

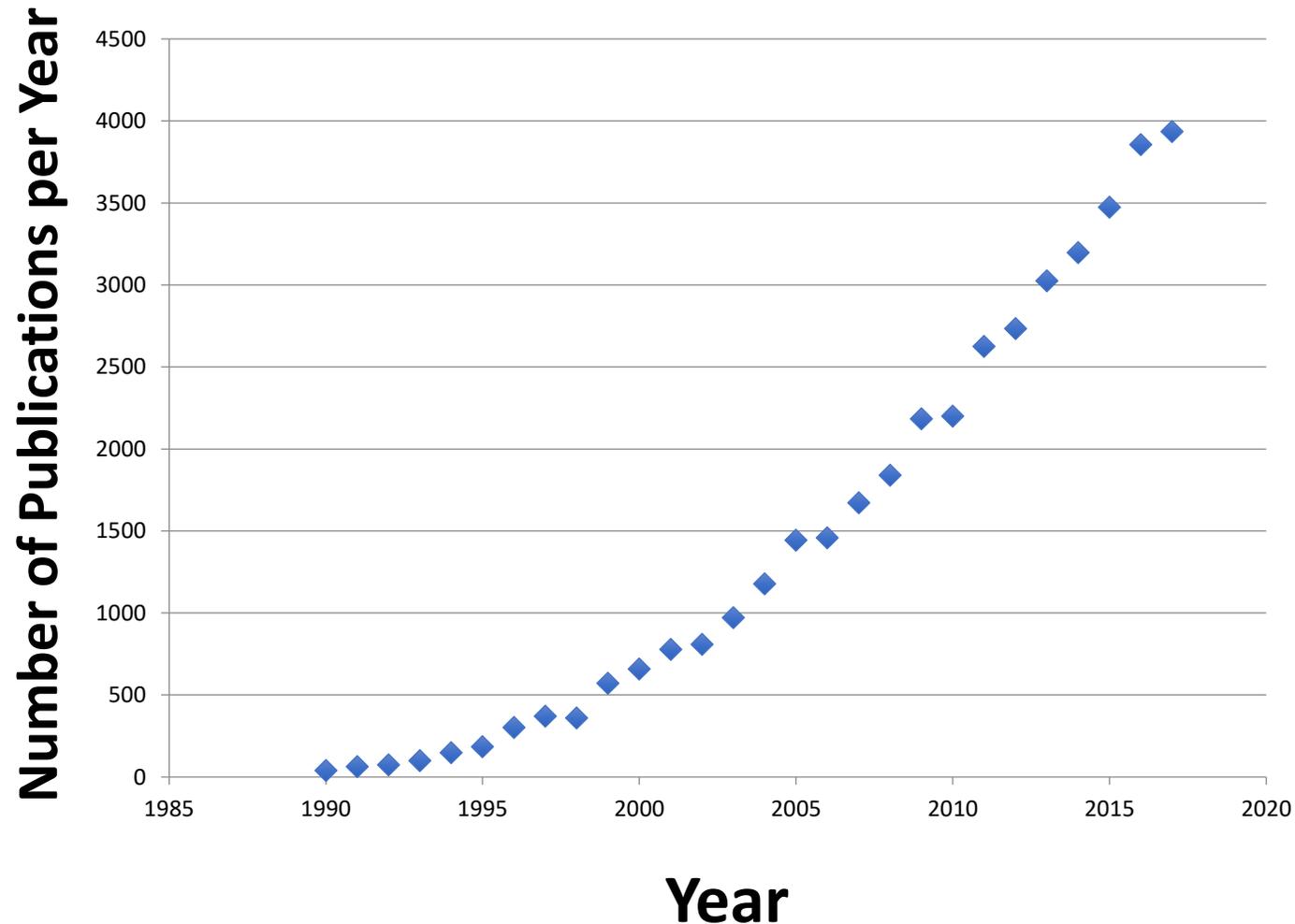
# Fishes as experimental animals

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Agricultural Sciences, Sweden

# Zebrafish (*Danio rerio*) one of the most important model organisms



**Next to mouse zebrafish is the most common experimental animal in Sweden**

**About 40 000 zebrafish/year**



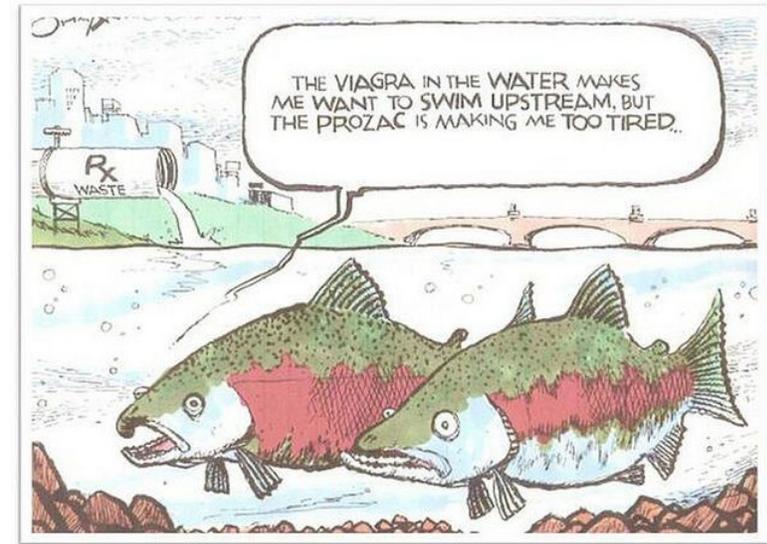
# The comparative approach



**August Krogh** (1874 – 1949),  
professor at the department  
of zoophysiology, Copenhagen  
University, Nobel Prize in  
Physiology or Medicine 1920

**Krogh's principle** states that "for such a large number of problems there will be some animal of choice, or a few such animals, on which it can be most conveniently studied."

