewart[b,c], Adam D. Collier[b,e], Aleksandra A. Kaluyeva[c], Shaomin Li[a], Yingcong Liu[a], Peirong Chen[a], JiaJia Wang[a], Lei Yang[a], Anisa Mitra[f], Subharthi Pal[f], Adwitiya Chaudhuri[f], Anwesha Roy[f], Missidona Biswas[f], Dola Roy[f], Anupam Podder[f], Manoj K. Poudel[b,c], Deepshikha P. Katare[g], Ruchi J. Mani[g], Evan J. Kyzar[b,h], Siddharth Gaikwad[i], Michael Nguyen[b,c], Cai Song[a,i], the International Zebrafish Neuroscience Research Consortium ZNRC



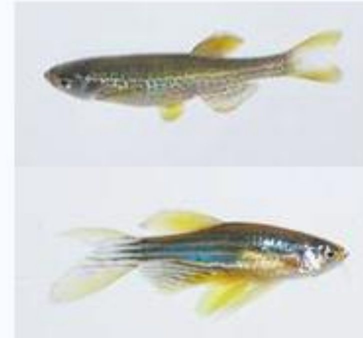


Photo Gallery Here!

2nd Zebrafish Behavioral Neuroscience and Neurophenotyping Workshop – ZB2N2012
New Orleans, LA

Dates (pre-SfN): October 11, 2012 (10 am-6 pm) OR
October 12, 2012 (10 am-6 pm)

Dates (post-SfN): October 18, 2012 (10 am-6 pm) OR
October 19, 2012 (10 am-6 pm)

Enhance your SfN2012 experience in New Orleans with a one day Zebrafish Behavioral Neuroscience and Neurophenotyping Workshop (ZB2N2012)

Topics

- Introduction to behavioral ecology of zebrafish
- Establishing a zebrafish colony in your lab
- General motor phenotypes
- Neurotoxicity models
- Anxiety and fear-related behaviors
- Social phenotypes: Manual and automated analyses of shoaling and social preference
- Memory and Learning
- Predator avoidance behavior
- Depression-like phenotypes
- Drug abuse and withdrawal
- Genetic/strain differences
- Aggression and boldness phenotypes
- Zebrafish behavioral syndromes
- Advanced video-tracking techniques
- Three-dimensional neurophenotyping approaches
- Using zebrafish in high-throughput small molecule screens
- Zebrafish models of psychoses
- Hallucinogenic, anxiolytic and antidepressant drugs' effects
- Measuring zebrafish body coloration: manual and automated analyses
- Physiological biomarkers: brain *c-fos* and *egr* expression, whole-body cortisol
- Ethograms
- Enhancing zebrafish neurobehavioral research with online databases and tools

General Introduction: As more and more labs are establishing zebrafish (*Danio rerio*) projects, zebrafish are rapidly becoming a popular model organism for neuroscience research, as more and more labs are establishing zebrafish projects. This day-long course consists of a series of lectures covering major neurobehavioral domains and advanced phenotyping techniques for probing normal and pathological behaviors in zebrafish. The lectures will be followed by

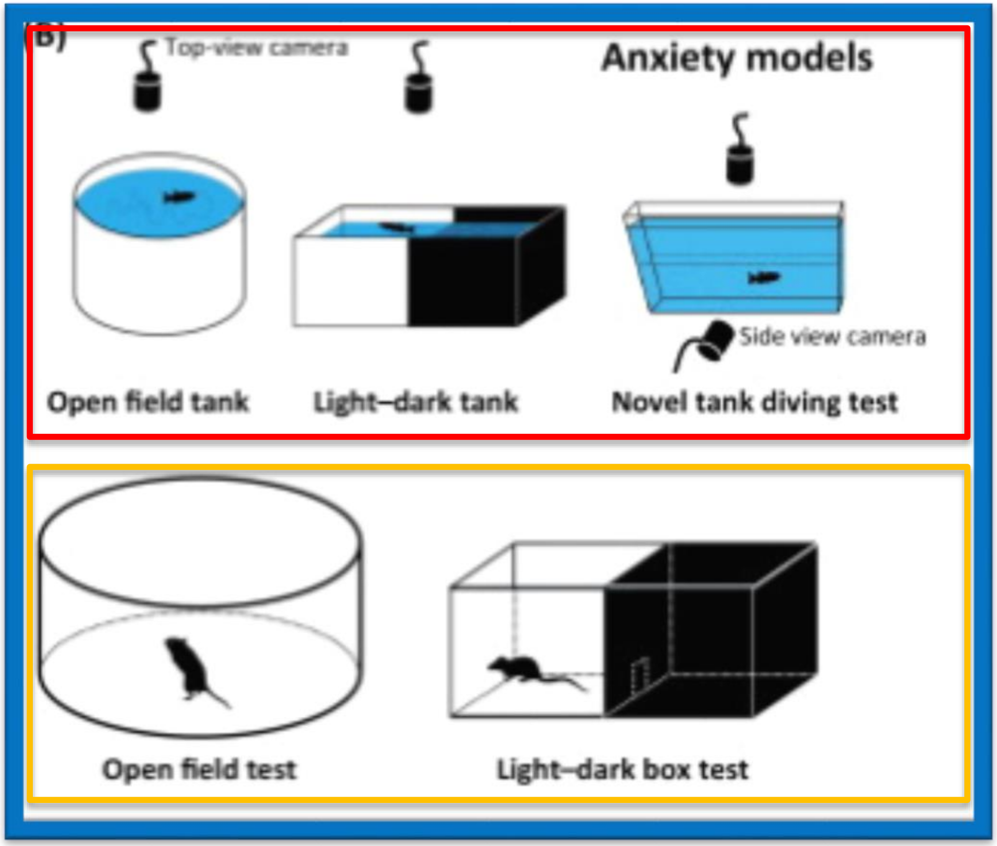
tools for neurobehavioural tests

neurobehavioral tests

exploration, anxiety and locomotion parallel traditionally used in rodents combined with automated video-tracking using top/side view cameras.

