

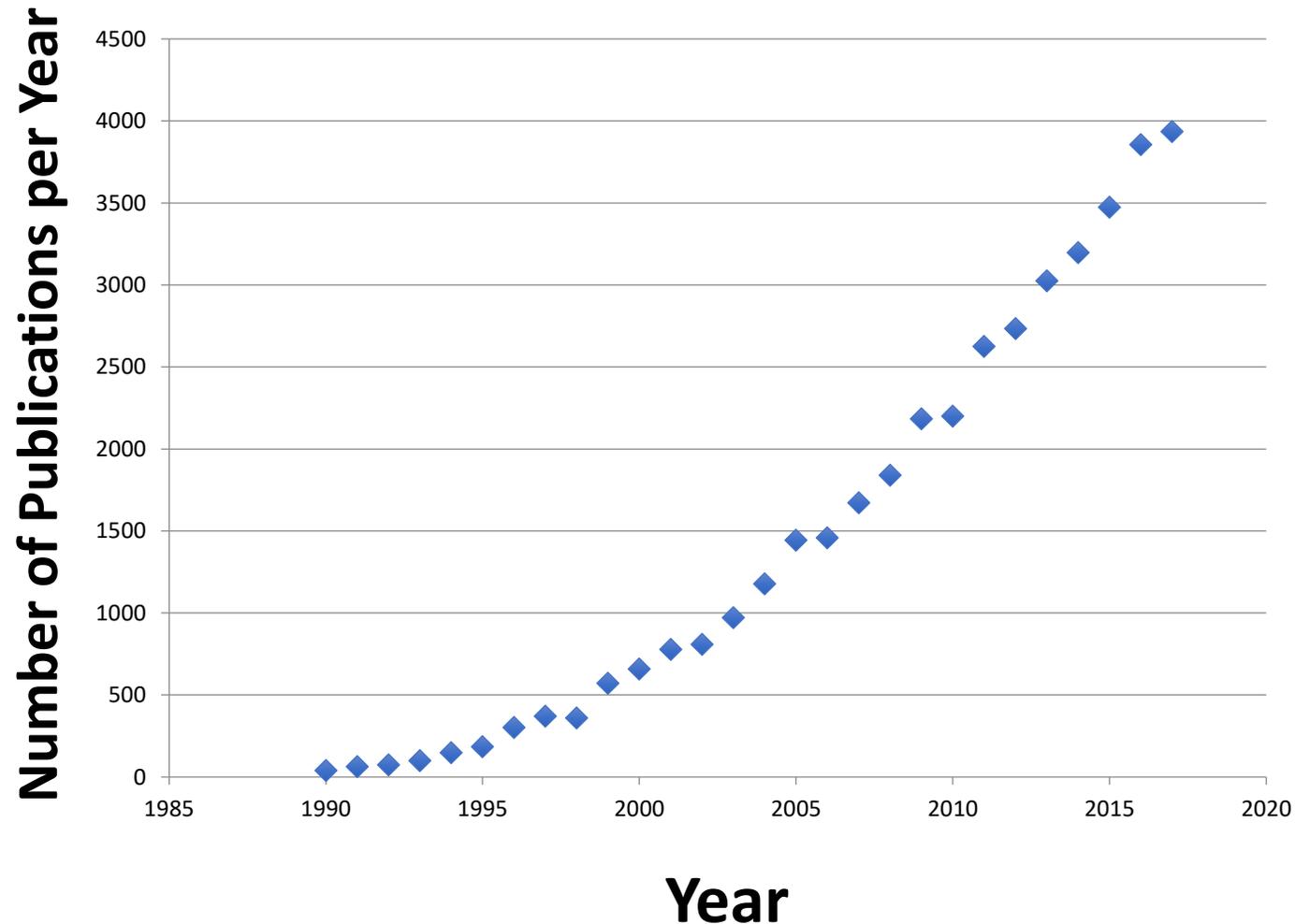
Fishes as experimental animals

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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



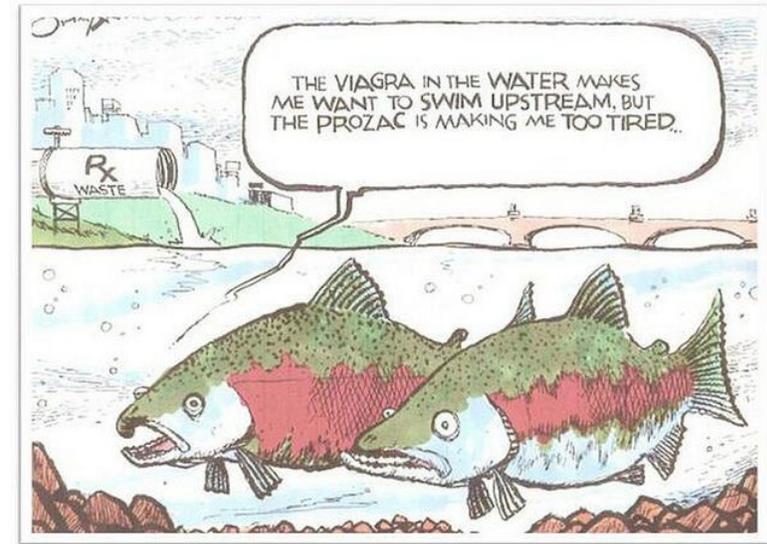
The comparative approach



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."