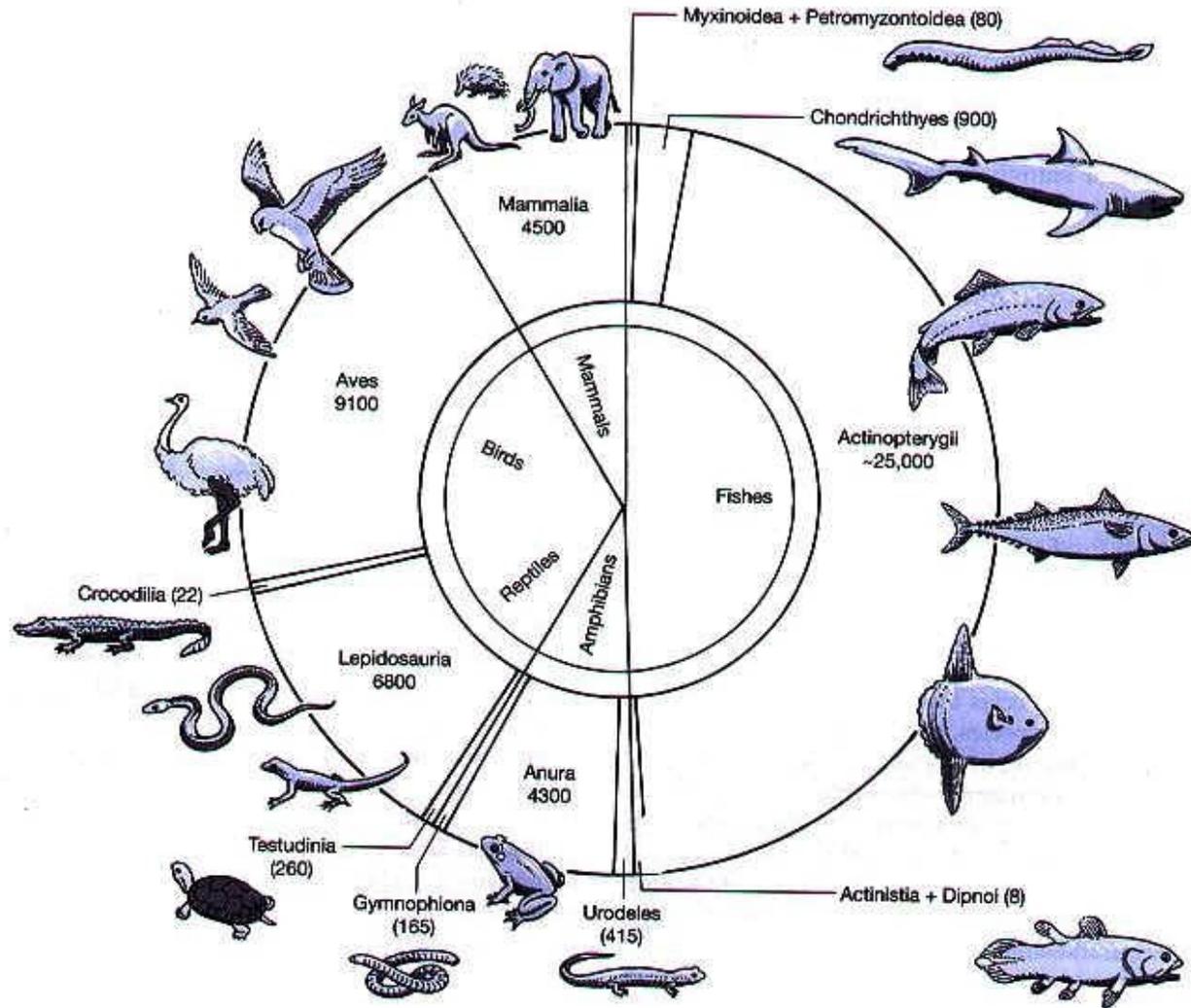
# Why fish?



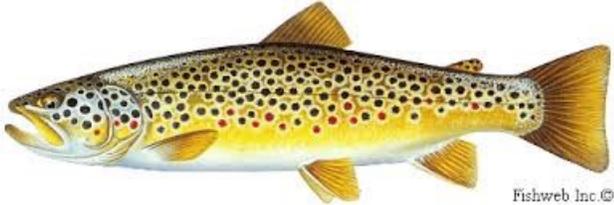
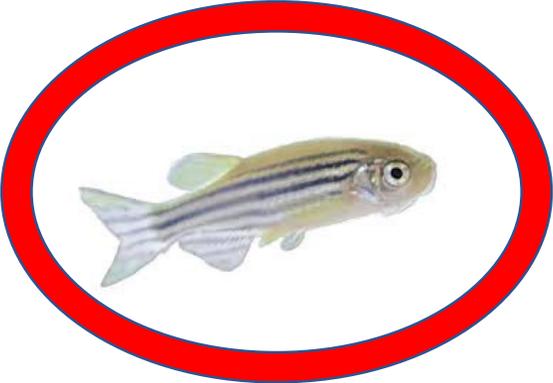
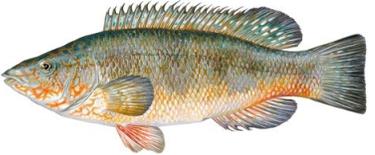
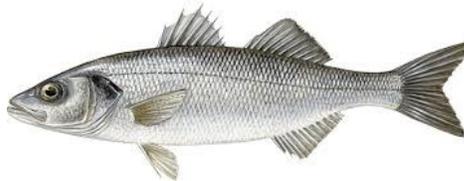
Fish as a model and fish as a fish



# The typical vertebrate has to be a fish



# Some of the species we worked on



## Three-spined stickleback (*Gasterosteus aculeatus*)



- An extremely well studied species
- Very popular in behavioral studies
- Becoming popular in toxicological studies
- Genome sequenced