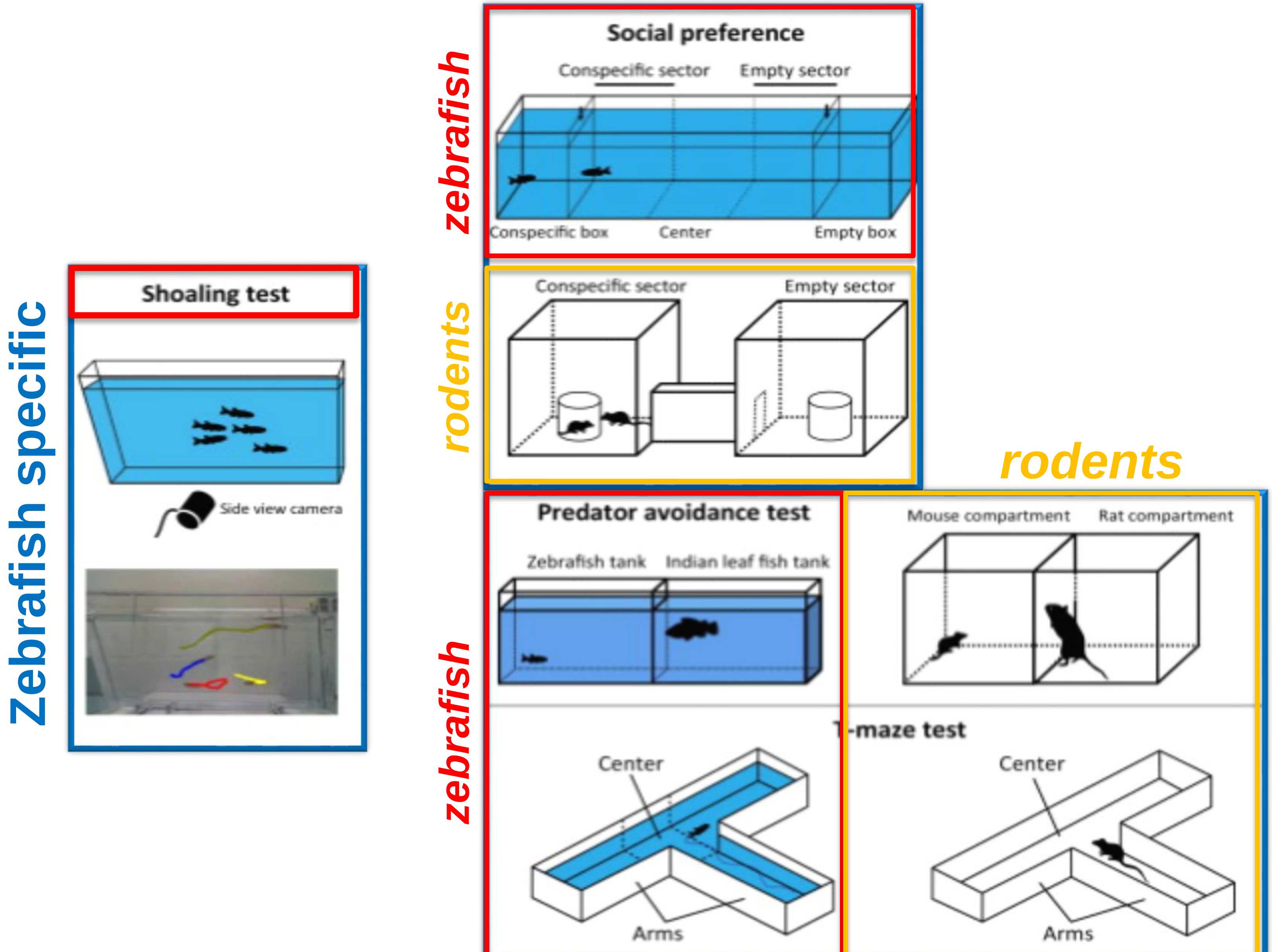
zebrafish

rodents



adapted by Kalueff et al., Trends Pharmacol Sci. (2014)

tools for neurobehavioural tests



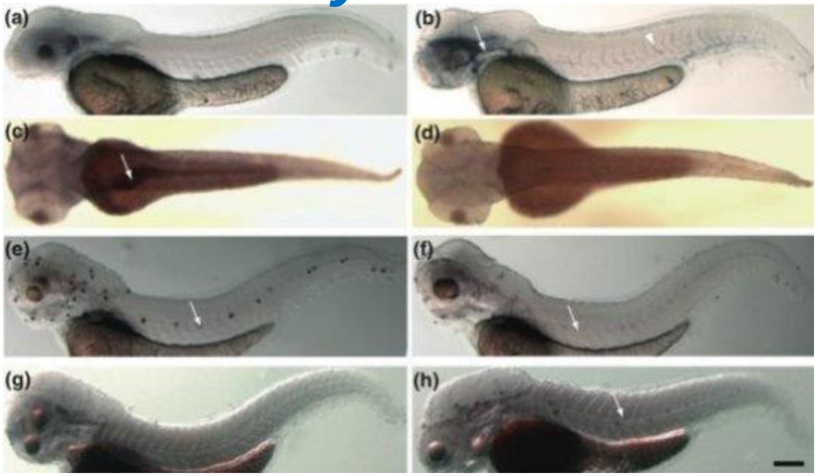
adapted by Kalueff et al., Trends Pharmacol Sci. (2014)

Zebrafish in Toxicology and Environmental Health

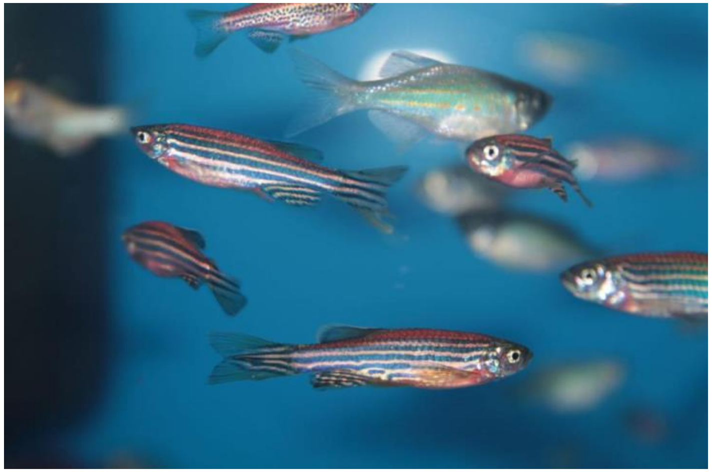
Zebrafish express a full range of *cytochrome P450* (*cyp*) genes required for xenobiotic metabolism and biotransformation

- *to detect toxins in water samples (combined actions of more stressors, i.e. temperature changes, combination of 2 or more toxicants)*
- *to investigate the mechanisms of action of environmental toxins and their related diseases*