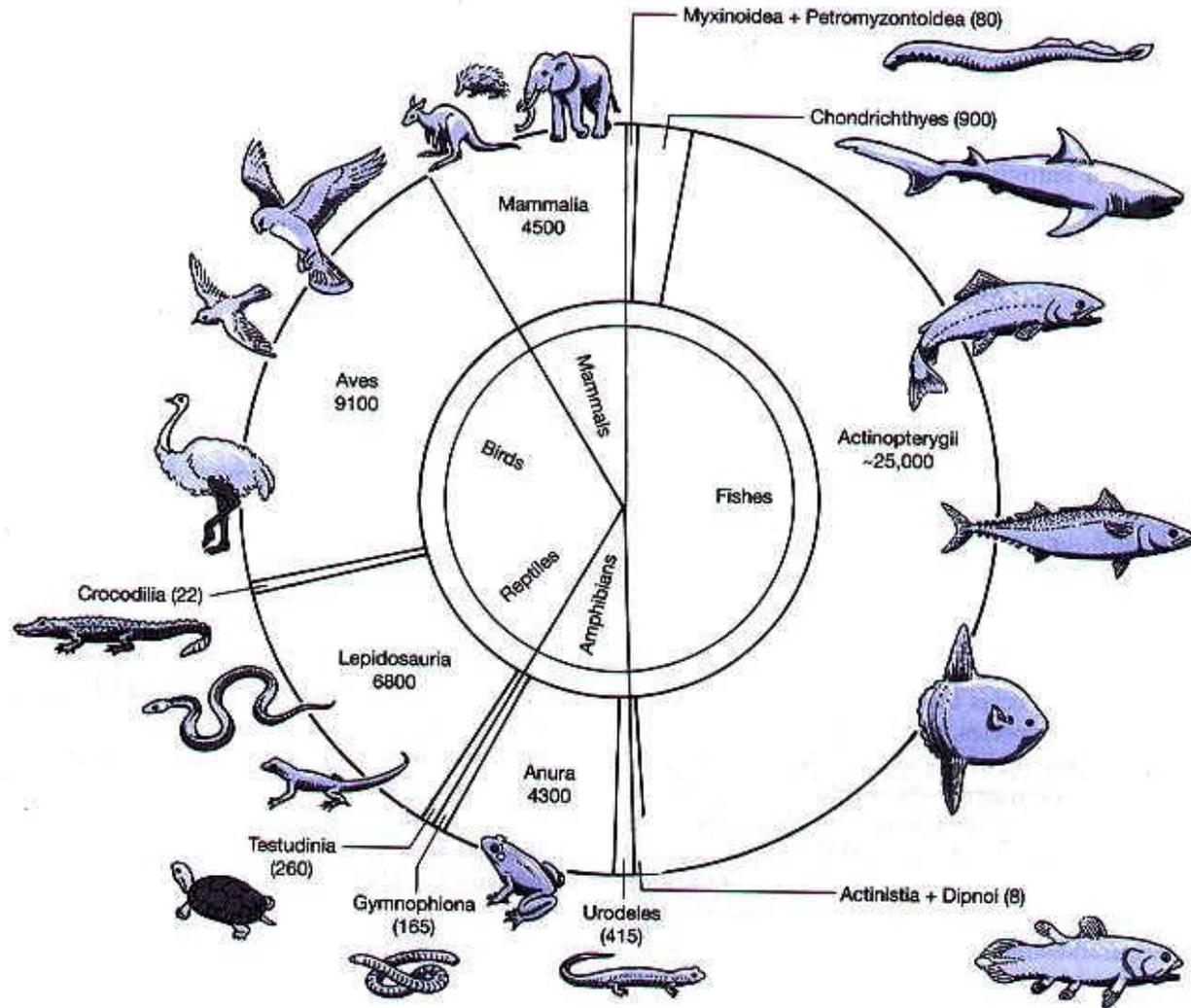
Why fish?