# Stickleback – the white rat of ethology

The percentage of papers using sticklebacks as subjects published in *Animal Behaviour* in each decade, classified by broad subject matter (Huntinford, Anim. Behav. (2003) 66, 409-415)

Decade	Aggression	Reproductive behavior	Foraging	Antipredator behavior
1950/60s	57	43		
1970s	40	34	13	13
1980s	24	34	12	30
1990s	23	26	23	28
2000s*	30	50	20	0

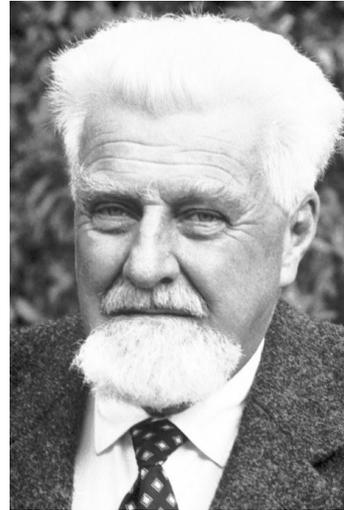
\*only 2000-2002

# The Nobel Prize in Physiology or Medicine 1973

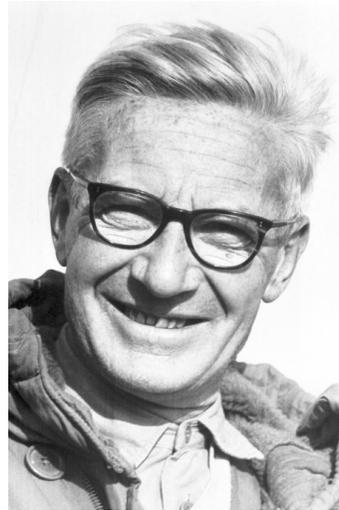
## The founders of ethology



Karl von Frisch



Konrad Lorenz



Niko Tinbergen



Stickleback male in spawning coloration  
(<https://www.uni-muenster.de/news/view.php?cmdid=9647>)

# Other interesting fish models

## Killifish



The African turquoise killifish, *Nothobranchius furzeri*, is with its short life span (3-9 months) an interesting model for aging, epigenetics, cancer etc

## Cichlids (>2000 species)



Tanganyika cichlid, *Astotilapia burtoni*. Complicated social behaviour. Social control of male sexual maturation. Broad care etc.

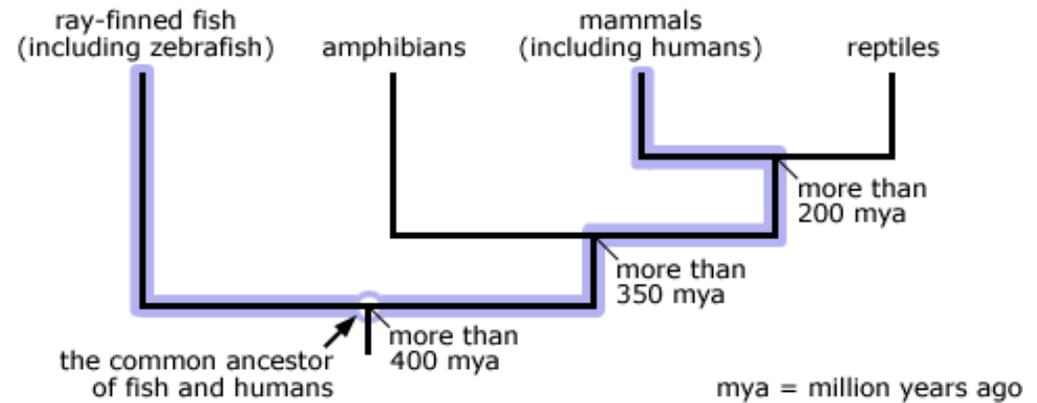


@Dily Sen Sarma

# Zebrafish and Human

## How similar are we?

- Both vertebrates
- Common ancestor 400 million years ago
- Still 70% identical at the gene level
- Large similarities in brain organisation and function



# Zebrafish in its natural environment

Fredrick Jutfelt, Associate Professor  
at the Department of Biology at NTNU, Norway



# Large zebrafish facilities



Uppsala University just opened a new zebrafish facility for 6 000 fish tanks, automatic feeding etc.

# Zebrafish welfare in large facilities

Water quality and nutrition is usually not a problem

- Barren environment
- Social stress
- Handling of fish

# Why enrichment?

- Obligated by law
- Increase environmental complexity
- Provide shelter
- Enriched environment preferred by the fish
- Appear to give increased welfare, i.e. higher survival, increased growth and reduced “anxiety-like” behaviour in novel tank diving test

Original Article

# What do zebrafish want? Impact of social grouping, dominance and gender on preference for enrichment

Paul Schroeder<sup>1</sup>, Soffia Jones<sup>2</sup>, Iain S Young<sup>2</sup> and Lynne U Sneddon<sup>2</sup>

Abstract

Laboratory Animals

limited

Laboratory Animals

2014, Vol. 48(4) 328–337

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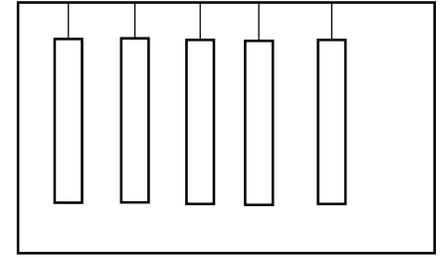
DOI: [10.1177/0023677214538239](https://doi.org/10.1177/0023677214538239) [la.sagepub.com](http://la.sagepub.com)



# Problems with enrichment

- Plastic enrichment may release toxic substances (plasticisers, metal ions) to the water
- Difficult to clean and exchange – bad water quality
- Easily monopolised by dominant fish – aggression and stress
- Disturbance of water circulation and possibly foraging