embryo/larvae

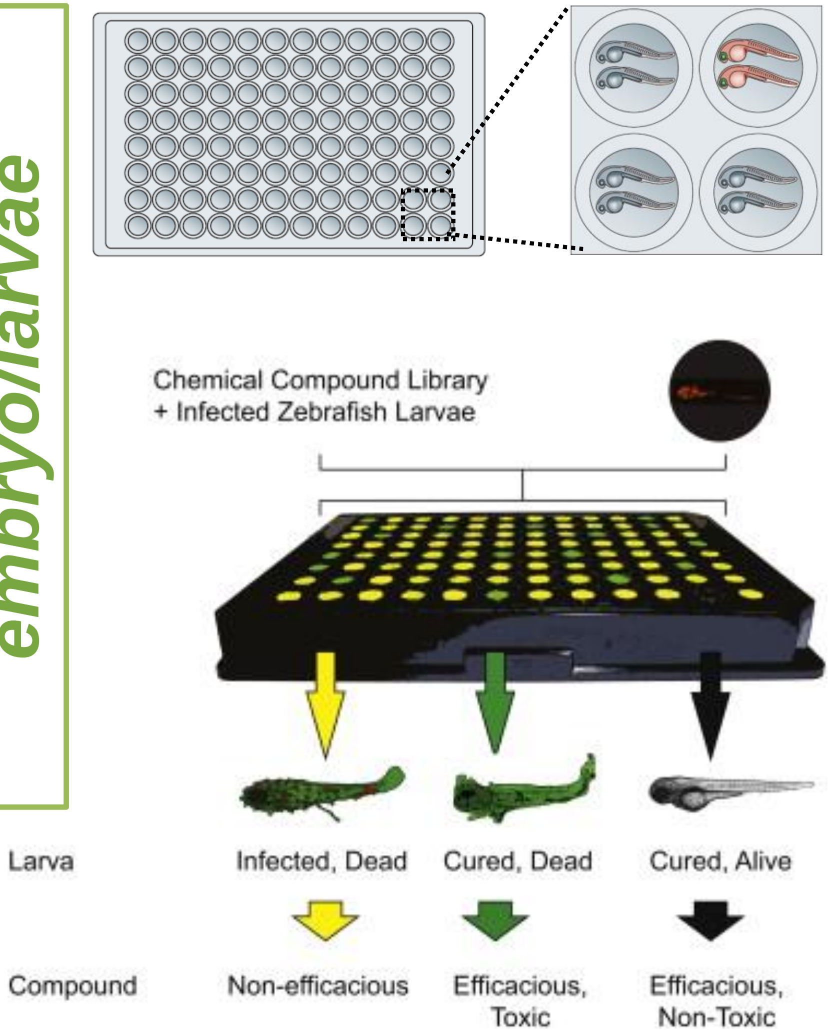


adults



Zebrafish in Toxicology and Environmental Health

embryo/larvae

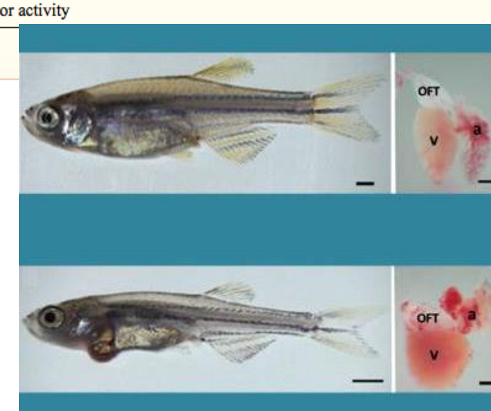


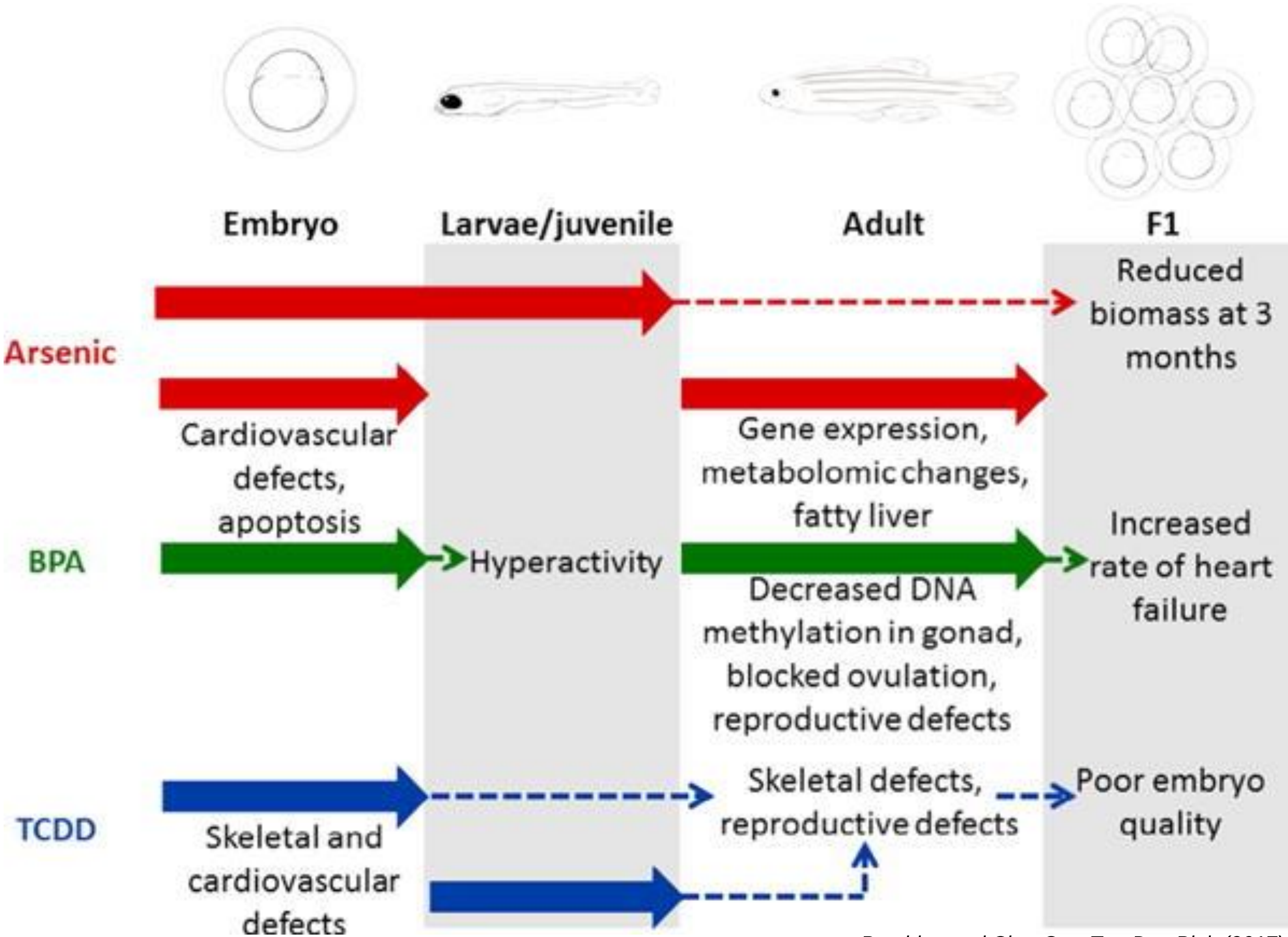
adults

Table 1

Transgenic Zebrafish Lines for Reporting Toxicant Exposure

Transgenic Line	Reporter for	Toxicants Tested	References
Tg(cyp1a:nls-gfp)	Cytochrome p450 Cyp1a	Aromatic hydrocarbons, dioxin-like compounds	Kim et al. (2013)
Tg(cyp1a:gfp)	Cytochrome p450 Cyp1a	Aromatic hydrocarbons, dioxin-like compounds	Xu et al. (2015)
Tg(mt:egfp)	Metallothionein	Heavy metals	Liu, Yan, Wang, Wu, and Xu (2016)
Tg(huORFZ:gfp)	Human CHOP	Heavy metals, endocrine disruptors	Lee et al. (2014)
TgBAC (hspb11:GFP)	Small heat shock protein hsbp11	Pesticides	Shahid et al. (2016)
Tg(5xERE:GFP)	Estrogen receptor activity	Estradiol, xenoestrogens, environmental water samples	Gorelick et al. (2014), Gorelick and Halpern (2011), and Gorelick, Pinto, Hao, and Bondesson (2016)
Tg(cyp19a1b:GFP)	Cytochrome p450 cyp19a1b, estrogen receptor activity	BPA, environmental water samples	Cano-Nicolau et al. (2016) and Sonavane et al. (2016)

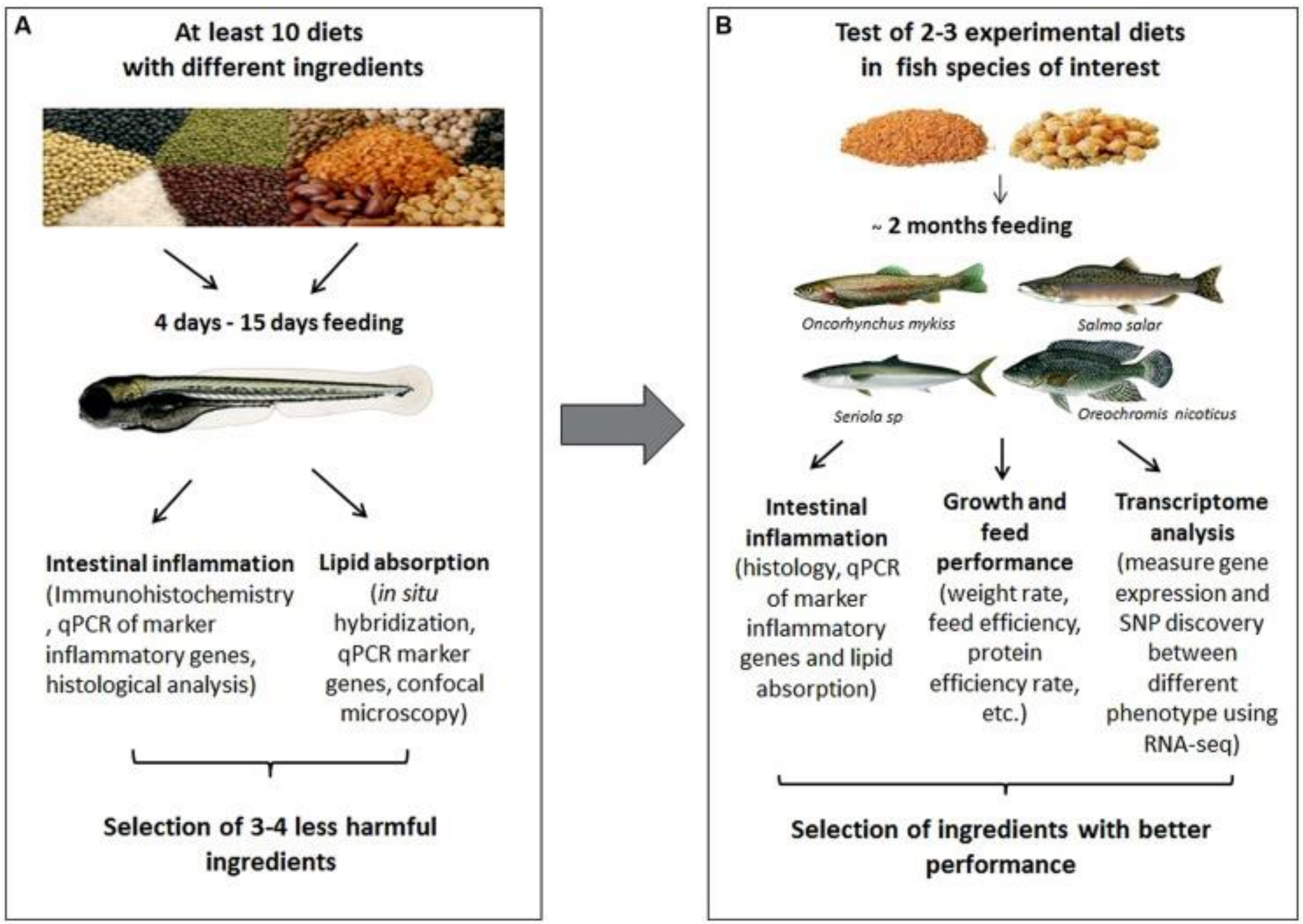




Bambino and Chu, Curr Top Dev Biol. (2017)