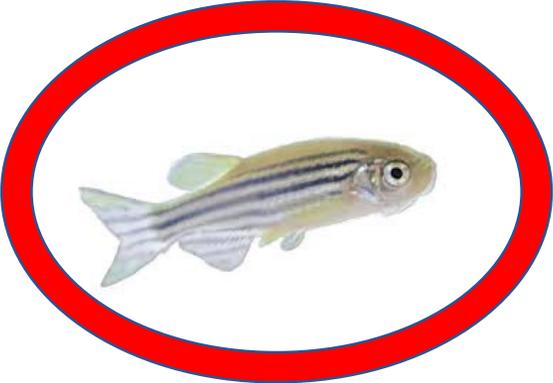
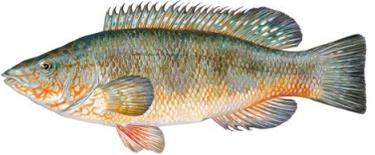
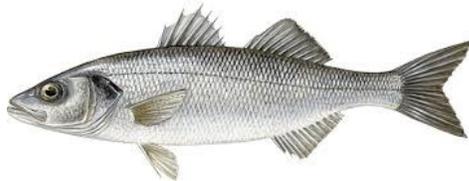
Fish as a model and fish as a fish



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

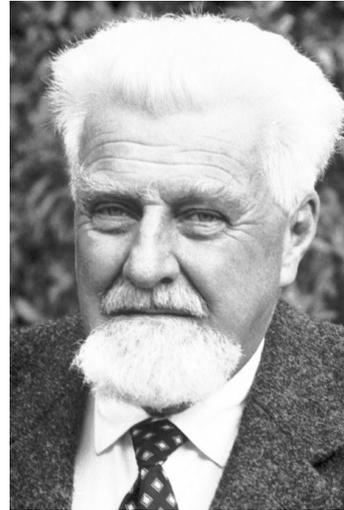
*only 2000-2002

The Nobel Prize in Physiology or Medicine 1973

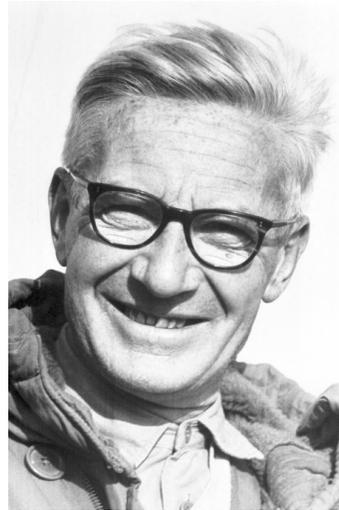
The founders of ethology



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.

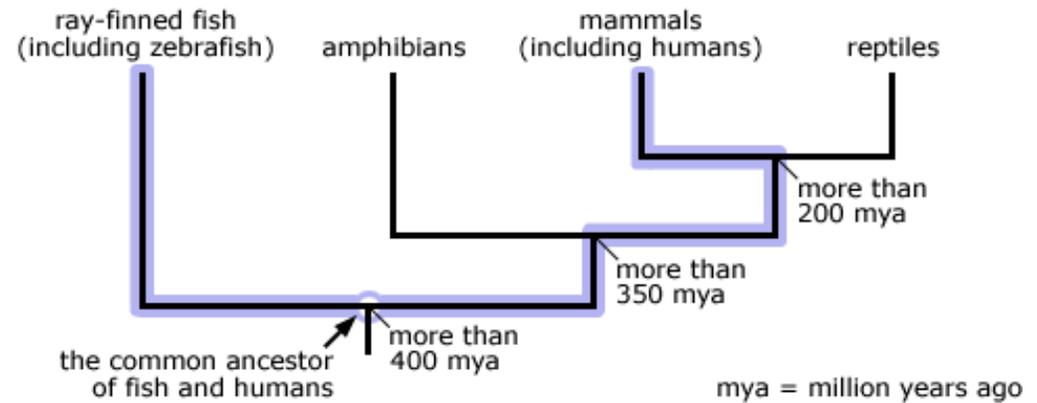


@Dily Sen Sarma

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 Schroeder¹, Soffia Jones², Iain S Young² and Lynne U Sneddon²

Abstract

Laboratory Animals

limited

Laboratory Animals

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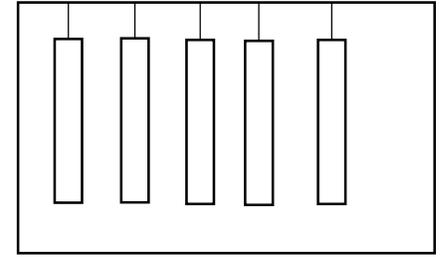
DOI: [10.1177/0023677214538239](https://doi.org/10.1177/0023677214538239) la.sagepub.com



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?