# Optimized enrichment



- Non-toxic material – medical grade silicone
- Sheets attached to the lid, leaving water surface and bottom free
- Will be impossible for dominant fish to monopolize
- How many sheets per tank?
- Large number of sheets more structure but may interfere with shoaling
- Colour of the sheets (green?). Semi-transparent sheets will make visual inspection easier

# How large tanks and how many fish in a tank?

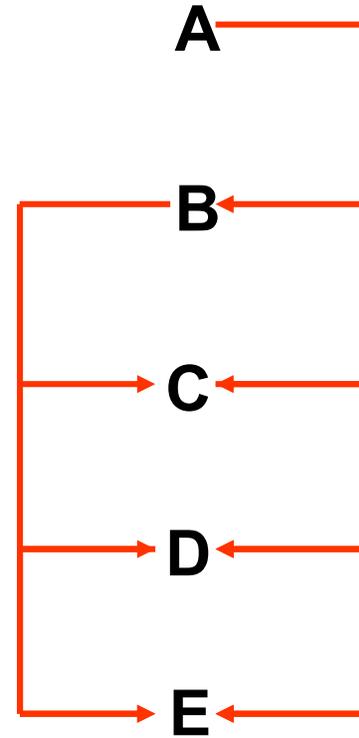
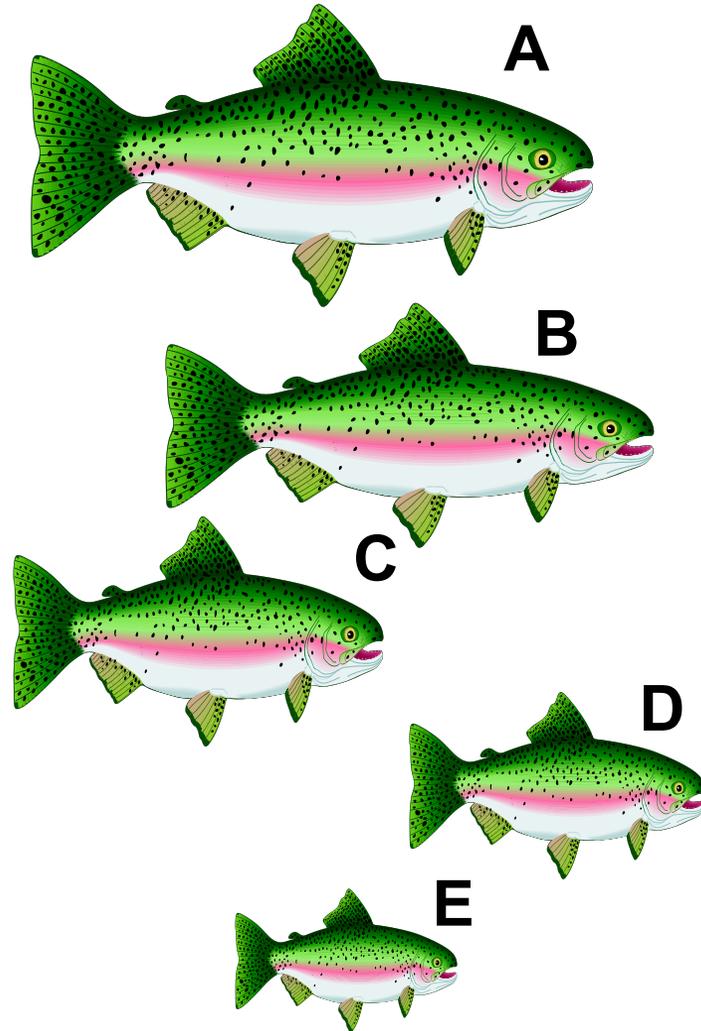


# Aggressive behavior in zebrafish



Clara Jeong

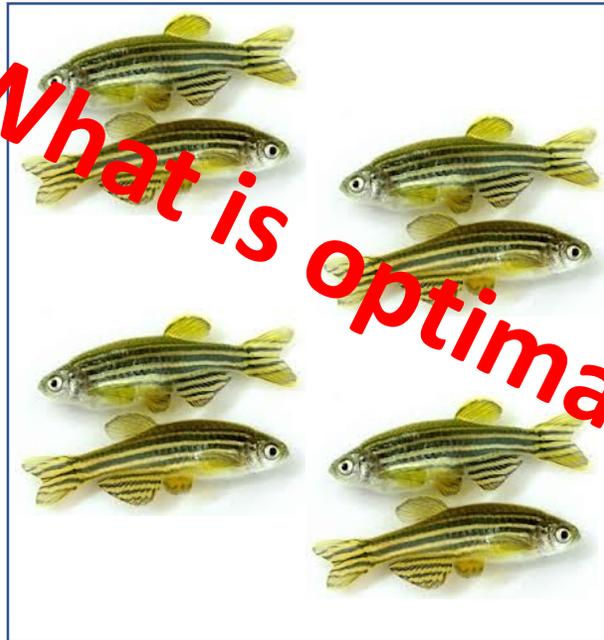
# Dominance hierarchies



# Social interactions



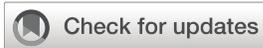
**Low density**  
**Aggressive behavior**  
**Intense stress**  
**Bad welfare**



**Optimal density**  
**Low aggression**  
**Good welfare**



**High density**  
**Low aggression**  
**Crowding stress**  
**Bad welfare**



#### OPEN ACCESS

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RECEIVED 11 April 2023

# Optimizing zebrafish rearing—Effects of fish density and environmental enrichment

Oly Sen Sarma<sup>1</sup>, Natalia Frymus<sup>1</sup>, Fredrik Axling<sup>2</sup>,  
Per-Ove Thörnqvist<sup>1</sup>, Erika Roman<sup>3,4</sup> and Svante Winberg<sup>1,3\*</sup>

<sup>1</sup>Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden, <sup>2</sup>Department of Surgical Sciences, Uppsala University, Uppsala, Sweden, <sup>3</sup>Department of Anatomy, Physiology, and Biochemistry, Swedish University of Agricultural Sciences, Uppsala, Sweden, <sup>4</sup>Department of Pharmaceutical Biosciences, Uppsala University, Uppsala, Sweden