Zebrafish as animal model for aquaculture nutrition research

Exploring molecular and cellular pathways that regulate responses to different diets



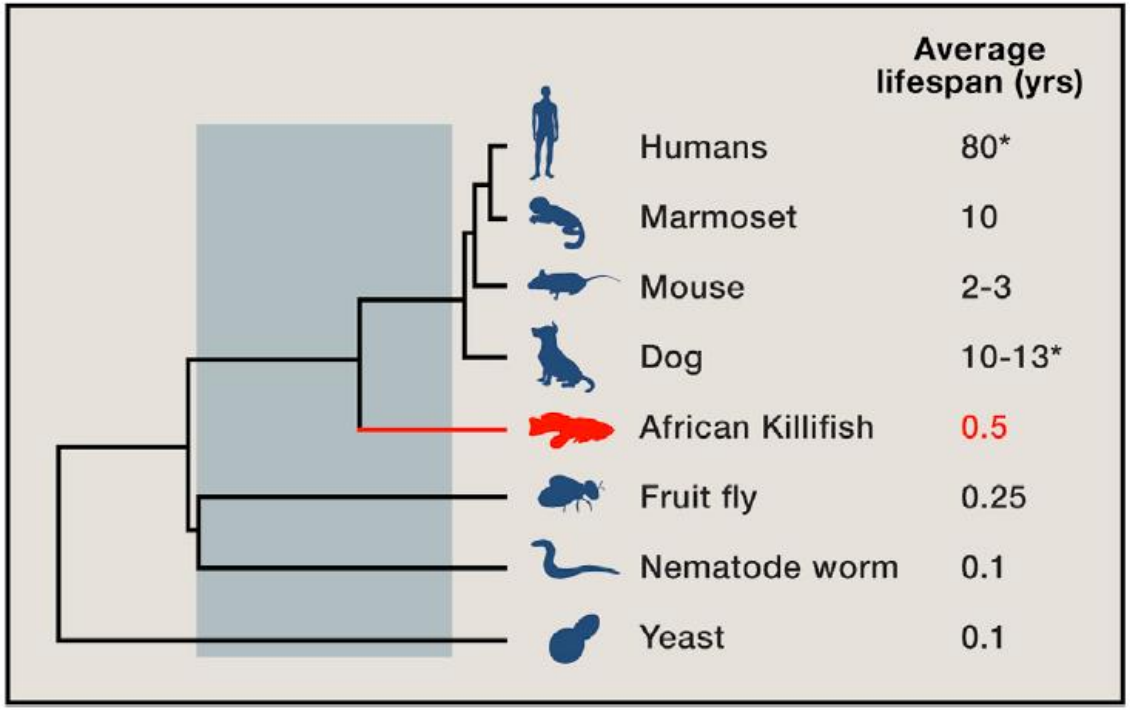
drawbacks

- **Not a mammal** (*some drugs may be metabolised in a different manner or, at least, at a different rate compared to mammals and this can alter their function*).
- **Genes duplicated**
- **Scarcity (or even absence) of inbred lines**
- **Drugs not water soluble can be problematic to administer by water immersion**
- **Species differences in blood-brain-barrier, which may affect the permeability of certain drugs**
- **Absence of parental care**

Nothobranchius furzeri: a colorful model system for human ageing

The African turquoise killifish (*Nothobranchius furzeri*), a teleost fish with a **natural lifespan** ranging between **4 and 9 months**, is emerging as a new promising model organism in ageing research.

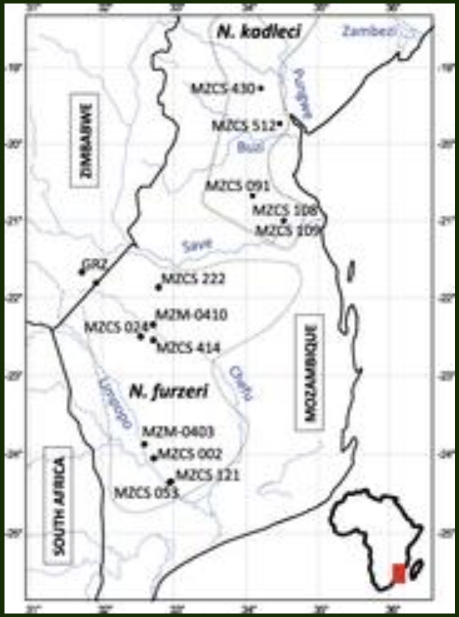
Nothobranchius furzeri: a colorful model system for human ageing



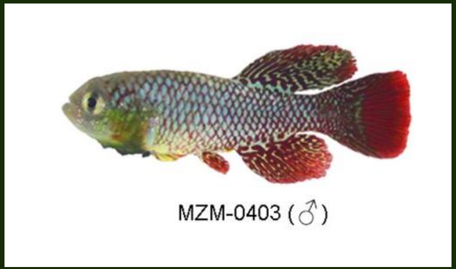
Wang et al., Cell (2015)

Evolutionary relationships between Humans and Organisms frequently used in ageing research