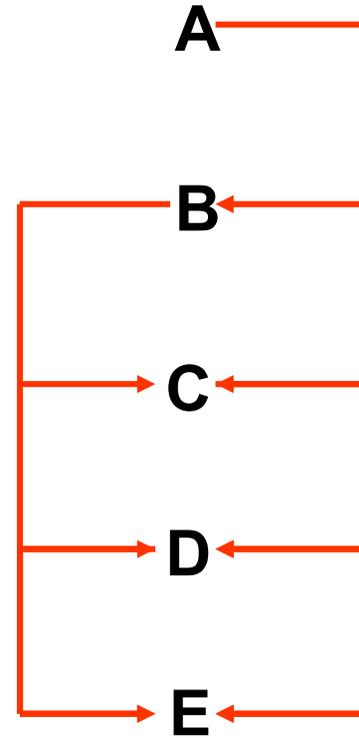
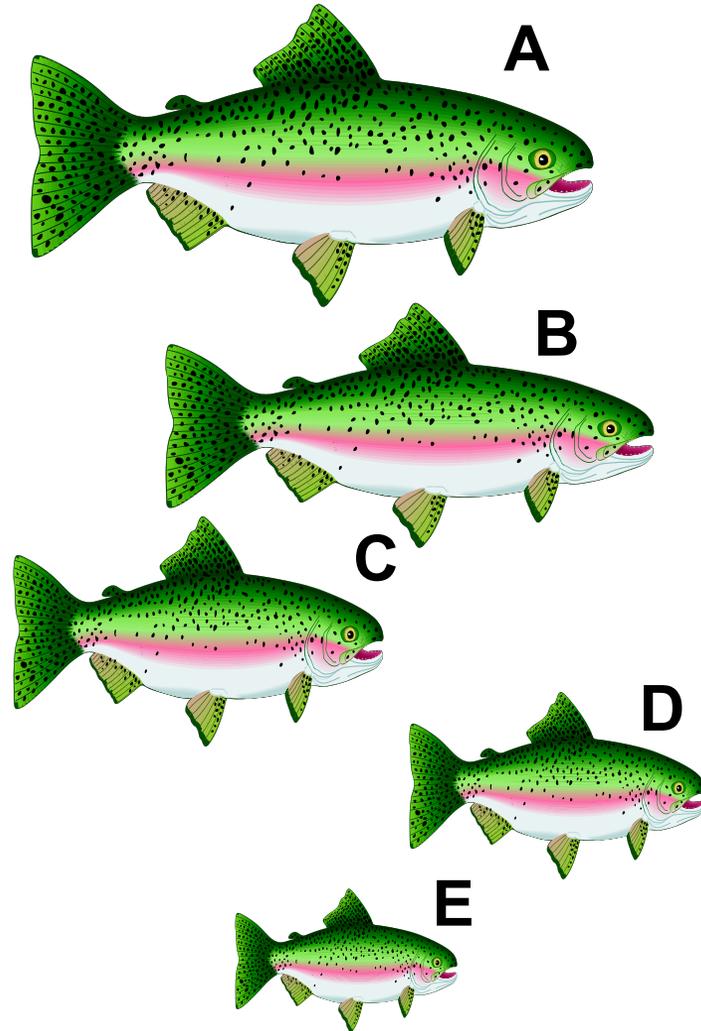


Aggressive behavior in zebrafish



Clara Jeong

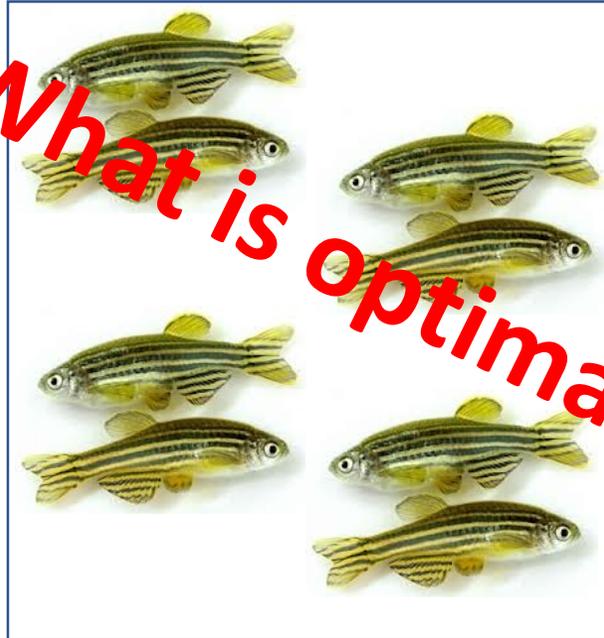
Dominance hierarchies



Social interactions



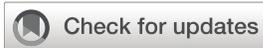
Low density
Aggressive behavior
Intense stress
Bad welfare



Optimal density
Low aggression
Good welfare



High density
Low aggression
Crowding stress
Bad welfare



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Optimizing zebrafish rearing—Effects of fish density and environmental enrichment

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Per-Ove Thörnqvist¹, Erika Roman^{3,4} and Svante Winberg^{1,3*}