# Stress responses

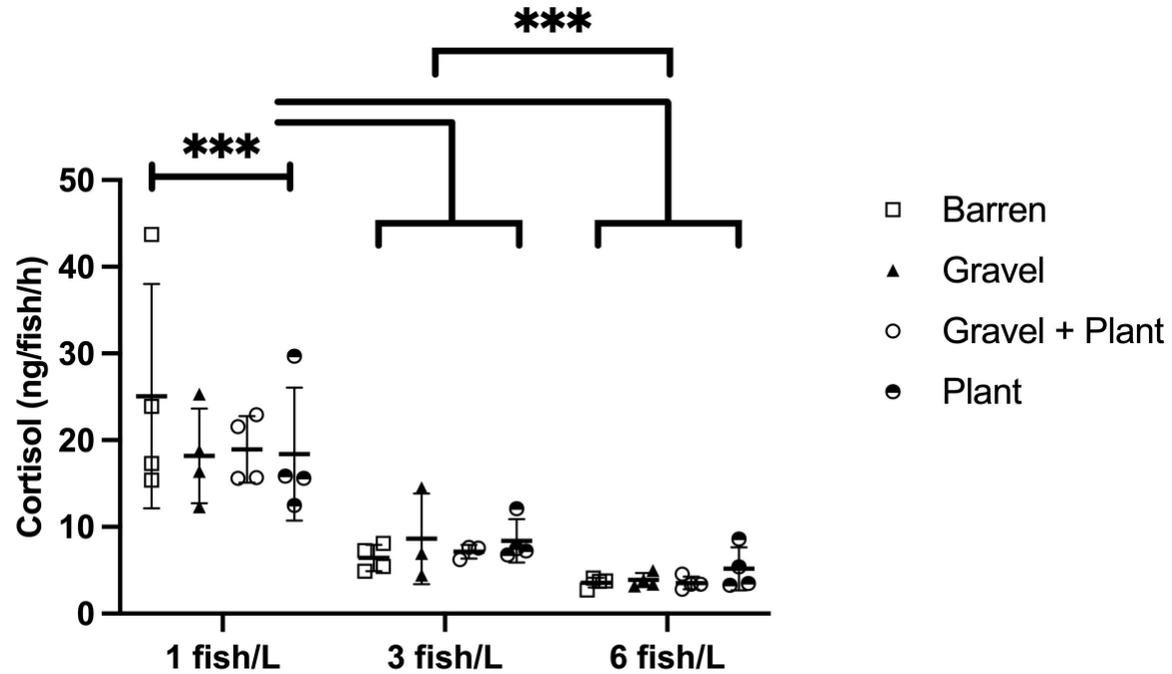
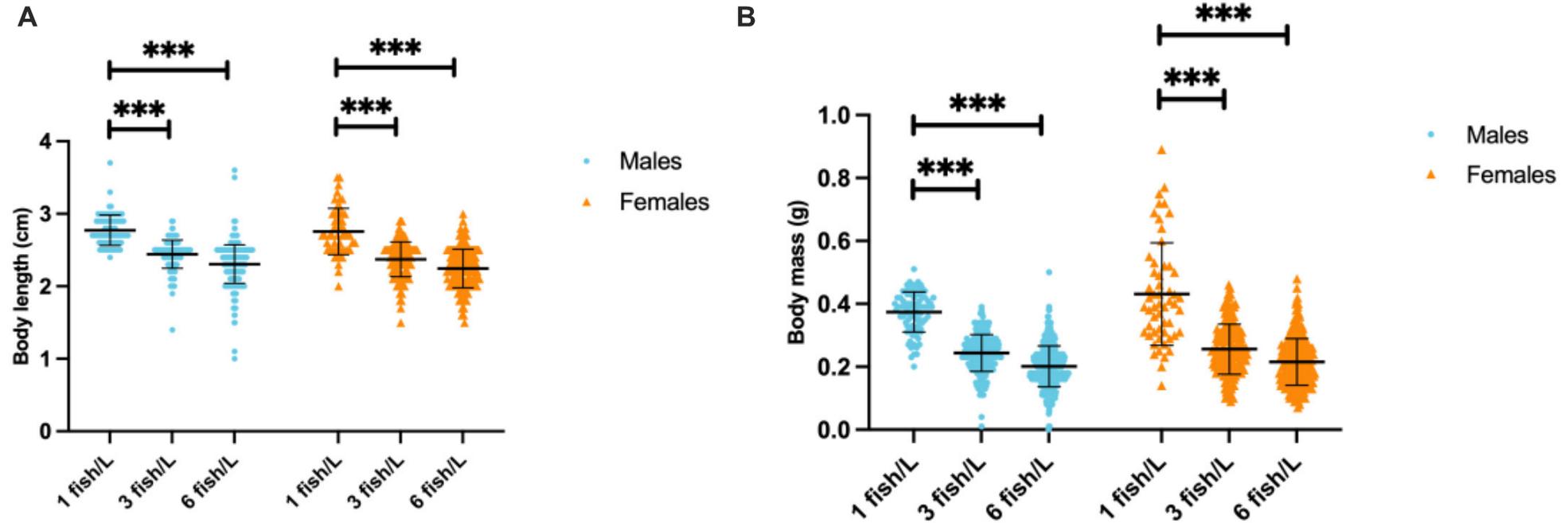


FIGURE 3

Cortisol secretion to surrounding water by zebrafish kept at different stocking densities with or without environmental enrichment. Groups of fish were subjected to confinement by lowering of the water level to 1 cm for 30 min. Values are shown as individual tanks with mean marked as line and SD in whiskers. \*\*\* $p < 0.001$  compared to fish kept at 3 or 6 fish/L.