Habitat

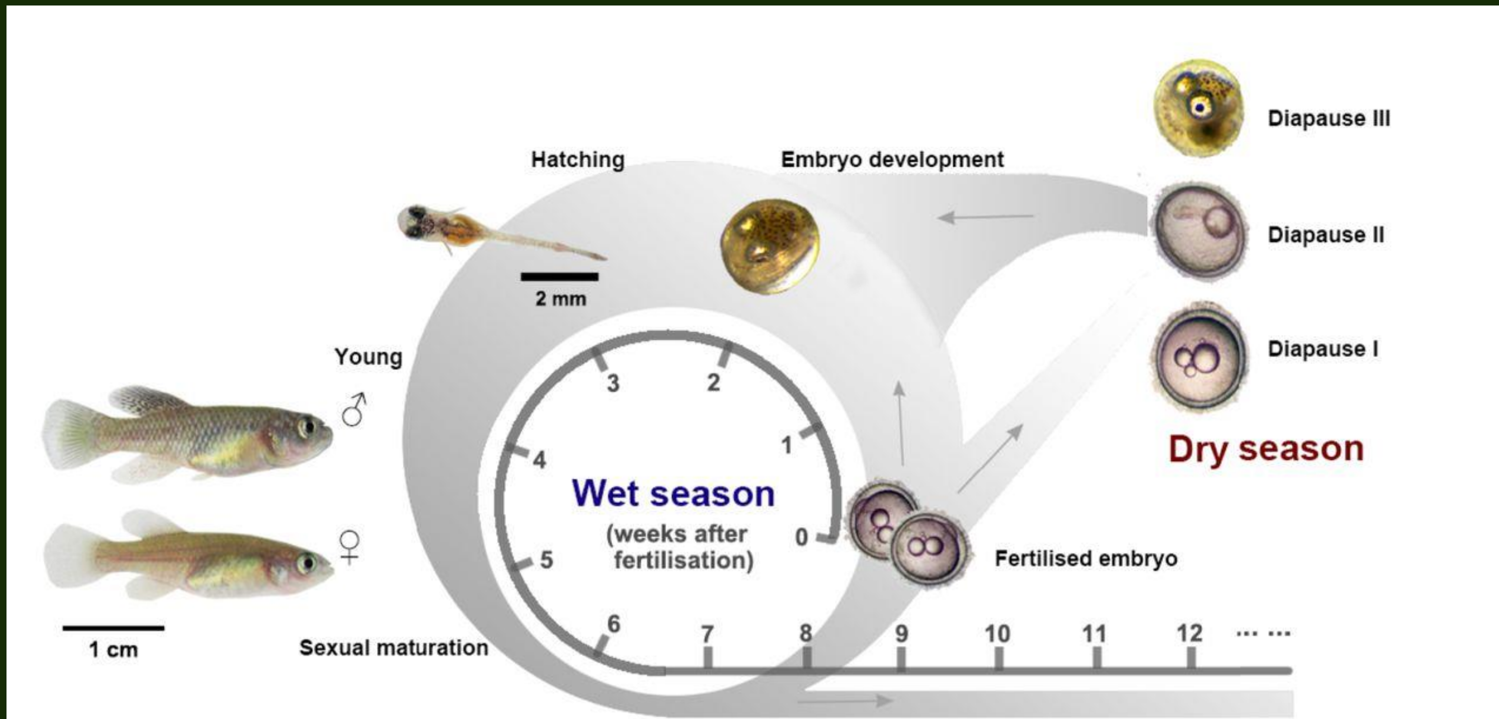


median lifespan ranging from 9 to 16 weeks



median lifespan ranging from 23 to 28 weeks

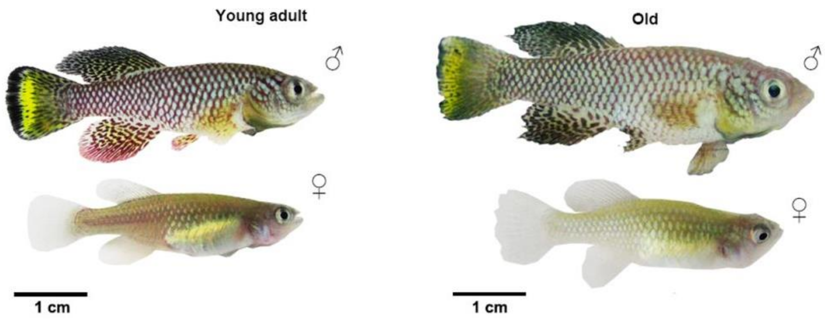
Life cycle of *N. furzeri*



Adapted by Kim et al., Disease Models and Mechanisms (2016)

Ageing phenotypes in *N. furzeri*

- progressively lose body and tail colour as well as their distinct patterning

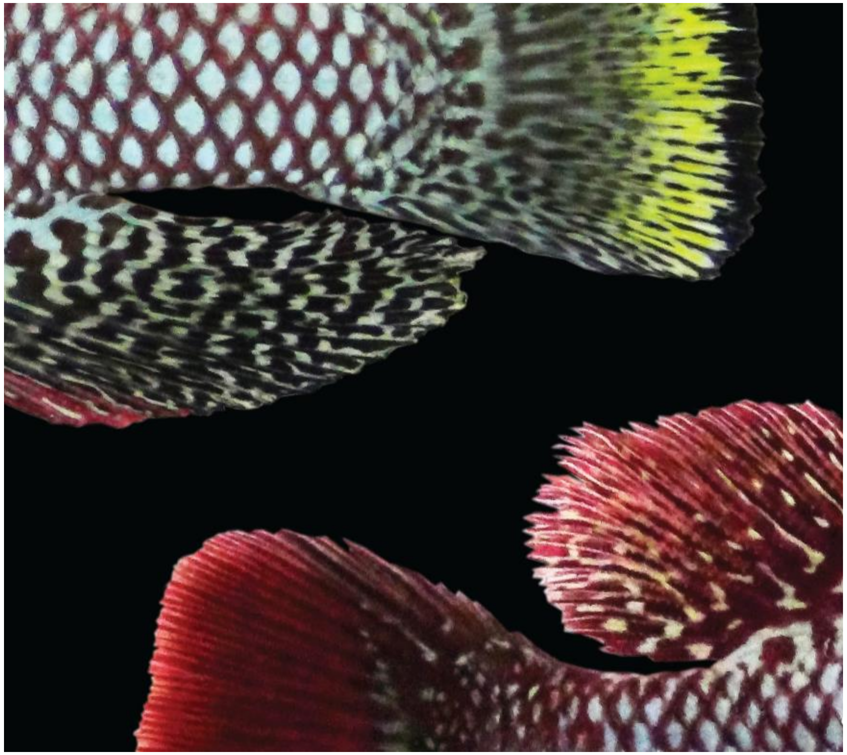


- abnormal spine curvature

- defective vision, fin structure deterioration, decreased spontaneous locomotion activity, learning impairment, emaciation

Ageing biomarkers

- accumulation of lipofuscin in the liver
- senescence-associated β-galactosidase (SA-β-gal) staining in the skin, a marker for cellular senescence and stress response in human cells
- telomeres shortening
- low regenerative capacity during ageing

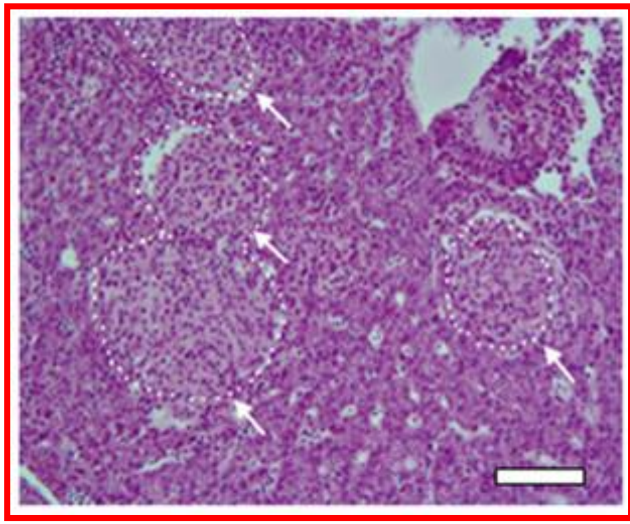


- spontaneos tumors, especially epatic tumors. Neoplastic lesions have been measured, using several tumour-associated proteins, including Bcl-2, cytokeratin-8, carcinoembryonic antigen and mutated p53

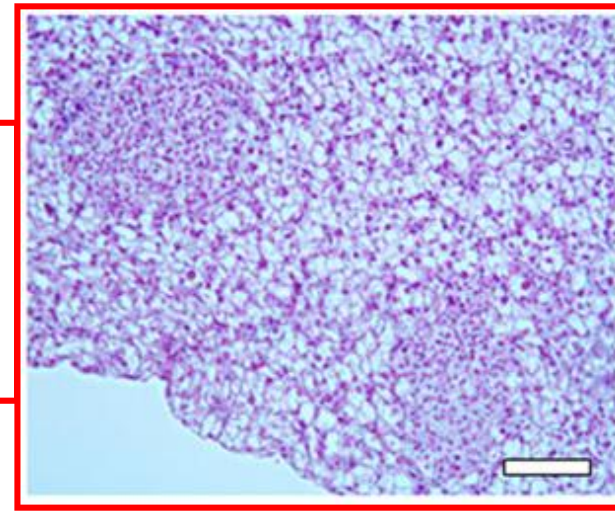
Cancer is the most common cause of death *N. furzeri*

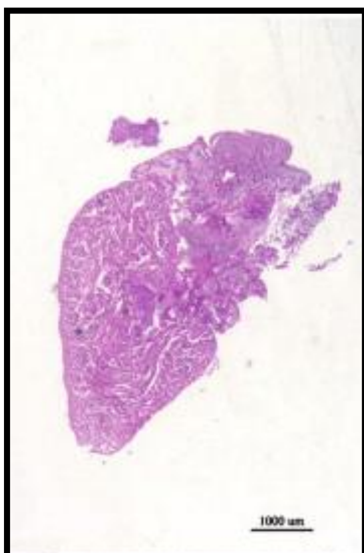
Ageing biomarkers

Degenerative lesions observed post mortem:

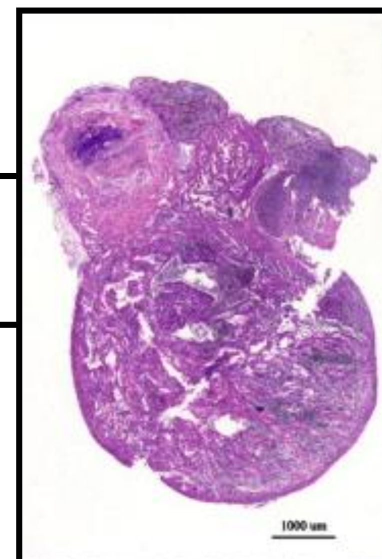


progressive liver degeneration up to steatosis



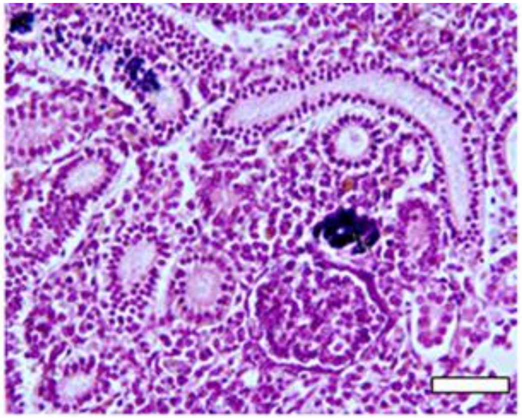
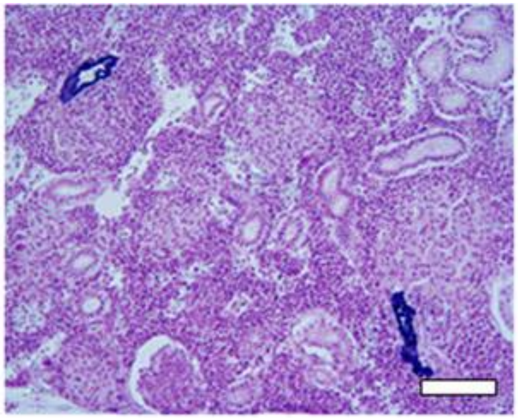


cardiac hypertrophy

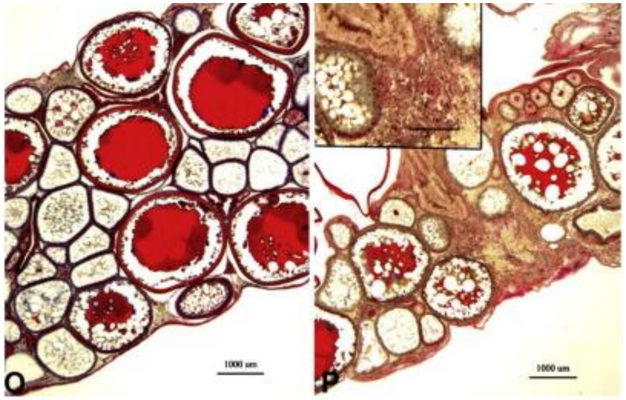


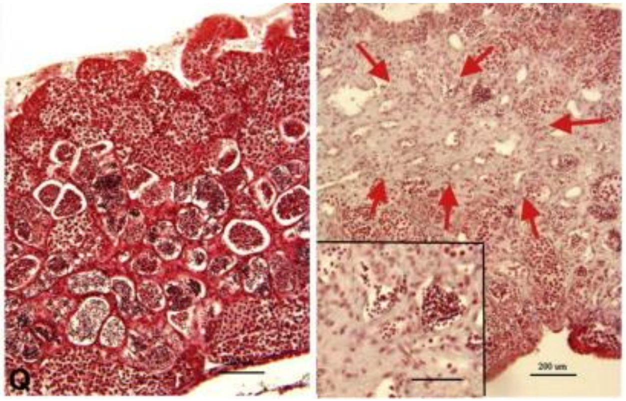
Ageing biomarkers

- nephrocalcinosis, tubular degeneration and tumors

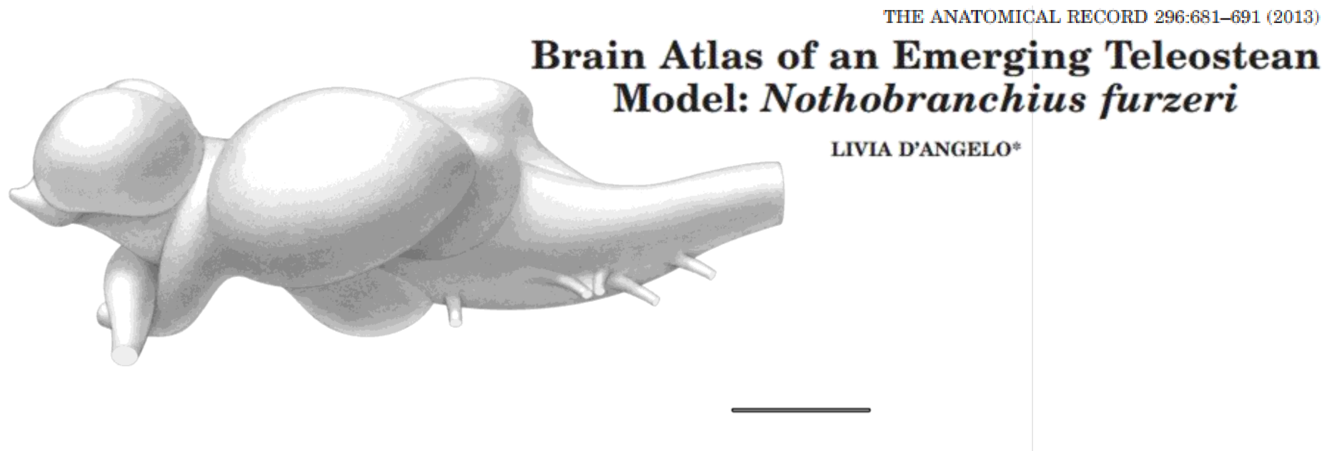
- ovary degeneration and interstitial fibrosis



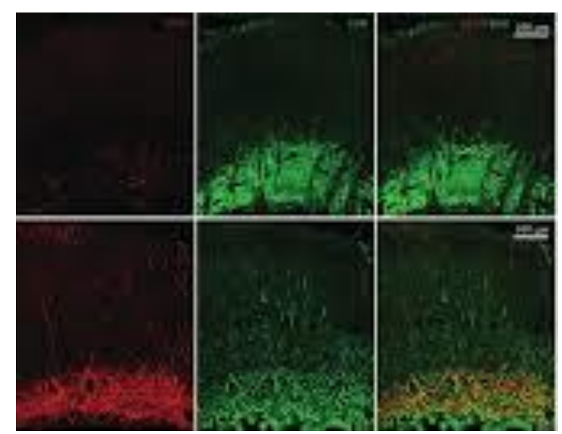


Ageing biomarkers

in the brain:

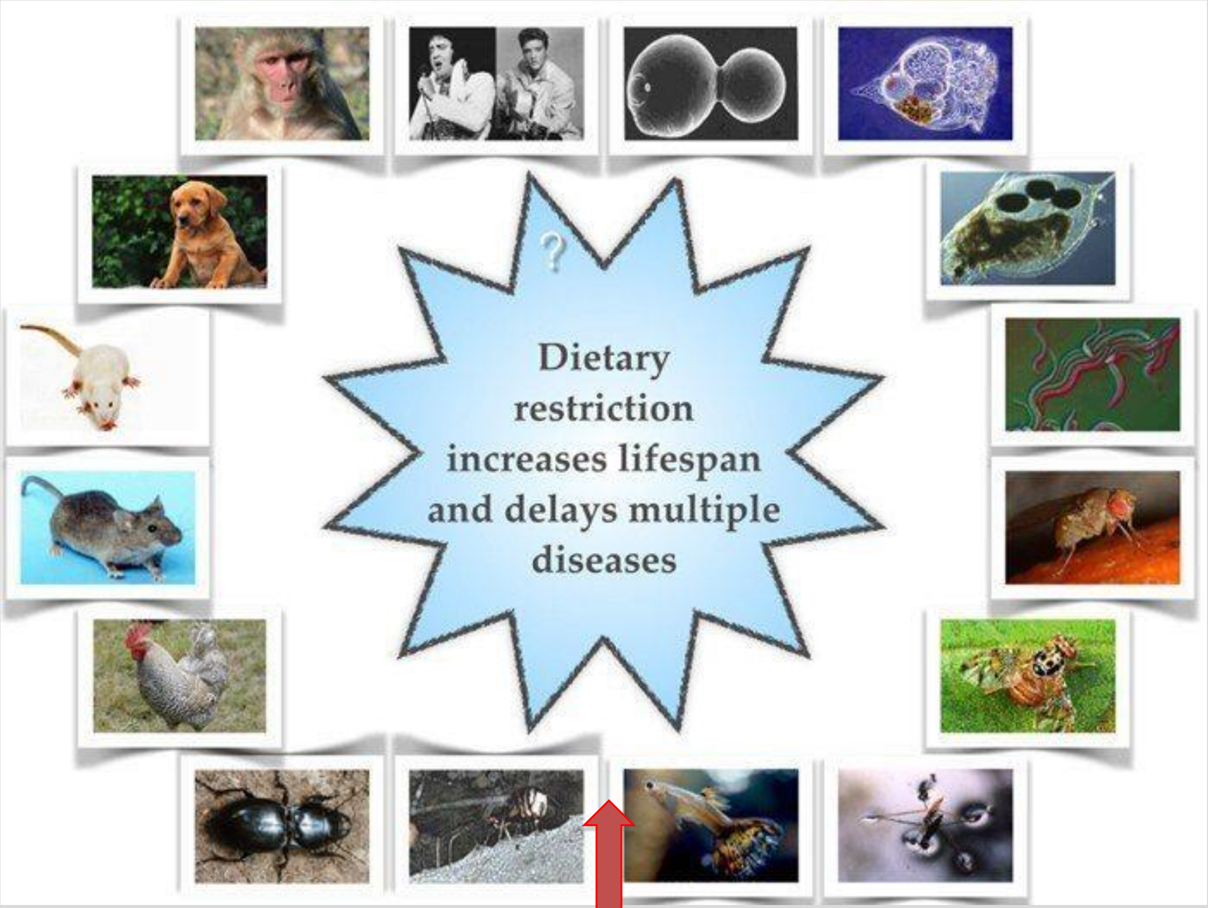


- age-related reduced mitotic activity of the neuronal progenitors
- up-regulation of GFAP, hallmark of gliosis
- neurodegeneration – measured by Fluoro-Jade B, which stains cell bodies, dendrites and axons of degenerating neurons
- accumulation of lipofuscin in the brain
- deposition of β-amyloid plaques



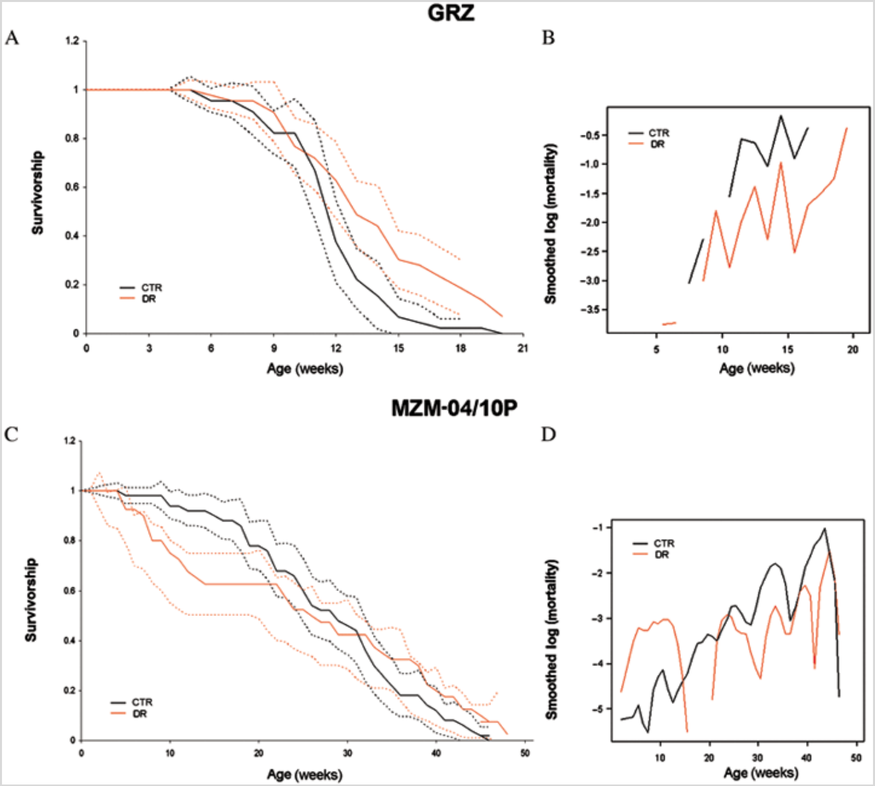
Terzibasi et al., Aging Cell (2012)

Experimental modulation of the lifespan



Experimental modulation of the lifespan

Effect of dietary restriction in N. furzeri



Terzibasi et al., Aging Cell (2009)

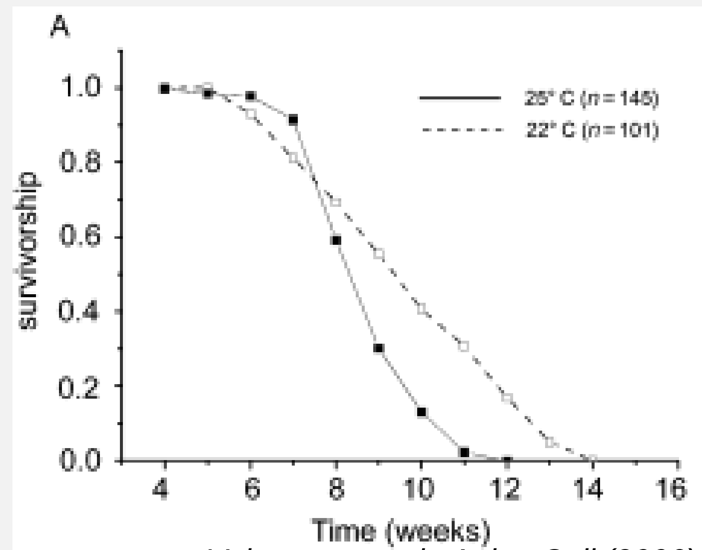
- prolonged lifespan in the short-lived GRZ strain,
- reduced neurodegeneration,
- slower accumulation of lipofuscin, improved learning performance, decreased occurrence of tumours.

Experimental modulation of the lifespan

Modulating both environmental and individual **<u>temperature</u>** has a significant impact on organism physiology and can modulate lifespan and ageing.

↓ water temperature

↑ median and maximum lifespan



Valenzano et al., Aging Cell (2006)

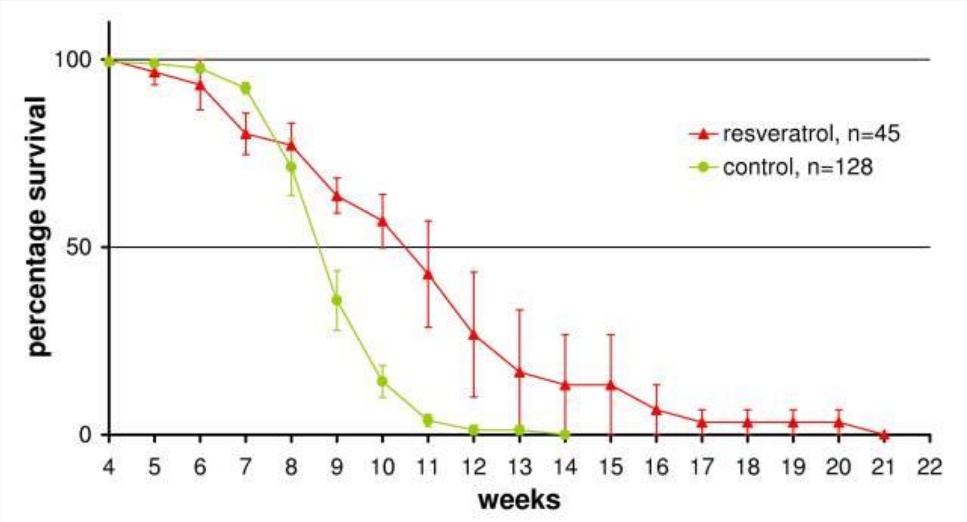
Several age-associated phenotypes
(*lipofuscin accumulation, spontaneous locomotor activity and learning performance*)
are also significantly improved in fish cultured at a lower temperature.