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Stress responses

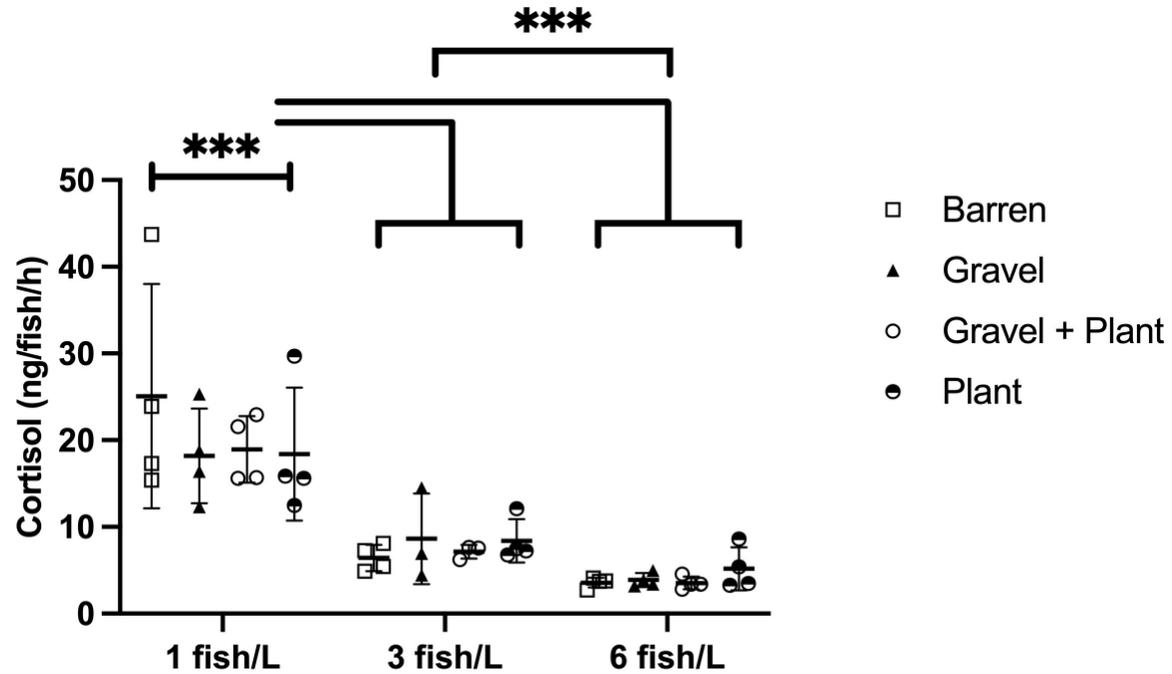
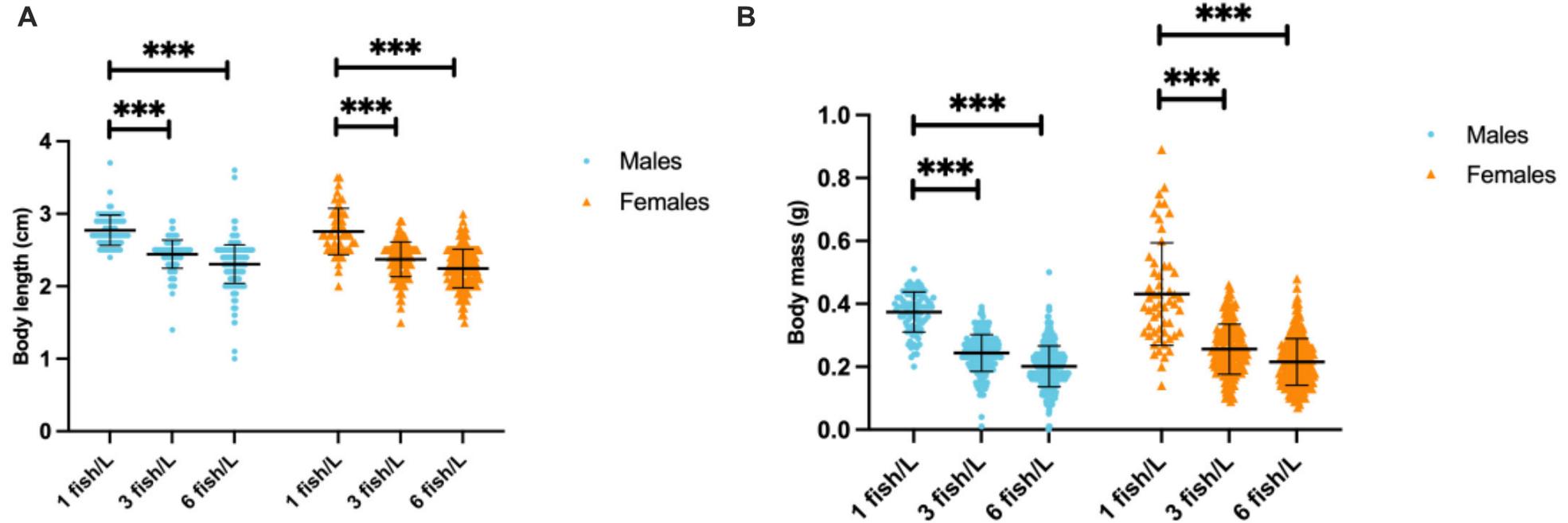


FIGURE 3

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?