# Lower growth at higher densities

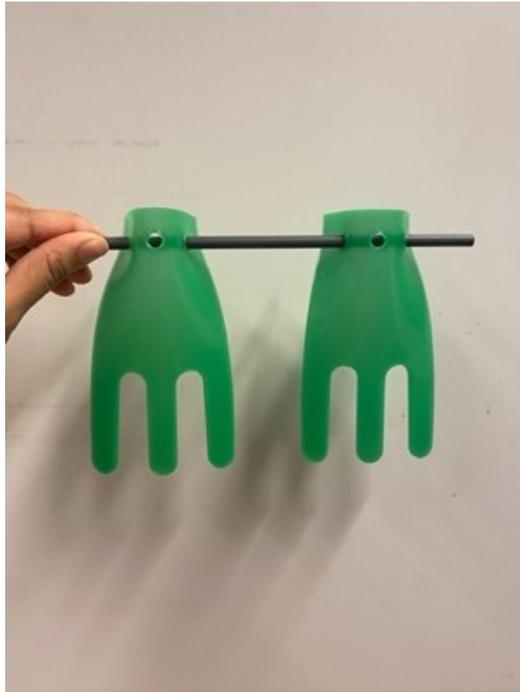


Sen Sarma et al. (2023) Front. Behav. Neurosci. DOI10.3389/fnbeh.2023.1204021

# How to design an environmental enrichment?

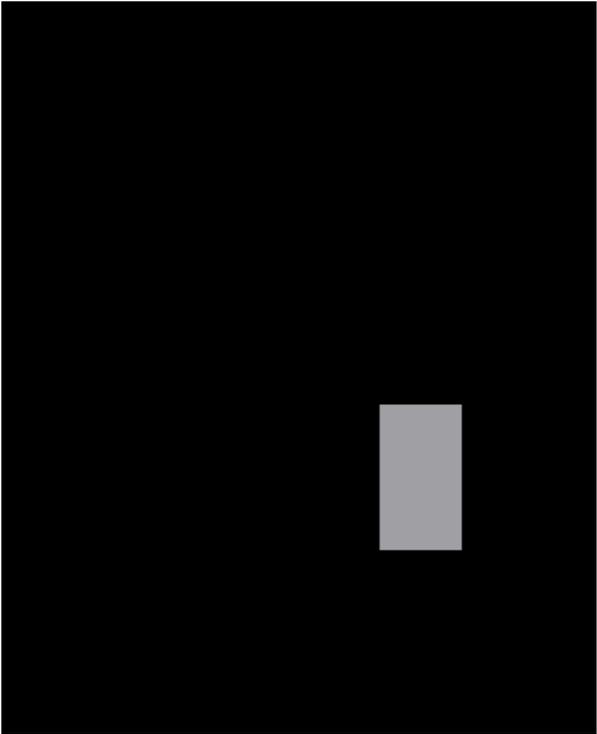
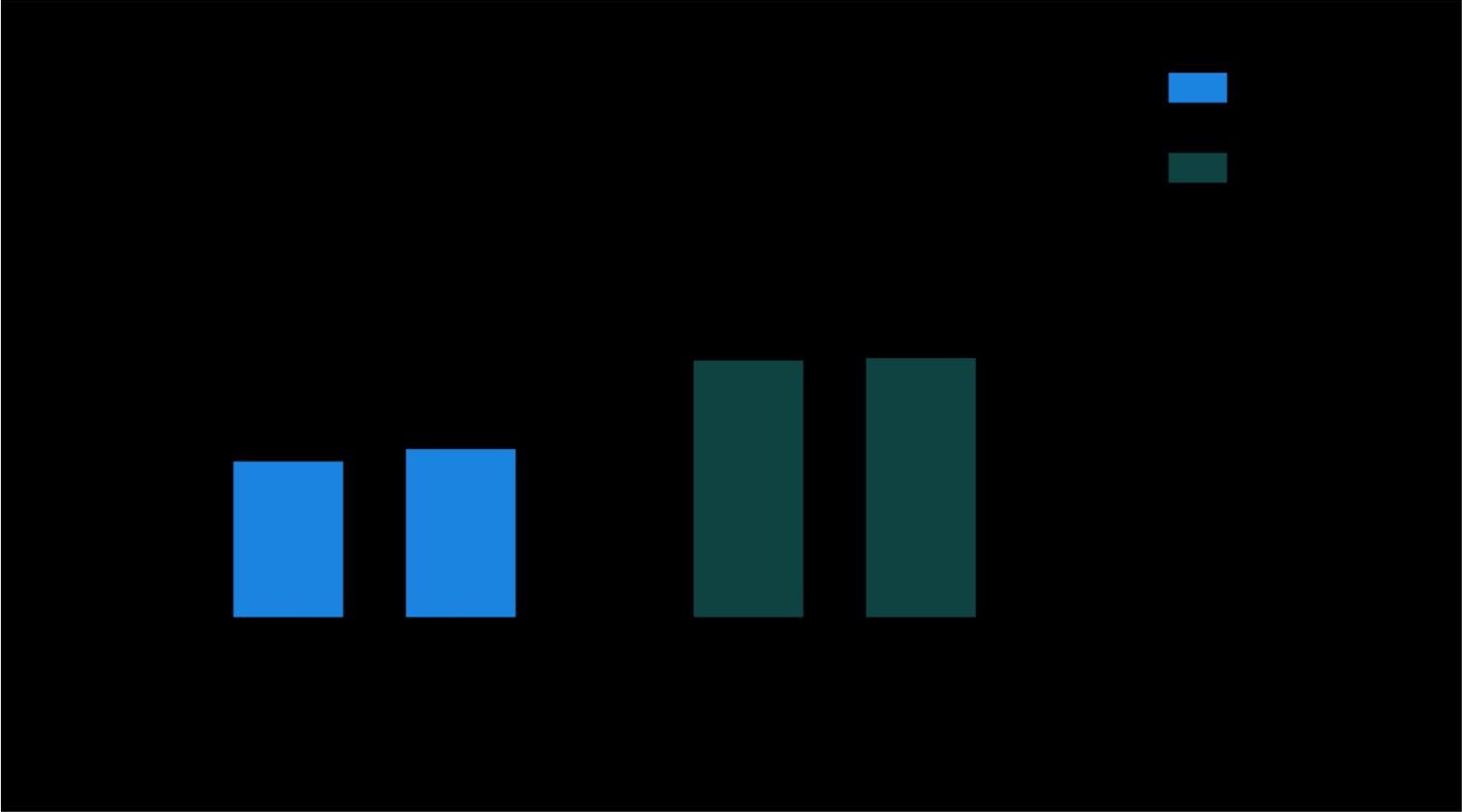
- Inert material that does not release any chemicals to the water
- Semitransparent to allowed observation of the fish
- Avoid monopolisation by dominant fish
- Adjusted according to fish density and tank size
- Easy to exchange and clean