Experimental modulation of the lifespan

The use of the natural polyphenol **resveratrol**.

This compound retards the age-dependent decline in *N. furzeri*.

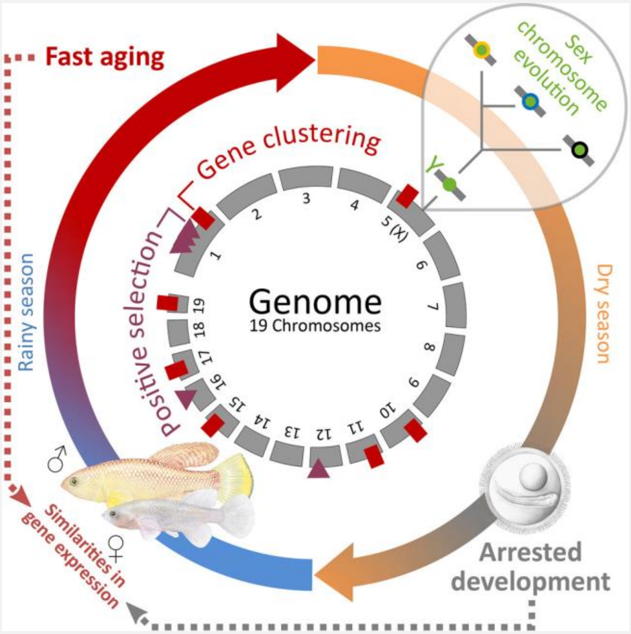
Increase median and maximum lifespan



- physically active for a longer time;
- better learning performance at later ages.

Genetic modifications

AVAILABLE ASSEMBLED GENOME and TRANSCRIPTOME



Resource

Cell

Insights into Sex Chromosome Evolution and Aging from the Genome of a Short-Lived Fish

Kathrin Reichwald,[1,14] Andreas Petzold,[1,14,16] Philipp Koch,[1,14] Bryan R. Downie,[1,14] Nils Hartmann,[1,14] Stefan Pietsch,[1] Mario Baumgart,[1] Domitille Chalopin,[2,17] Marius Felder,[1] Martin Bens,[1] Arne Sahm,[1] Karol Szafranski,[1] Stefan Taudien,[1] Marco Groth,[1] Ivan Arisi,[3] Anja Weise,[4] Samarth S. Bhatt,[4] Virag Sharma,[5,6] Johann M. Kraus,[7] Florian Schmid,[7,8] Steffen Priebe,[9] Thomas Liehr,[4] Matthias Görlach,[1] Manuel E. Than,[1] Michael Hiller,[5,6] Hans A. Kestler,[1,7,10] Jean-Nicolas Volff,[2] Manfred Schartl,[11,12] Alessandro Cellerino,[1,13,15] Christoph Englert,[1,10,15] and Matthias Platzer[1,15,*]

Resource

Cell

The African Turquoise Killifish Genome Provides Insights into Evolution and Genetic Architecture of Lifespan

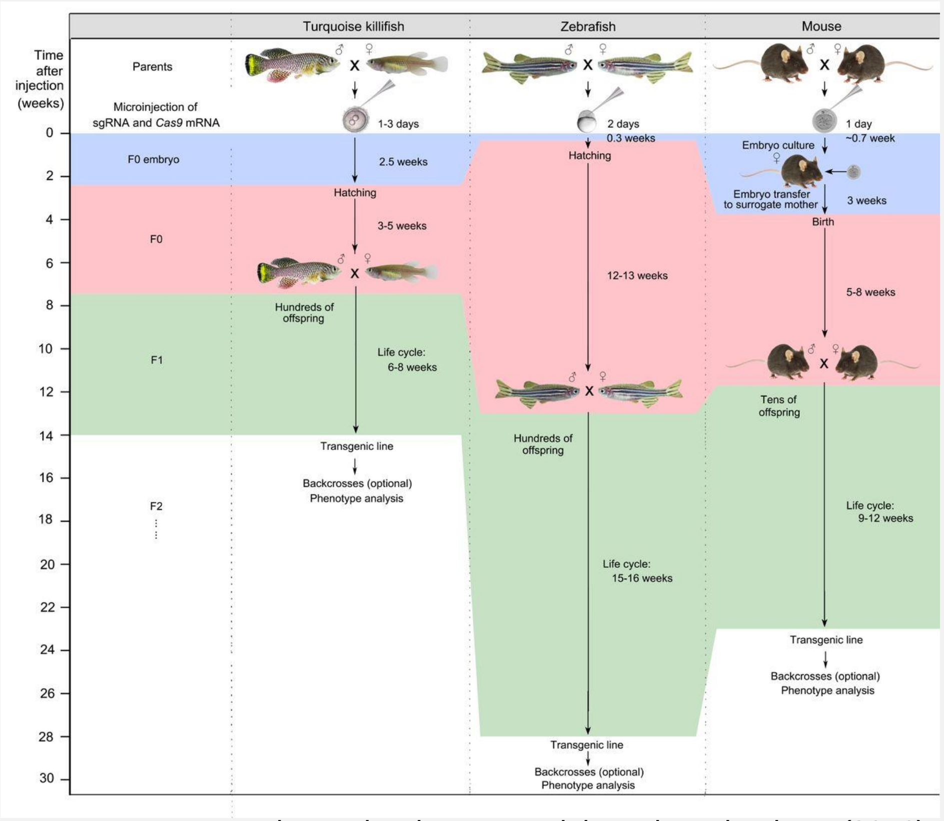
Dario Riccardo Valenzano,[1,7,8,*] Bérénice A. Benayoun,[1,7] Param Priya Singh,[1,7] Elisa Zhang,[1] Paul D. Etter,[2] Chi-Kuo Hu,[1] Mathieu Clément-Ziza,[3] David Willemsen,[4] Rongfeng Cui,[4] Itamar Harel,[1] Ben E. Machado,[1] Muh-Ching Yee,[1,9] Sabrina C. Sharp,[1] Carlos D. Bustamante,[1] Andreas Beyer,[5] Eric A. Johnson,[2] and Anne Brunet[1,6,*]

Genetic modifications

Two methods successfully developed to modify *N. furzeri* genome:

I. random genome integration through the **Tol2 DNA transposase**
II. targeted genome editing using **CRISPR/Cas9** nuclease

Both methods require microinjection into the one-cell-stage embryo.



Kim et al., Disease Models and Mechanisms (2016)

Looking forward: potential applications and….

- **Time saving in routinely laboratory activities and consequently research achievements.**
- **Uniquely combines a short lifespan and life cycle with vertebrate-specific features, missing from the currently used non-vertebrate model organisms.**
- **Genomic and transcriptome data analysis have revealed many orthologous genes to humans and other model organisms.**
- **Several age-related pathways between *N. furzeri* and humans are conserved.**
- **Fish models are cheaper, less space demanding and much more prolific than murine models (studies involing 1000s of adult animals are affordable to „normal“ labs)**

….limitations of *N. furzeri* model

- **Lack of important organs (lungs, uterus, mammary glands...).**
- **Quality of genome reference sequences inferior to mice.**
- **Genome duplication! About 30% of the human genes have two orthologs in fish.**
- **Husbandry and management require good knowledge of the biology of species.**