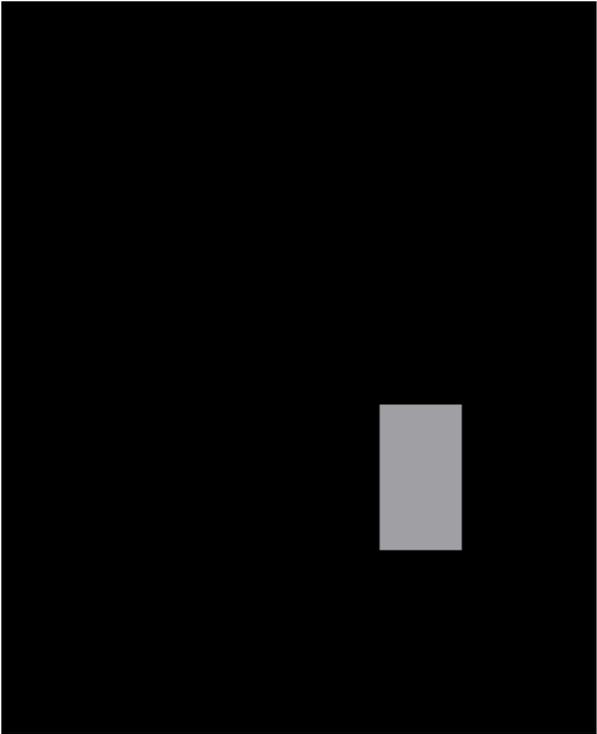
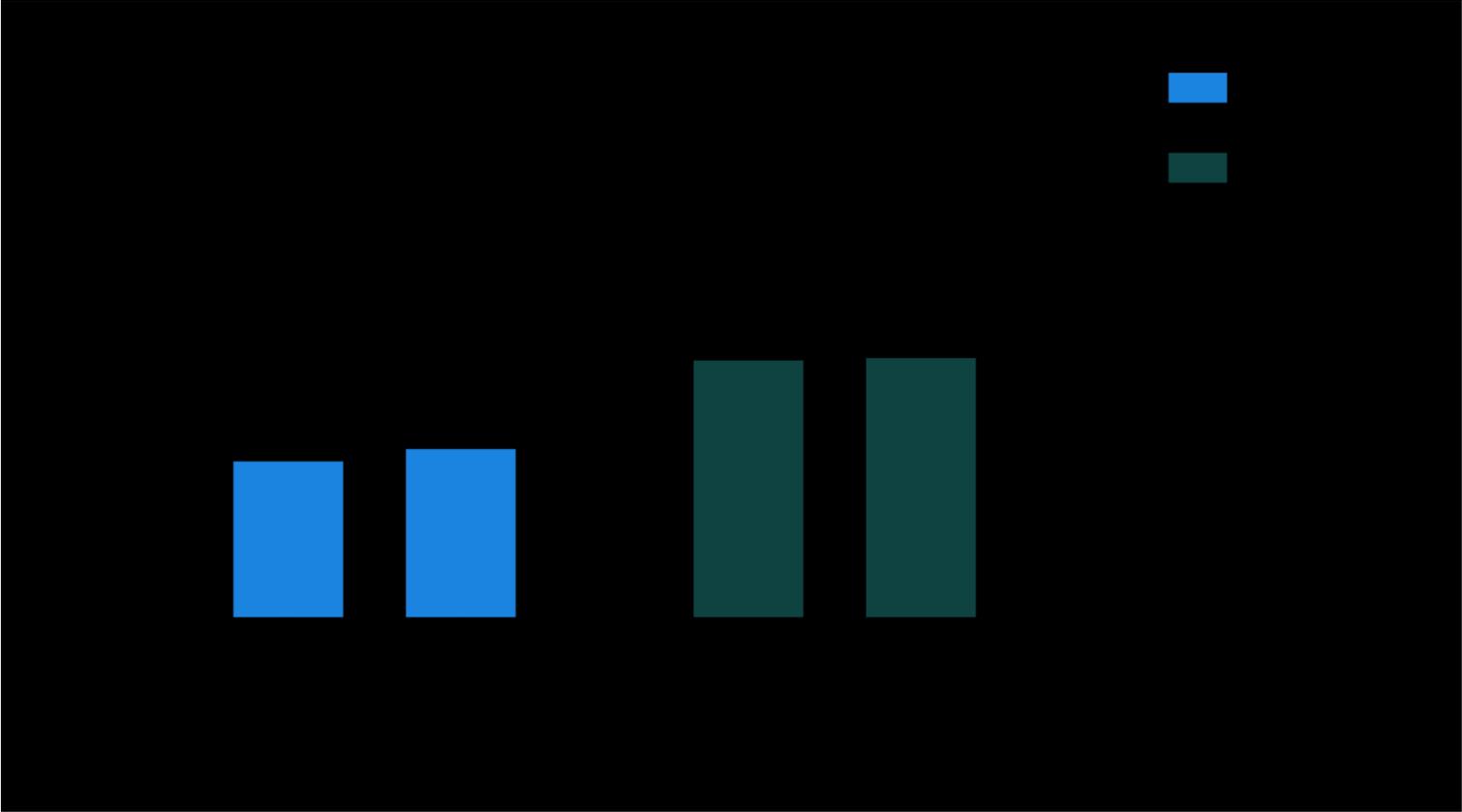
- Inert material that does not release any chemicals to the water
- Semitransparent to allowed observation of the fish
- Avoid monopolisation by dominant fish
- Adjusted according to fish density and tank size
- Easy to exchange and clean