# Environmental enrichment made of medical silicone



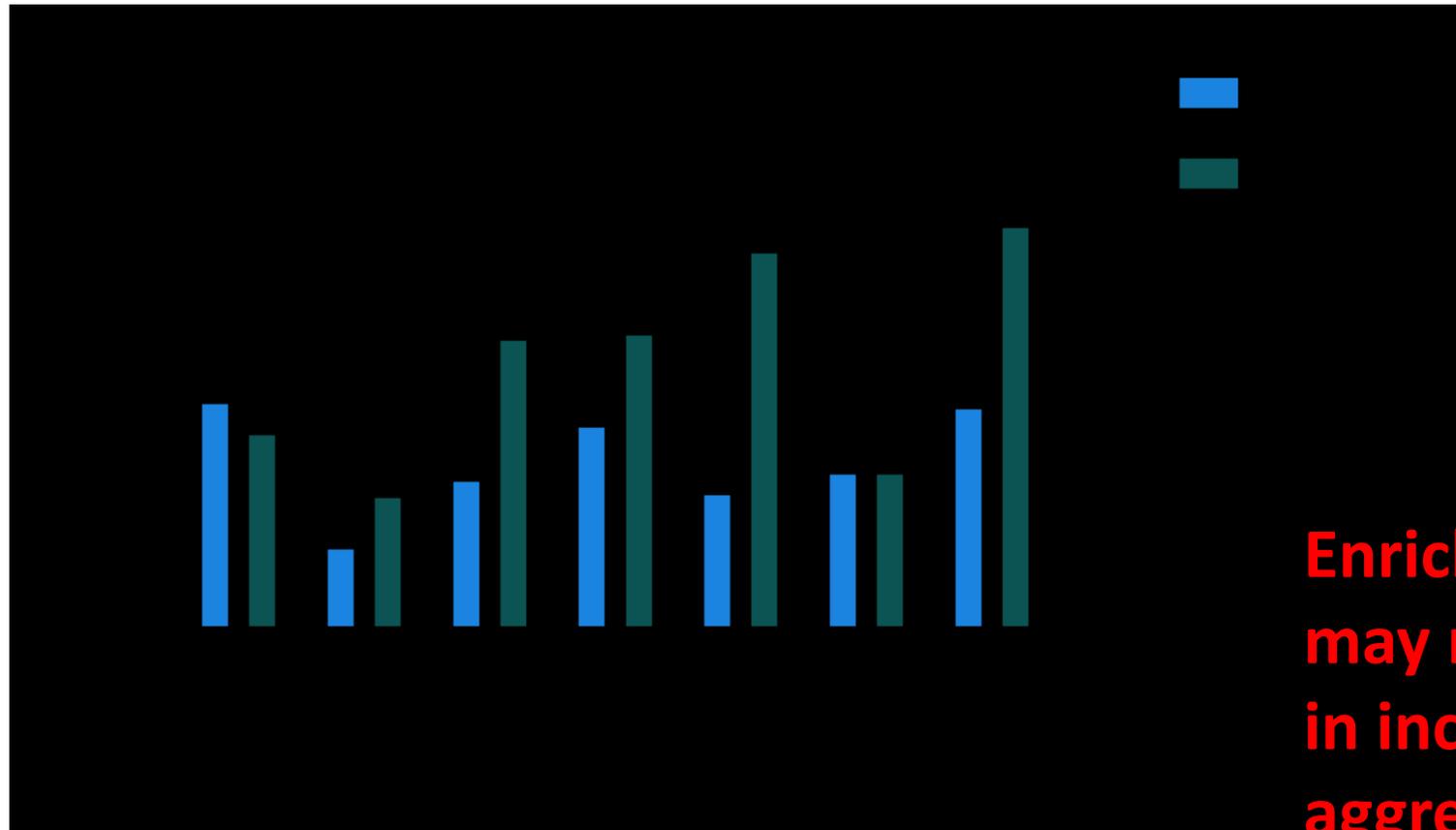
Sen Sarma et al. unpublished

# Tank size and enrichment



Sen Sarma et al. unpublished

# Environmental enrichment and aggression



**Enrichment  
may result  
in increased  
aggression**

Sen Sarma et al. unpublished

# Isolation of zebrafish

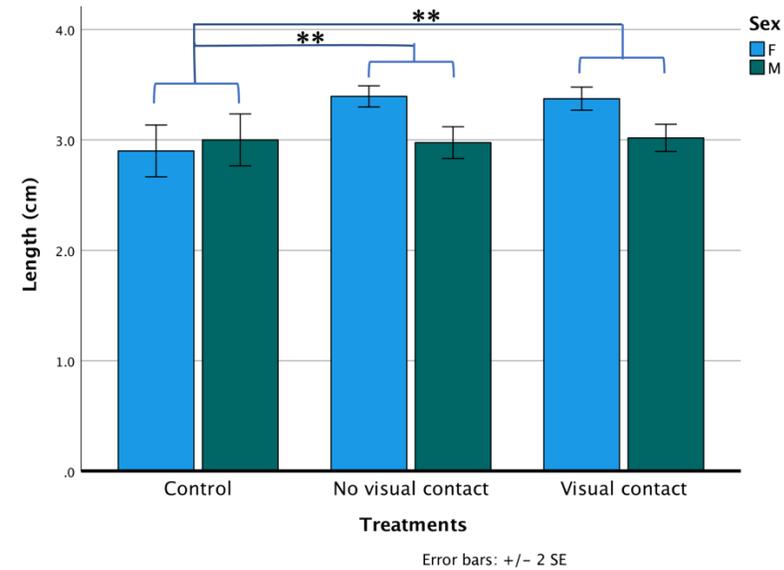
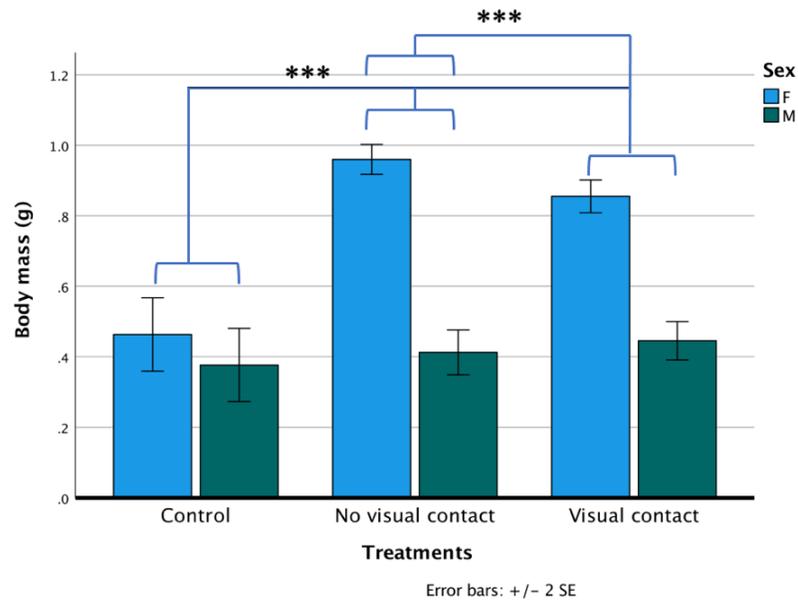
- Behaviour of individual fish
- Sampling, DNA etc
- Treatment etc



**Isolation with  
or without  
visual contact**



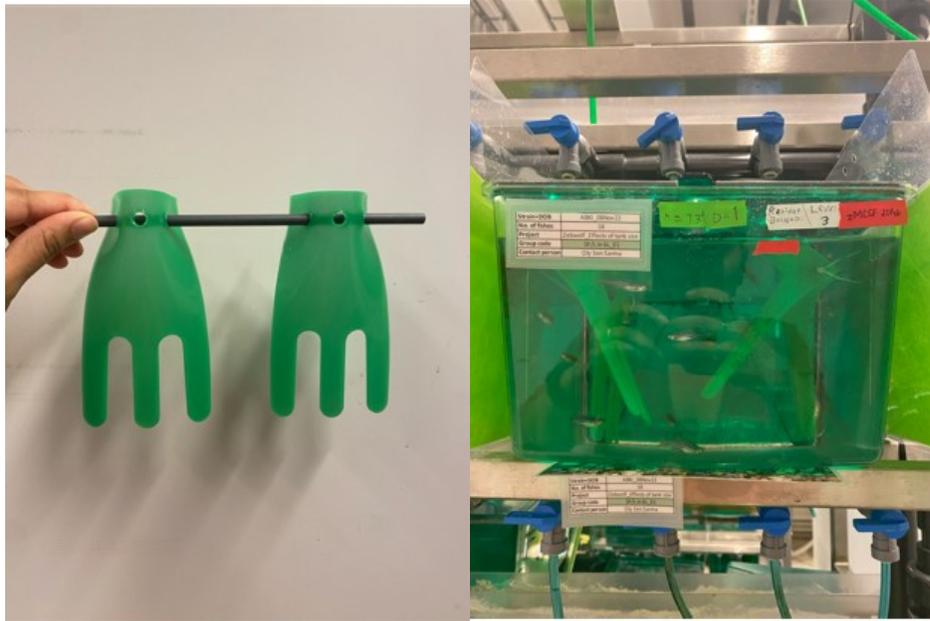
# Long-term isolation



Sen Sarma et al. unpublished

**Isolation resulted in increased growth**

# New 3R project: Optimizing environmental enrichment for zebrafish



**Medical silicone**



**Enrichment manufactured by  
Tecniplast**

**Erika Roman, SLU, Lars Bräutigam, KI; Lynne Sneddon, GU  
Sofie Kromann and Giordani Rubegni, Scanbur;**

# Comercially available enrichment



Datesand group, UK

# Summary

- Zebrafish is rapidly becoming the most important vertebrate model organism
- Most likely the importance of other fish species as experimental animals will increase in the near future
- In modern zebrafish facilities water quality, hygiene, nutrition is optimised and do not challenge fish welfare
- However, welfare can be compromised by barren environment, crowding, social interaction (low density)
- Environmental enrichment should be made of inert materials and designed to allow easy cleaning
- Enrichment must be adjusted to tank size, stocking density and fish behaviour

# Acknowledgements

Pavla Hubena Post doc

Oly Sen Sarma PhD-student

Professor Erika Roman, SLU



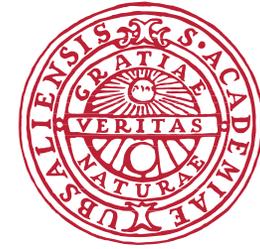
## The Swedish Research Council



## The Facias Foundation



Thank you for listening  
Questions?



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