Environmental enrichment made of medical silicone



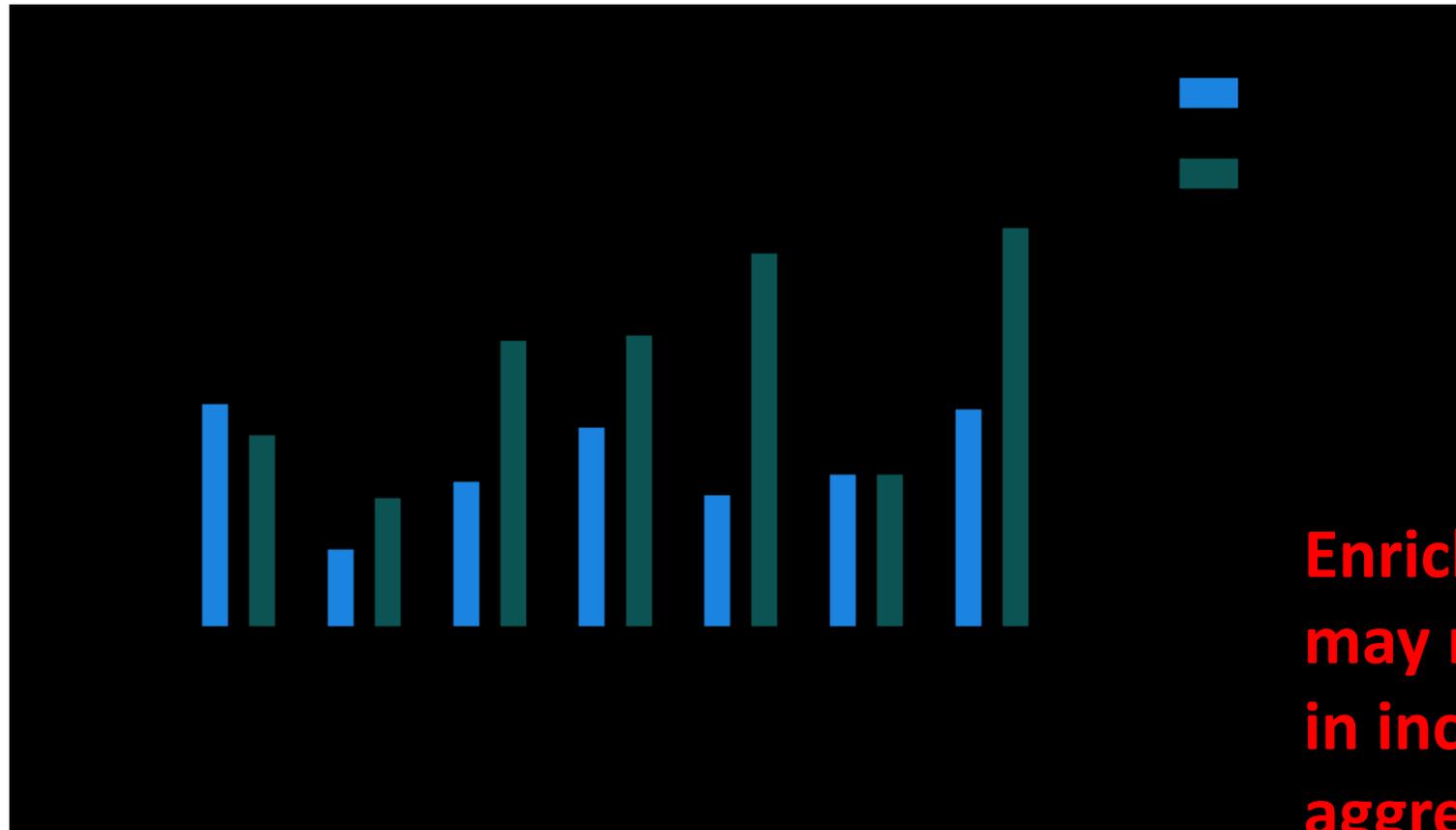
Sen Sarma et al. unpublished

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

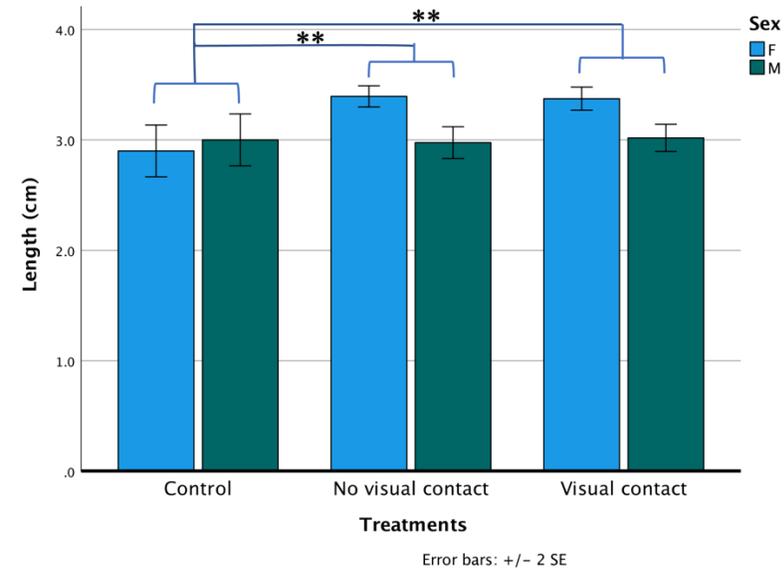
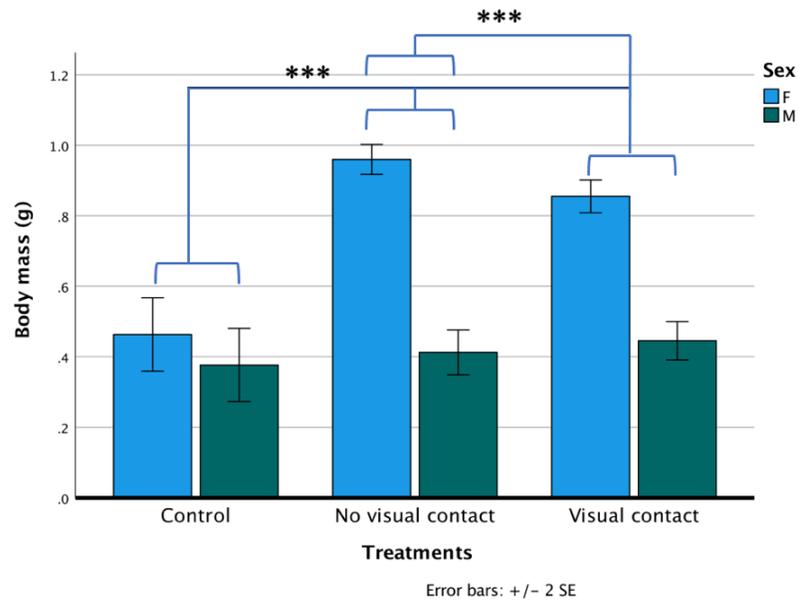
- Behaviour of individual fish
- Sampling, DNA etc
- Treatment etc



**Isolation with
or without
visual contact**



Long-term isolation



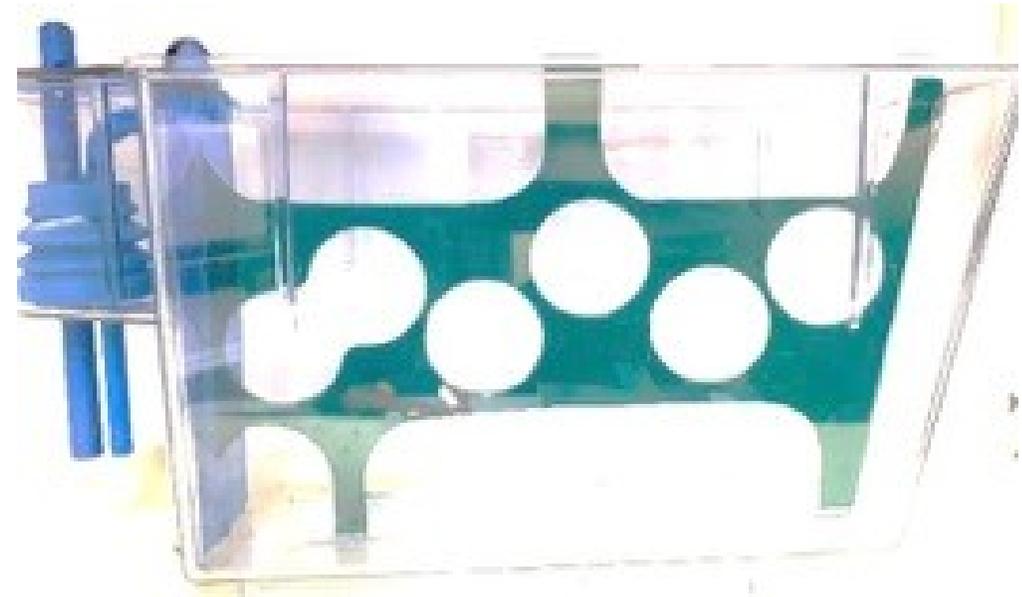
Sen Sarma et al. unpublished

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;**

Comersially 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

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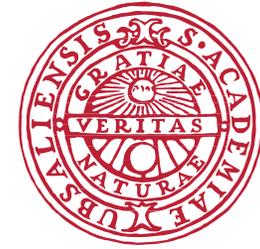
The Swedish Research Council



The Facias Foundation



Thank you for listening
Questions?



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